Next-Generation Voltage Imaging Sensor

Development of New-Generation Microbial Rhodopsins with Enhanced Voltage-Sensitivity using Molecular Dynamics Simulations

S. Hwang, C. Demidova, T. Utesch, H. Sun, *Technische Universität Berlin, and Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)*

In Short

- Molecular dynamics simulations applying membrane potentials
- Investigation of voltage-dependent conformational changes of microbial rhodopsins
- Investigation of the effect of retinal isomerization
- Rational design of microbial rhodopsin-derived voltage sensors for optogenetic application

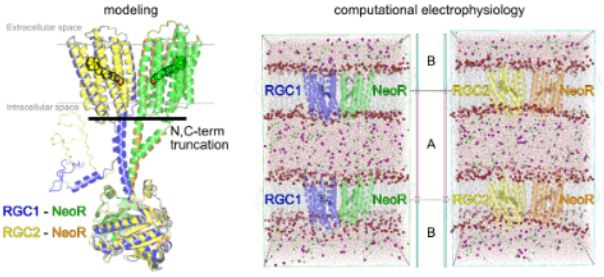

Figure 1: *Computational electrophysiology of RGC1-NeoR and RGC2-NeoR: The heterodimer RGC1 (blue cartoon) - NeoR (green cartoon) and the heterodimer RGC2 (yellow cartoon) - NeoR (orange cartoon) were predicted using AlphaFold2-Multimer [4]. The covalently bound retinal to a lysine in the NeoR is represented by sphere model. (right panel) The system used in the computational electrophysiology simulations, consisting of two lipid bilayers (grey sticks and red spheres), each including RGC1/RGC2-NeoR heterodimers, surrounded by water (red lines), potassium ions (purple spheres), and chloride ions (green spheres). Periodic boundary conditions create two compartments, A and B, with distinct charge imbalance. Thus, a transmembrane voltage gradient is established across each membrane.*

The application of light-sensitive proteins has revolutionized the neurosciences since 2005. Up to now the main players have been light-gated ion channels that catalyze the transport of ion across membranes (vectorial catalysis).[1] In 2014 the first enzyme rhodopsin has been discovered, the rhodopsin-guanylyl-cyclase (RGC) of *Blastocladiella emersonii*.[2] These RGCs have not only defined a completely new and totally uncharacterized family of proteins, but they also bear great potential for optogenetic applications as they can be used to optically trigger cGMP-dependent signaling processes and/or open cGMP-gated ion channels in different cell types. The fact that rhodopsin can modulate the activity of a C-terminal enzymatic unit was totally unexpected discovery. Hegemann and collaborators have identified and characterized different RGCs including a heterodimeric RGC with a green rhodopsin as light sensor and NeoR as light modulator.[3] NeoR is the rhodopsin with most near-infrared (NIR) sensitivity, hence highly interesting for any in vivo applications. It is designed to fine tune the catalytic activity by a so far unknown mechanism. This highly unusual NeoR needs to be characterized in order to capitalize their properties for NIR optogenetics.

In this regard, molecular dynamics (MD) simulation is an important theoretical approach for understanding functional dynamics of the heterodimeric RGCs-NeoR at the atomic scale. Within this project, we will use this method to investigate retinal *cis* isomerization-induced conformational changes of the rhodopsins and effect of mutation in influencing integrity of retinal binding pocket. As voltage-dependent modulation of NeoR fluorescence has not been observed to date, it is highly desirable to develop new NeoR variants with considerably voltage-sensitivity. As our previous theoretical and experimental studies has already contributed significantly to the understanding of voltage-sensitivity in archaerhodopsins (**project ID: bec00212**).[5] We will extend this integrative approach here for designing new NeoR variants with desired voltage-sensitivity. Furthermore, it should be noted that previous simulations of archaerhopdopsins were solely carried out with retinal in the all-*trans* configuration [5], which is thought to be the ground state in the rhodopsin photocycle. Further studies on the effect of transmembrane voltages on other intermediates of the photocycle in archaerhodopsins using atomistic MD simulations will be highly interesting. Here we will employ MD-based computational electrophysiology [6] (Figure 1) and alchemical free-energy calculations [7] (Figure 2) to predict relative free-energy changes upon mutation of the rhodopsins or conformational transition of the retinal at different applied membrane voltages. These predicted free-energy changes offer a quantitative evaluation of the stability of the chromophore binding pocket which is most likely to be coupled to the fluorescence quantum yield. The advantage of this state-of-the-art computational method is to avoid extremely long and computational unfeasible simulations, e.g. the transition between all-*trans* and 13-*cis* retinal. Experimentally these insights are very difficult to obtain but

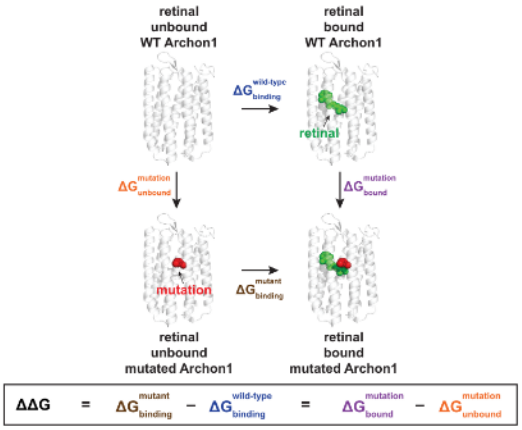

Figure 2: *Thermodynamic cycle for alchemical free-energy calculation: Relative free energy calculations of the binding of retinal between Archon1 and Archon1 mutant.*

are essential for the understanding of the underlying mechanism.

As a summary, within this project we have three major objectives on different microbial rhodopsins: (a) Understand the effect of membrane voltages on the retinal *cis-trans* isomerization in archaerhodopsins, (b) predict new-generation archaerhodopsins with enhanced voltage-sensitivity using alchemical free-energy calculations, and (c) induce voltage sensitivity to a rhodopsin-guanylyl cyclase NeoR and investigate the mechanism of how membrane voltage regulates enzyme function using atomistic MD simulations.

WWW

https://www.leibniz-fmp.de/sun

More Information

[1] K. Deisseroth, P. Hegemann, *Science* **357**, eaan5544 (2017). doi:10.1126/science.aan5544

[2] G. M. Avelar, R. I.Schumacher, P. A. Zaini, G. Leonard, T. A. Richards, S. L. Gomes, *Curr. Biol.* **24**, 12341240 (2014). doi: 10.1016/j.cub.2014.04.009

[3] M. Broser, A. Spreen, P. E. Konold, E. Peter, S. Adam, V. Borin, I. Schapiro, R. Seifert, J. T. M. Kennis, Y. A. B. Sierra, P. Hegemann, *Nat. Communications* **11**, 5682 (2020). doi: doi.org/10.1038/s41467-020-19375-8

[4] R. Evans, M. O'Neill, A. Pritzel, N. Antropova, A. Senior, T. Green,A. Zidek, R. Bates, S. Blackwell, J. Yim, O. Ronneberger, S. Bodenstein, M. Zielinski, A. Bridgland, A. Potapenko, A. Cowie, K. Tunyasuvunakool, R. Jain, E. Clancy, P. Kohli, J. Jumper, D. Hassabis, *bioRxiv* doi:10.1101/2021.10.04.463034

[5] A. Silapetere, S. Hwang, Y. Hontani, R. F. 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, P. Hegemann, *Nat. Communications*, accepted with minor corrections (2022).

[6] C. Kutzner, H. Grubmller, B. L. de Groot, U. Zachariae, *Biophys. J.* **101**, 809-817 (2011). doi:10.1016/j.bpj.2011.06.010

[7] M. Aldeghi, B. L. de Groot, V. Gapsys, *Methods Mol. Biol.* **1851**, 19-47 (2019). doi: 10.1007/978-1-4939-8736-8_2

Project Partners

(1) Experimental Biophysics Group, Humboldt-Universitt 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