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Time-resolved serial crystallography at synchrotrons and XFELs

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Chloride transport is an essential process maintaining ion balance across cell membranes, cell growth, and neuronal action potentials. However, the molecular mechanism of the transport remains elusive. Among chloride transporters, light-driven rhodopsins have gained attention as optogenetic tools to manipulate neuronal signaling. We combined time-resolved serial crystallography (SwissFEL and SLS synchrotron) to provide a comprehensive view of chloride-pumping rhodopsin's structural dynamics and molecular mechanism throughout the transport cycle from 10 ps to 50 ms [1]. We traced transient anion binding sites, obtained evidence for the mechanism of light energy utilization in transport, and identified steric and electrostatic molecular gates ensuring unidirectional transport. These structural insights provided the basis for mutagenesis and functional study of the mechanistic features enabling finely controlled chloride transport across the cell membrane.

Furthermore, I will present insights into the photochemistry and selectivity of retinal isomerization in proton-pumping rhodopsin [2] and show that, in favorable cases, even larger structural changes can be captured in crystals [3].

Our recent study of a distinct photoreceptor, Light-Oxygen-Voltage (LOV) domain, will be introduced [4,5]. The first insights into the structural dynamics of LOV photoactivation will be presented, providing the basis for proposing a molecular mechanism of a covalent thioether bond formation between a flavin mononucleotide cofactor and a reactive cysteine, Cys57 (unpublished).

References

1. Mous, S. et al. *Science* 375 (2022) 845.
2. Nogly P et al., *Science* 361 (2018), eaat0094.
3. Weinert et. al., *Science* 365 (2019), 61.
4. Gotthard et al. *IUCrJ* (2024), 749.
5. Gotthard et al. *IUCrJ* (2024), 792.

Primary author: NOGLY, Przemysław (Jagiellonian University in Krakow)

Presenter: NOGLY, Przemysław (Jagiellonian University in Krakow)

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