Next-generation optogenetic tools

Development of new-generation microbial rhodopsins with enhanced voltage-sensitivity and ion selectivity using molecular dynamics simulations and QM/MM based vertical excitation energy calculations

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In Short

- Molecular dynamics simulations applying computational electrophysiology method
- Investigation of ion permeation and selectivity in channelrhodopsins
- QM/MM refinement and calculation of vertical excitation energies to characterize channelrhodopsins

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 favorable characteristics, such as adapted photo excitation properties, bistability, or specific ion selectivity.[7]

Recently, light gated potassium-selective channelrhodopsins (KCRs) were discovered in *Hyphochytrium catenoides* (Hc), which allow for precise optogenetic silencing.[8,9] Interestingly, these channelrhodopsins discriminate K^+ from Na^+, i.e. they are potassium selective, without the conventional selectivity filter formed by the "signature sequence" of classical tetrameric potassium channels.[10] In addition, the selectivity of HcKCR1, on of the discovered potassium selective channelrhodopsin in the genome of Hc, was attributed to just two transmembrane helices and three key

residues, which due to the low complexity of the structural basis provides an excellent basis for an in depth investigation of the ion conduction mechanism and selectivity. In addition, in 2022 targeted mutation was used to engineer the first calcium selective channelrhodopsins, with a sufficiently high selectivity for Ca^{2+} to be employed as "genetically encoded calcium actuators".[11]

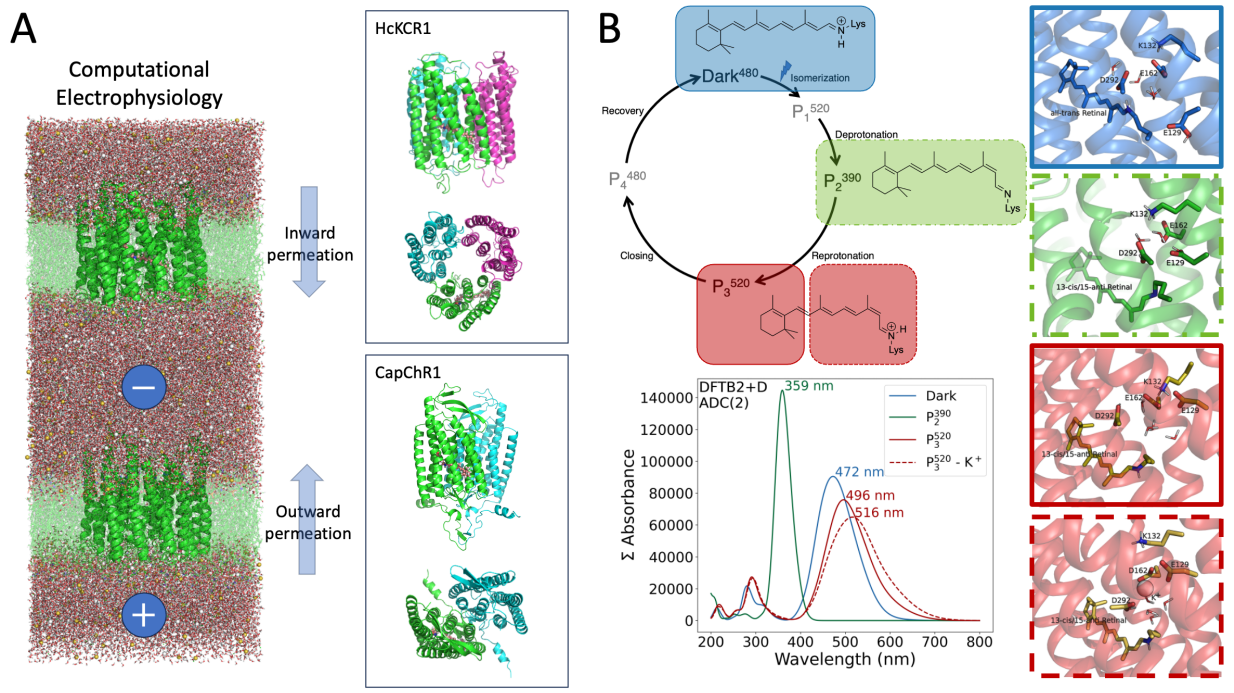

Figure 1: (A) exemplification of computational electrophysiology simulation setup for potassium selective channelrhodopsin HcKCR1, with depiction of inward and outward permeation direction, as well as front and top views of channelrhodopsins HcKCR1 (trimeric quaternary structure) and CapCR1 (dimeric quaternary structure). (B) Photocycle and experimental UV-Vis absorption exemplified for channelrhodopsin C1C2, accompanied by examples of QM/MM refined structures and preliminary DFTB2+D//ADC(2)/cc-pVDZ calculated UV-Vis Spectrum (3 trajectories for dark and P_3^{520} states, 1 trajectory for P_2^{390} state) .

This project encompasses two main objectives. The first aims to unravel the ion conduction mechanisms and ion selectivity in these newly discovered and engineered channelrhodopsins using molecular dynamics simulations under transmembrane potentials. That is, the simulations will be performed using Computational Electrophysiology method allowing a simultaneous simulation of in- and outward ion conduction within a single MD run, as exemplified in Figure 1A. This method allows for an unbiased exploration of ion conduction in various naturally occurring and engineered channelrhodopsins. The second objective is to characterize the photo properties of the investigated channelrhodpsins by hybrid quantum mechanic/molecular mechanics simulations and calculation of vertical excitation energies by high level post-Hartree–Fock methods. These calculations should allow us to better quantify the hydrogen bonding network, protonation states of titratable residues close to the retinal chromophore and water occupancy in the chromophore binding pocket, as these excitations are extremely sensitive to the electrostatic embedding by the opsin environment, as also highlighted by the photocycle and preliminary UV-Vis spectra for channelrhodopsin C1C2 (Figure 1B).

References

(1) Zhang, C.; Vierock, J.; Yizhar, O.; Fenno, L. E.; Tsunoda, S.; Kianianmomeni, A.; Prigge, M.; Berndt, A.; Cushman, J.; Polle, J.; J. Magnuson, P. H.; Deisseroth, K. The microbial opsin family of optogenetic tools. *Cell* **2011**, *147*, 1446–1457.

(2) Kim, C. K.; Adhikari, A.; Deisseroth, K. Integration of optogenetics with complementary methodologies in systems neuroscience. *Nat. Rev. Neurosci.* **2017**, *18*, 222–235.

(3) Sahel, J.; Boulanger-Scemama, E.; Pagot, C.; Arleo, A.; Galluppi, F.; Martel, J. N.; Esposti, S. D.; Delaux, A.; Aubert, J. S.; Montleau, C.; Gutman, E.; Audo, I.; Duebel, J.; Picaud, S.; Dalkara, D.; Blouin, L.; Taiel, M.; Roska, B. Partial recovery of visual function in a blind patient after optogenetic therapy. *Nat. Med.* **2021**, *27*, 1223–1229.

(4) Sengupta, A.; Chaffiol, A.; Macé, E.; Caplette, R.; Desrosiers, M.; Lampič, M.; Forster, V.; Marre, O.; Lin, J. Y.; Sahel, J.; Picaud, S.; Dalkara, D.; Duebel, J. Red-shifted channelrhodopsin stimulation restores light responses in blind mice, macaque retina, and human retina. *EMBO Mol. Med.* **2016**, *8*, 1248–1264.

(5) Nagel, G.; Ollig, D.; Fuhrmann, M.; Kateriya, S.; Musti, A. M.; Bamberg, E.; Hegemann, P. Channelrhodopsin-1: A Light-Gated Proton Channel in Green Algae. *Science* **2002**, *296*, 2395–2398.

(6) Nagel, G.; Szellas, T.; Huhn, W.; Kateriya, S.; Adeishvili, N.; Berthold, P.; Ollig, D.; Hegemann, P.; Bamberg, E. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *PNAS* **2003**, *100*, 13940–13945.

(7) Deisseroth, K.; Hegemann, P. The form and function of channelrhodopsin. *Science* **2017**, *357*, eaan5544.

(8) Govorunova, E. G.; Sineshchekov, O. A.; Brown, L. S.; Bondar, A.-N.; Spudich, J. L. Structural Foundations of Potassium Selectivity in Channelrhodopsins. *mBio* **2022**, *13*, e03039–22.

(9) Tajima, S.; Kim, Y. S.; Fukuda, M.; Jo, Y.; Wang, P. Y.; Paggi, J. M.; Inoue, M.; Byrne, E. F.; Kishi, K. E.; Nakamura, S.; Ramakrishnan, C.; Takaramoto, S.; Nagata, T.; Konno, M.; Sugiura, M.; Katayama, K.; Matsui, T. E.; Yamashita, K.; Kim, S.; Ikeda, H.; Kim, J.; Kandori, H.; Dror, R. O.; Inoue, K.; Deisseroth, K.; Kato, H. E. Article Structural basis for ion selectivity in potassium-selective channelrhodopsins. *Cell* **2023**, *186*, 4325–4344.

(10) MacKinnon, R. Potassium Channels and the Atomic Basis of Selective Ion Conduction (Nobel Lecture). *Angew. Chem. Int. Ed.* **2004**, *43*, 4265–4277.

(11) Fernandez-Lahore, R. G.; Pampaloni, N. P.; Schiewer, E.; Heim, M.-M.; Tillert, L.; Vierock, J.; Oppermann, J.; Walther, J.; Schmitz, D.; Owald, D.; Plested, A. J. R.; Rost, B. R.; Hegemann, P. Calcium-permeable channelrhodopsins for the photocontrol of calcium signalling. *Nat. Commun.* **2022**, *13*, 7844.