Nogly group
Institute of Molecular Biology and Biophysics

Studying a light-driven chloride pump at SLS and SwissFEL

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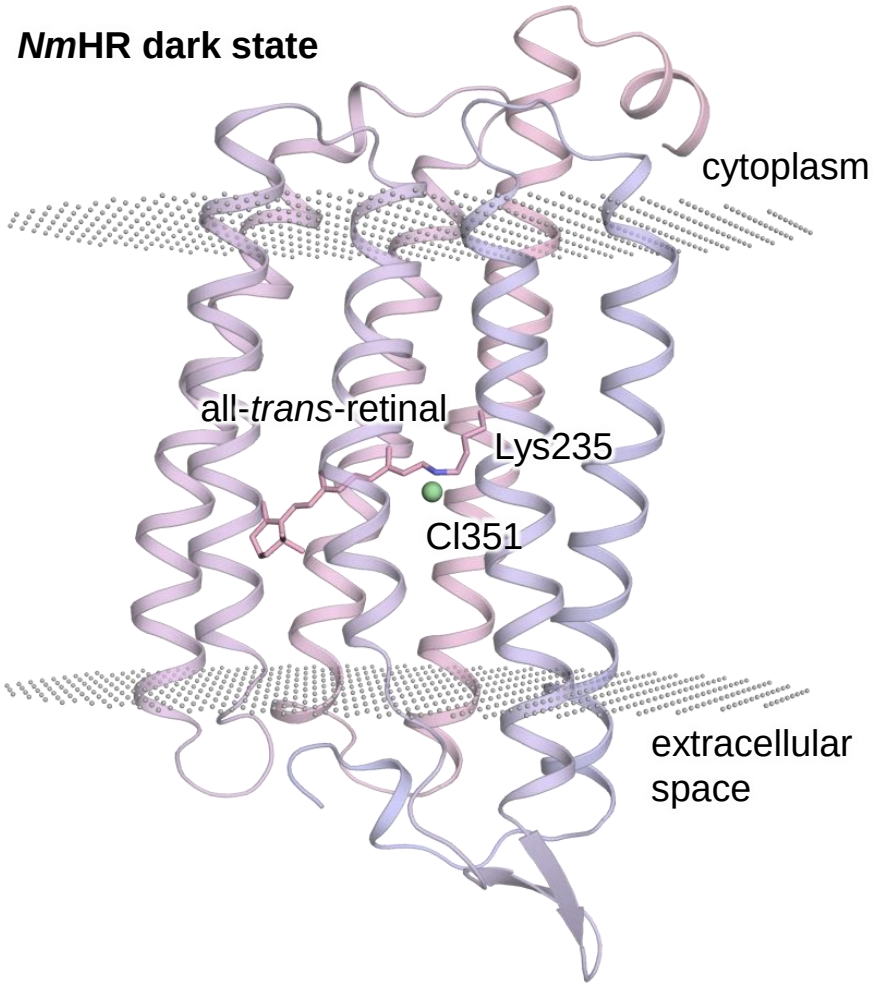
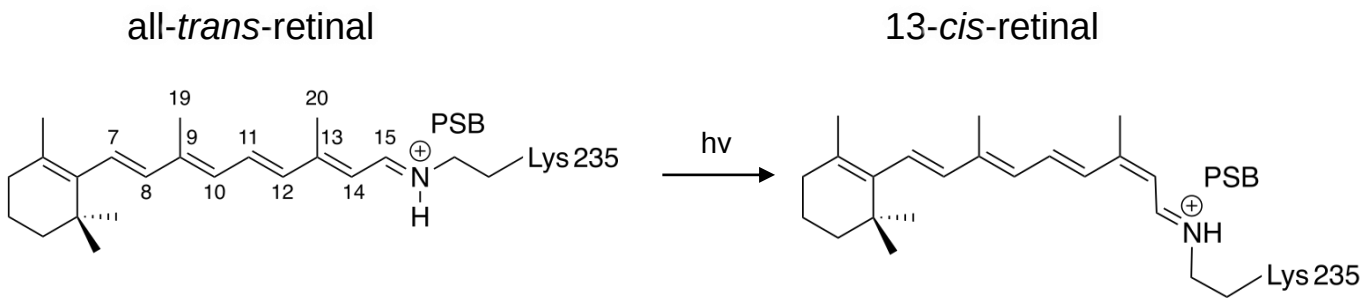
MX user meeting
1st of March, 2022

An introduction to light-driven chloride transport

- *Nonlabens marinus* halorhodopsin (*Nm*HR) is an inward chloride pumping rhodopsin
- Characteristic 7 transmembrane α-helices
- Retinal chromophore covalently bound via a positively charged protonated Schiff base (PSB) to a lysine residue
- Retinal photoisomerization followed by conformational changes in the protein and ion transport
- Chloride is bound in the dark state and released after photoactivation





Yoshizawa *et al.* (2014) *PNAS* **111**: 6732-6737. Kim *et al.* (2016) *Nat Comm* **7**: 12677. Hosaka *et al.* (2016) *J Biol Chem* **291**: 17488-17495.

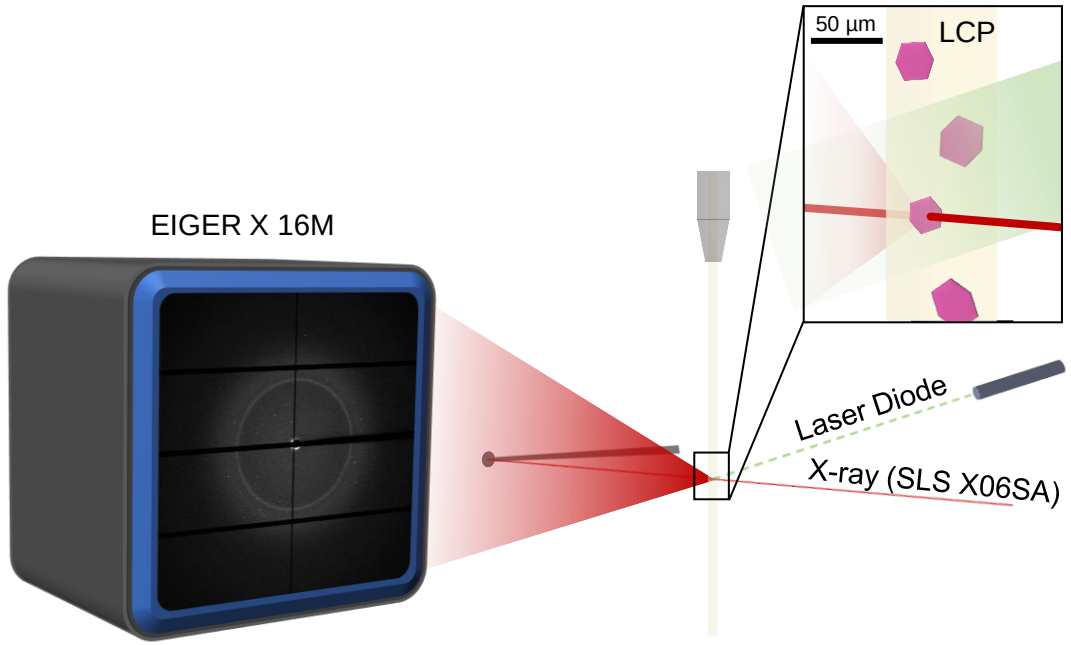
Open questions about chloride transport by *Nm*HR

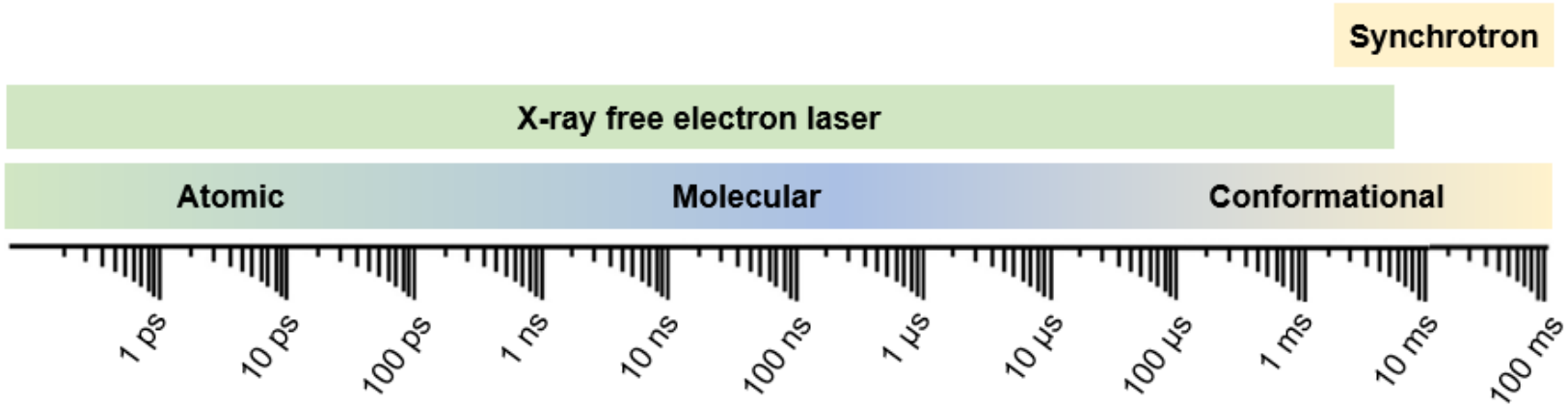
- What is the anion transport pathway?
- How is photon energy converted to drive transport?
- How can chloride pass the steric bottleneck that is encountered at the retinal chromophore?
- How does *Nm*HR control a unidirectional anion flow?

→ Time-resolved serial crystallography

Advantages of serial crystallography

- Serial crystallography
  - Multiple smaller crystals
  - Room temperature measurement
  - Time-resolved studies with a pump-probe setup
- Performed at the X-ray free electron laser (XFEL) or synchrotron light source
- Mechanism can be probed in a wide time range

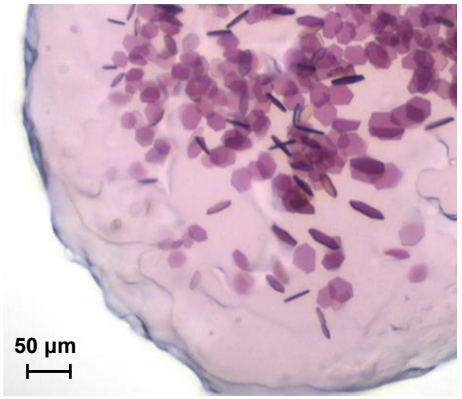




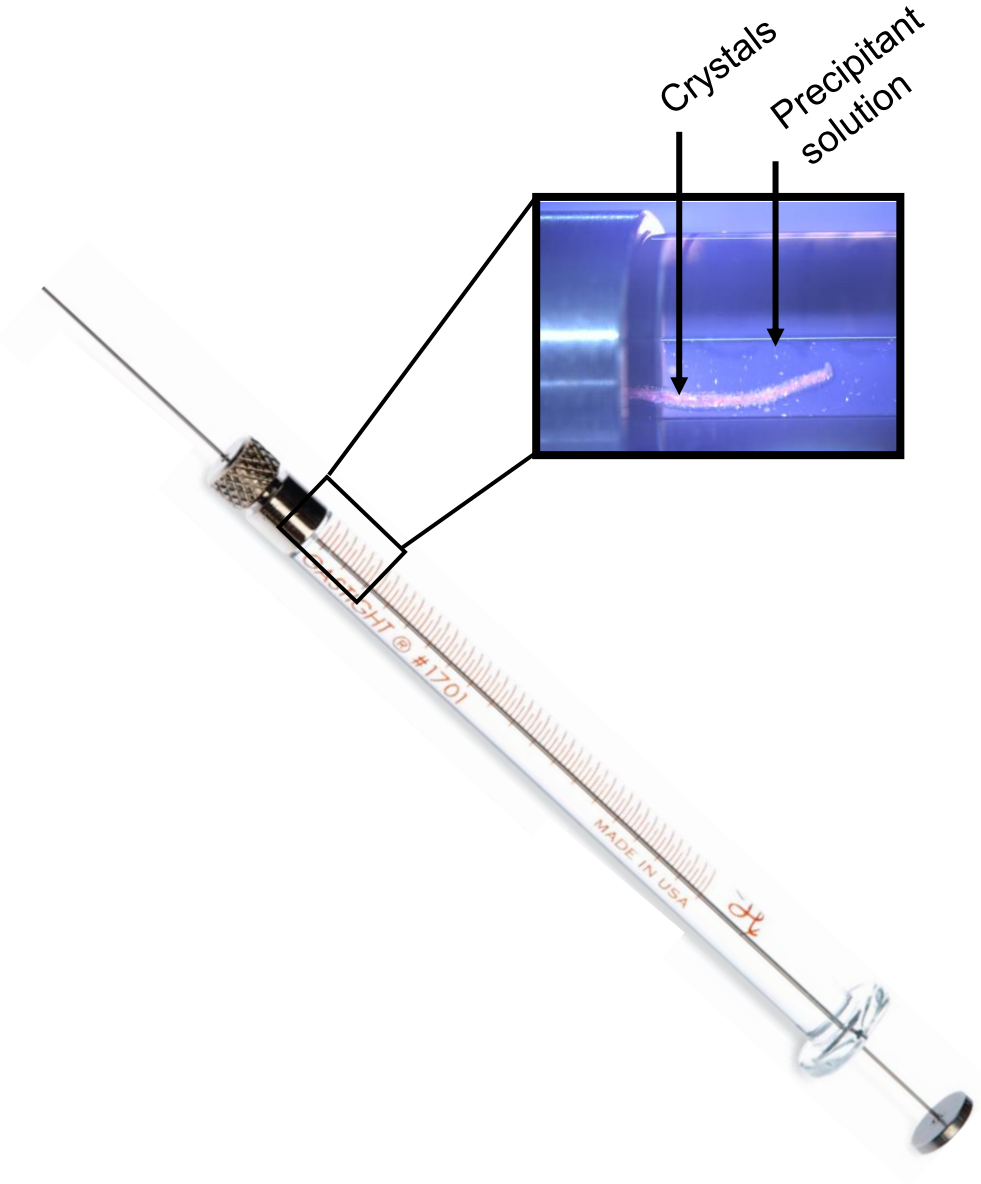
Time scale of motion as published by Standfuss (2019) *Curr Op Struc Biol* 57: 63-71

Sample challenges

- Reproducible expression and purification
- High protein sample purity for successful crystallization
- Homogeneous crystalline sample with a defined crystal size

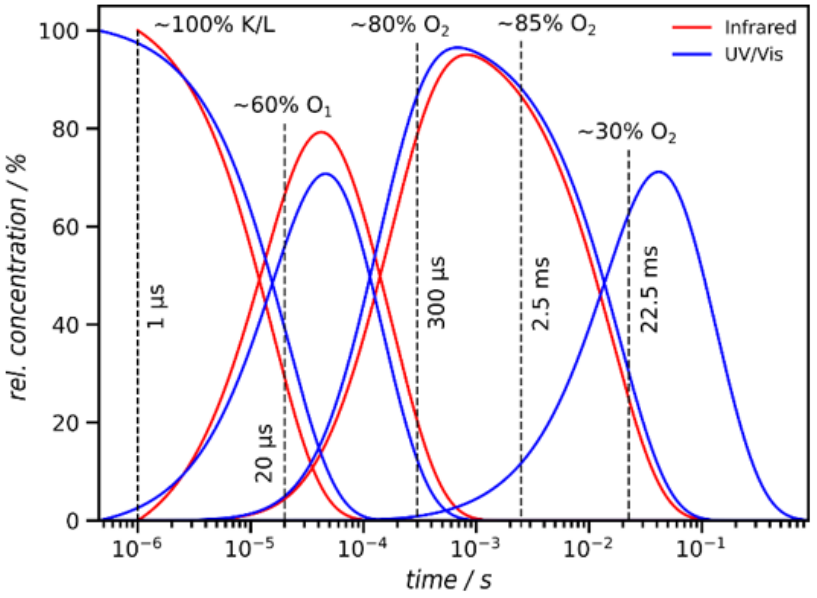


*Nm*HR crystals in
lipidic cubic phase

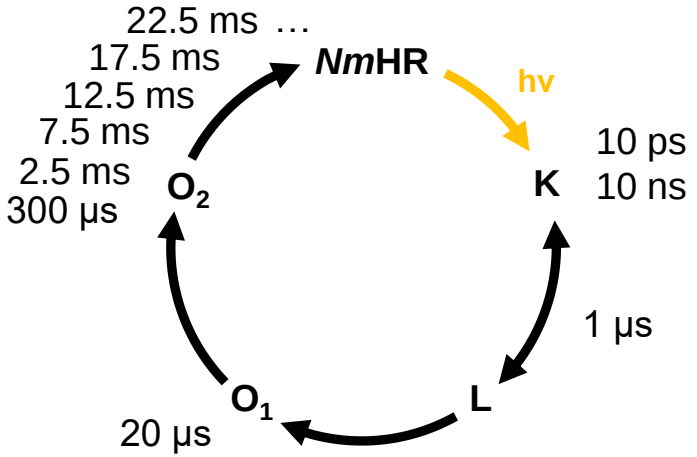


- Large volume of crystalline sample required
  - XFEL: 4 ml of crystals per 24 hours
  - Synchrotron: 120 µl of crystals per 24 hours

Probing the *Nm*HR photocycle



- Probed the *Nm*HR photocycle with UV-Vis and infrared crystal spectroscopy
- Determined the active state structure up to microseconds after photoactivation at SwissFEL and in the millisecond time domain at SLS X06SA



Mous *et al.* (2022) *Science* 375: 845-851. Time-resolved spectroscopy performed in collaboration with the Heberle group, FU Berlin.

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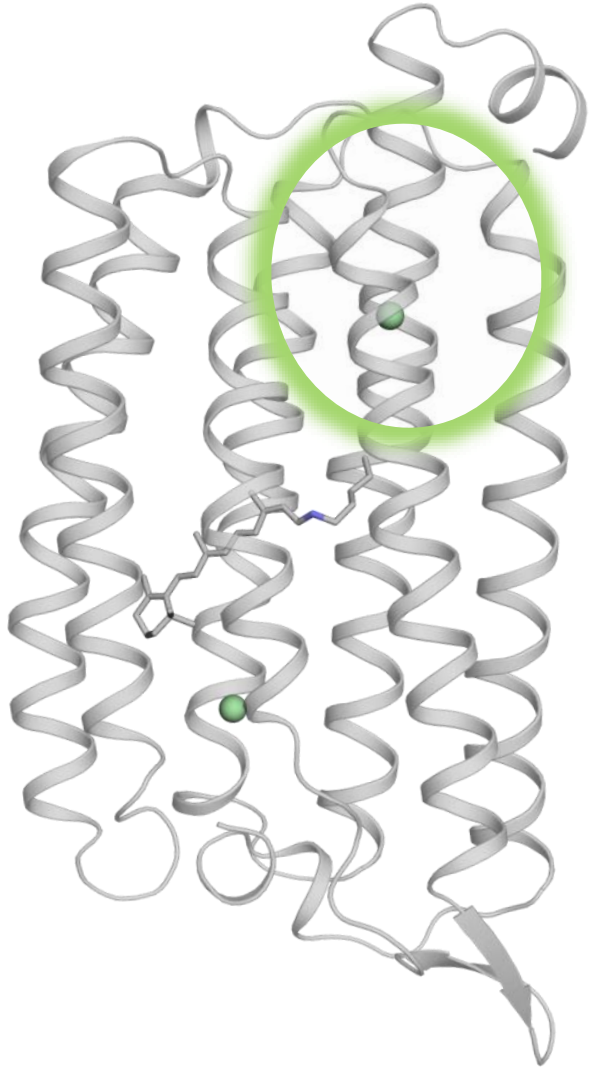
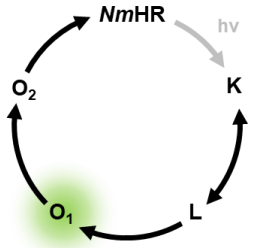
Chloride release

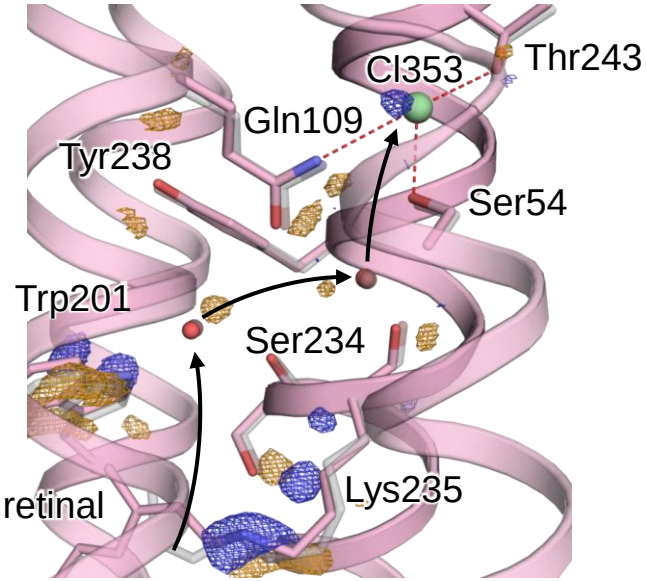
Δt = 20 µs



Chloride release at Δ*t* = 20 µs



Difference Fourier map
$F_{obs,20\ \mu s} - F_{obs,\ dark}$ at 3.0 σ



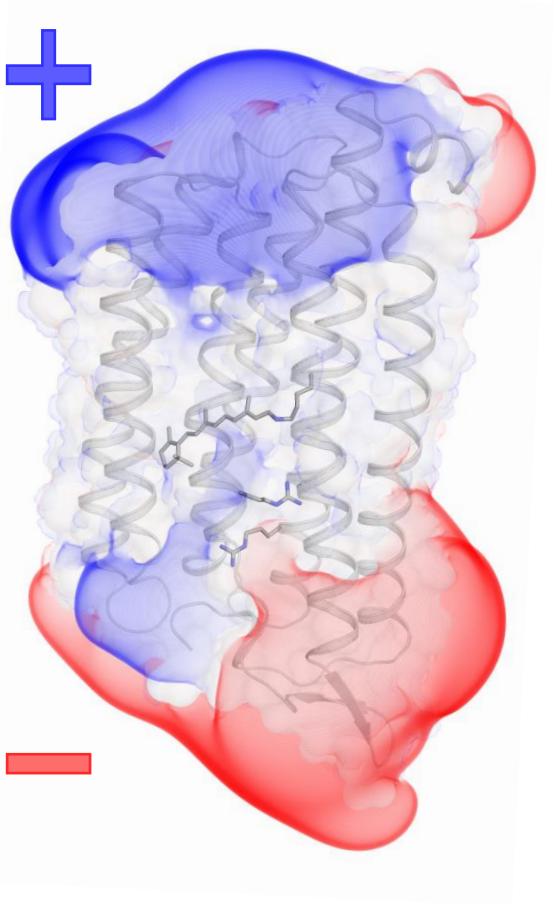
Positive density difference

Negative density difference

Δ*t* = 20 µs

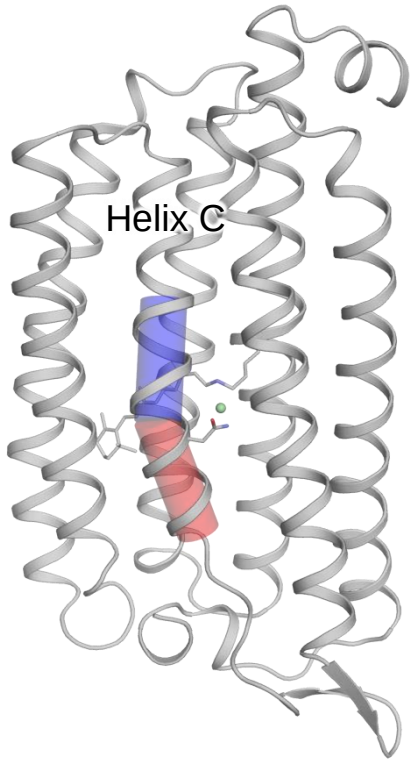
Dark state

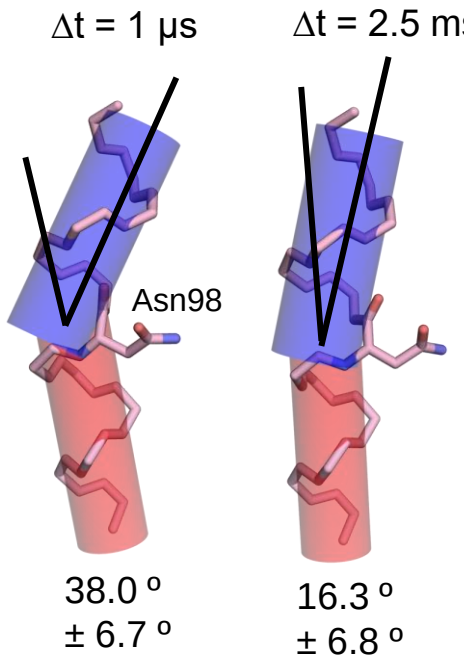
Electrostatic isopotential contour map at ± 1 k_bT/e_c

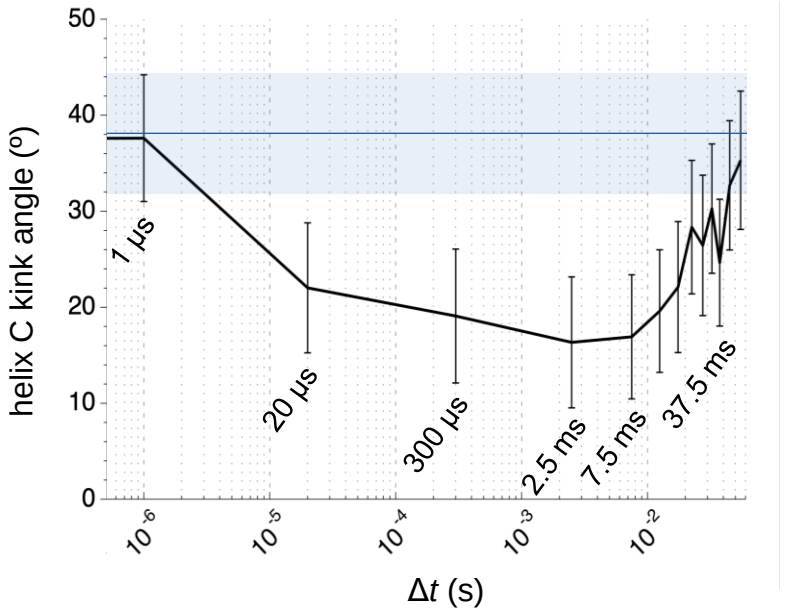


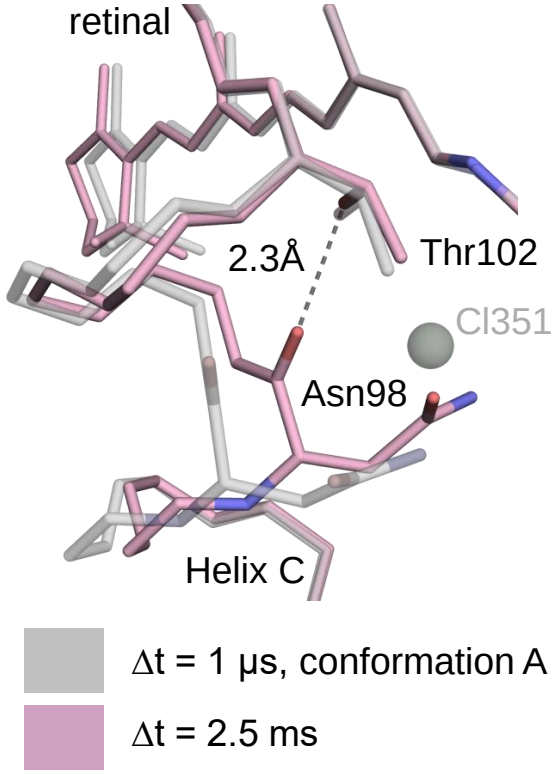
- The transient Cl353 binding site is formed in the exit tunnel at Δ*t* = 20 µs
- Release of the anion into the cytoplasm is driven by a macroscopic dipole across the protein

Mechanism for a unidirectional anion flow: the steric gate









- Evolution of helix C conformation following Cl351 depletion
- Straightening of helix C brings the Asn98 side chain into the depleted Cl351 binding site, closing the steric gate

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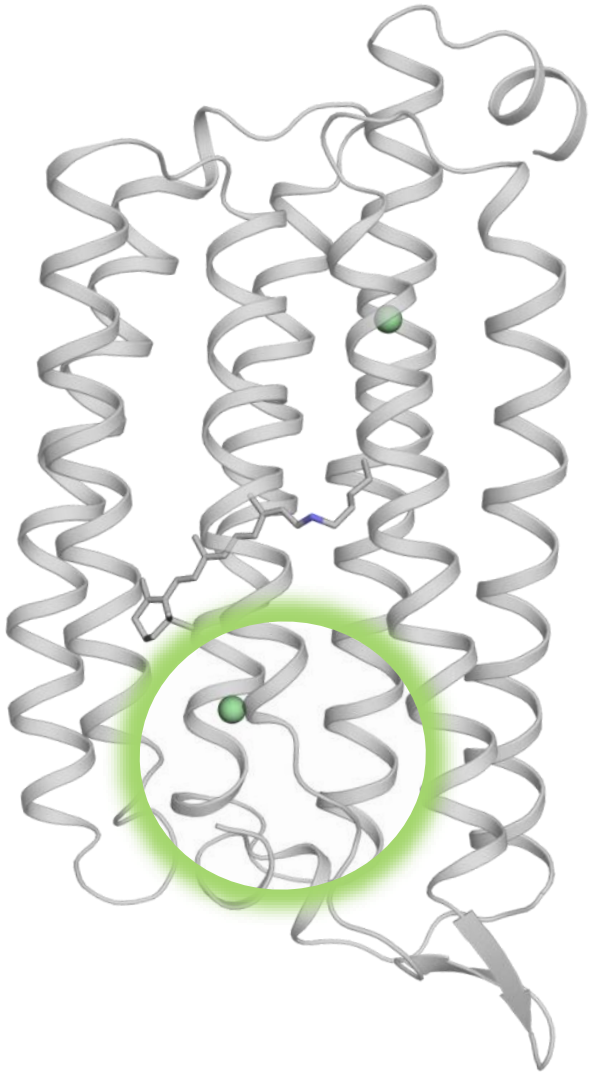
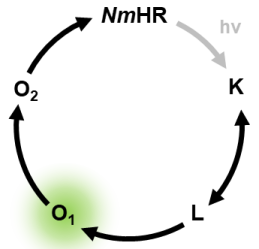
Chloride uptake

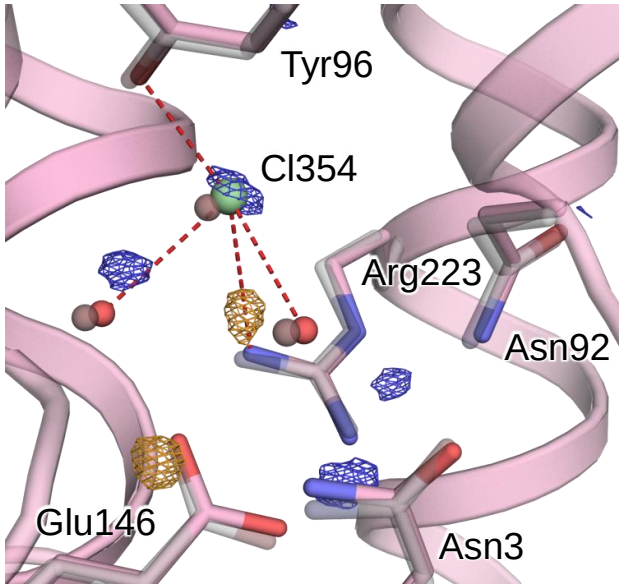
$\Delta t \geq 20$ µs



Chloride uptake at Δt = 20 µs



Difference Fourier map
$F_{obs,20\ \mu s} - F_{obs,\ dark}$ at 3.0 σ



Positive density difference

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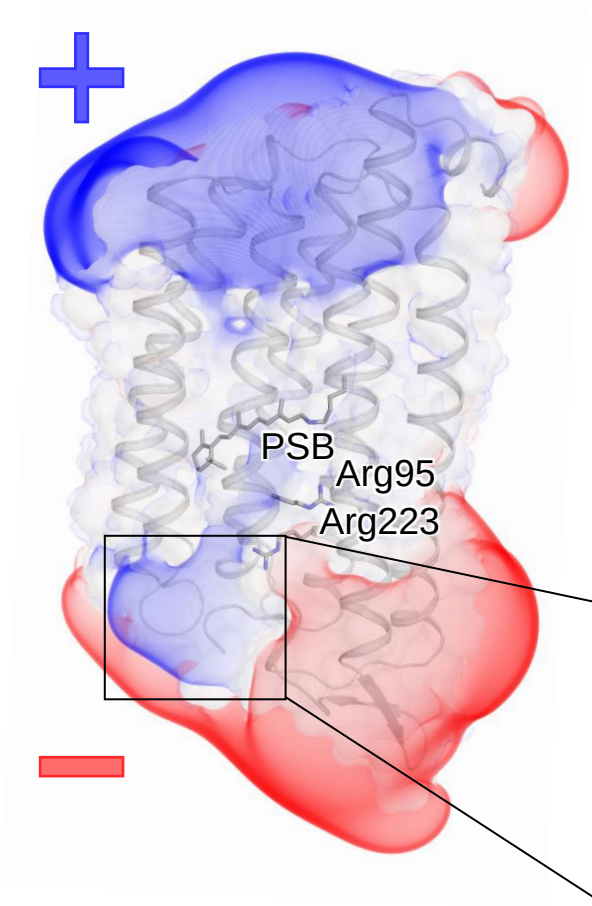
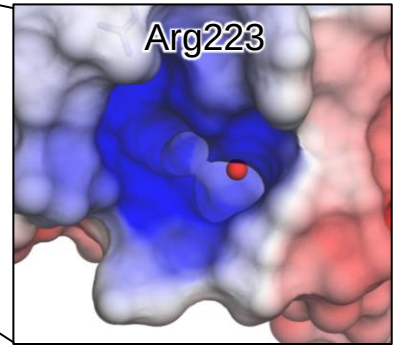
Dark state

Electrostatic isopotential contour map at ± 1 k_bT/e_c



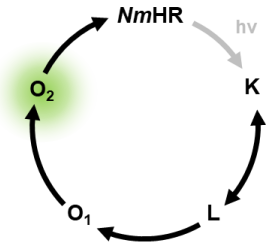
Entrance cavity

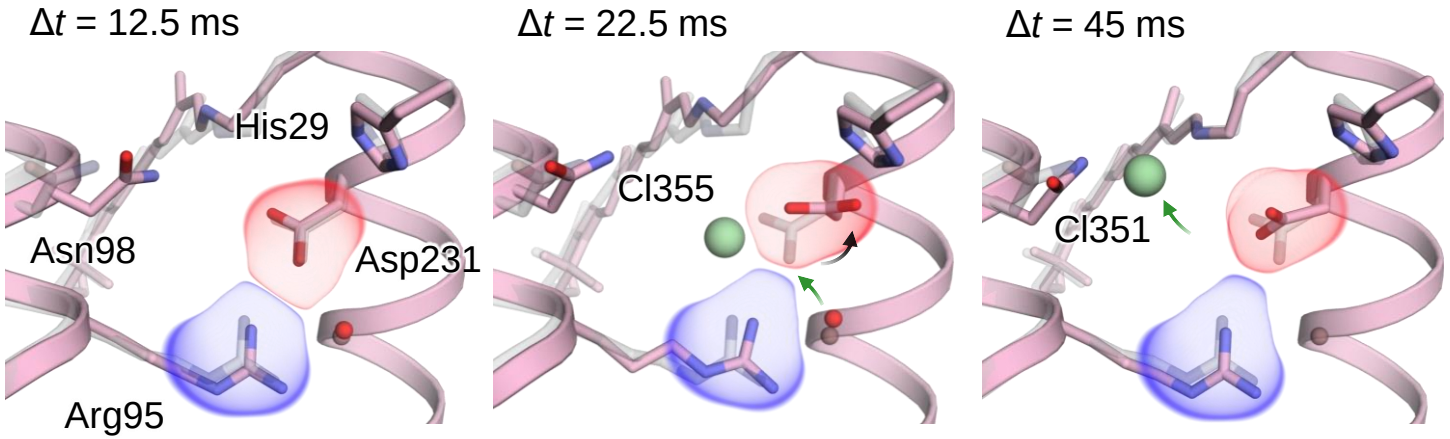


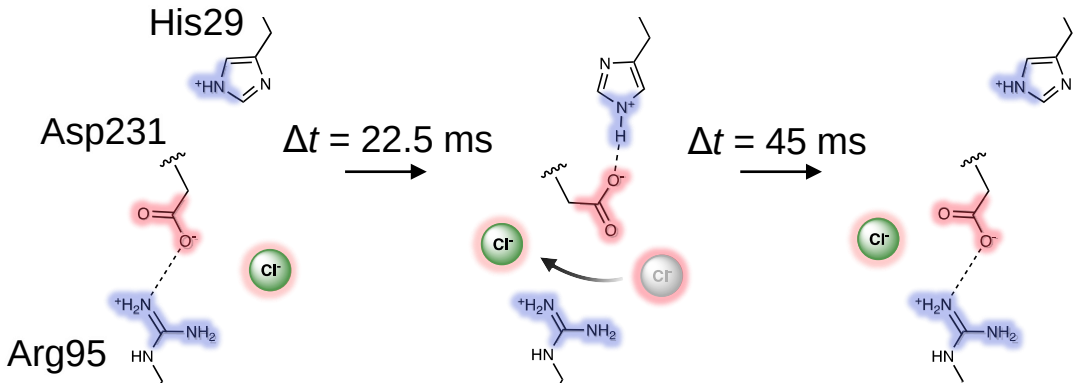
- The transient Cl354 binding site is formed in the triple water cluster at Δt = 20 µs
- Uptake of the anion into the cytoplasm is directed by the positively charged patch and driven by Arg223, Arg95, and the PSB

Mous *et al.* (2022) *Science* 375: 845-851.

Ensuring a unidirectional anion flow: the electrostatic gate



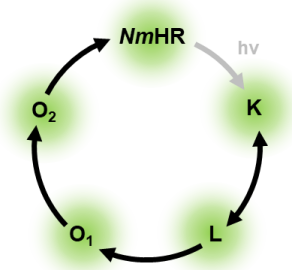


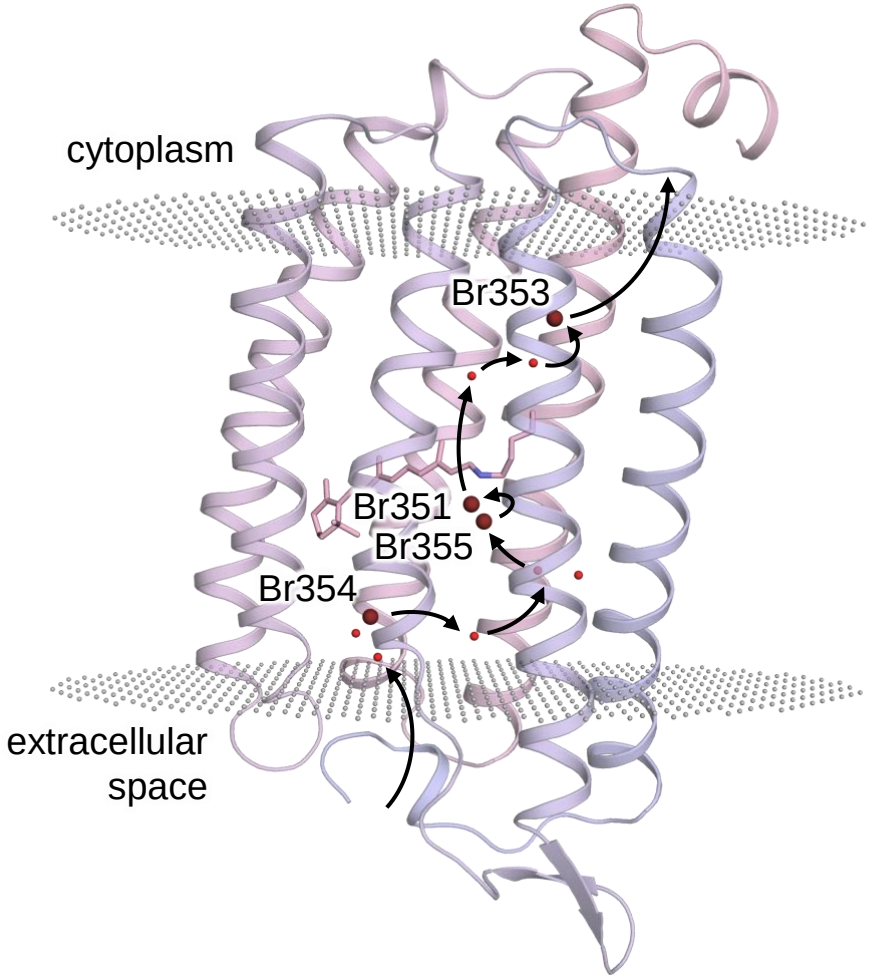


- The anion encounters a salt bridge between Arg95 and Asp231 in the uptake channel, the electrostatic gate
- After a conformational change in the Asp231 side chain at Δ*t* = 22.5 ms, the transient Cl355 binding site is formed
- The nearby dark state Cl351 binding site is regenerated at Δ*t* = 45 ms and the gate closes

Mous *et al.* (2022) *Science* 375: 845-851.

New approach to trace transient binding sites

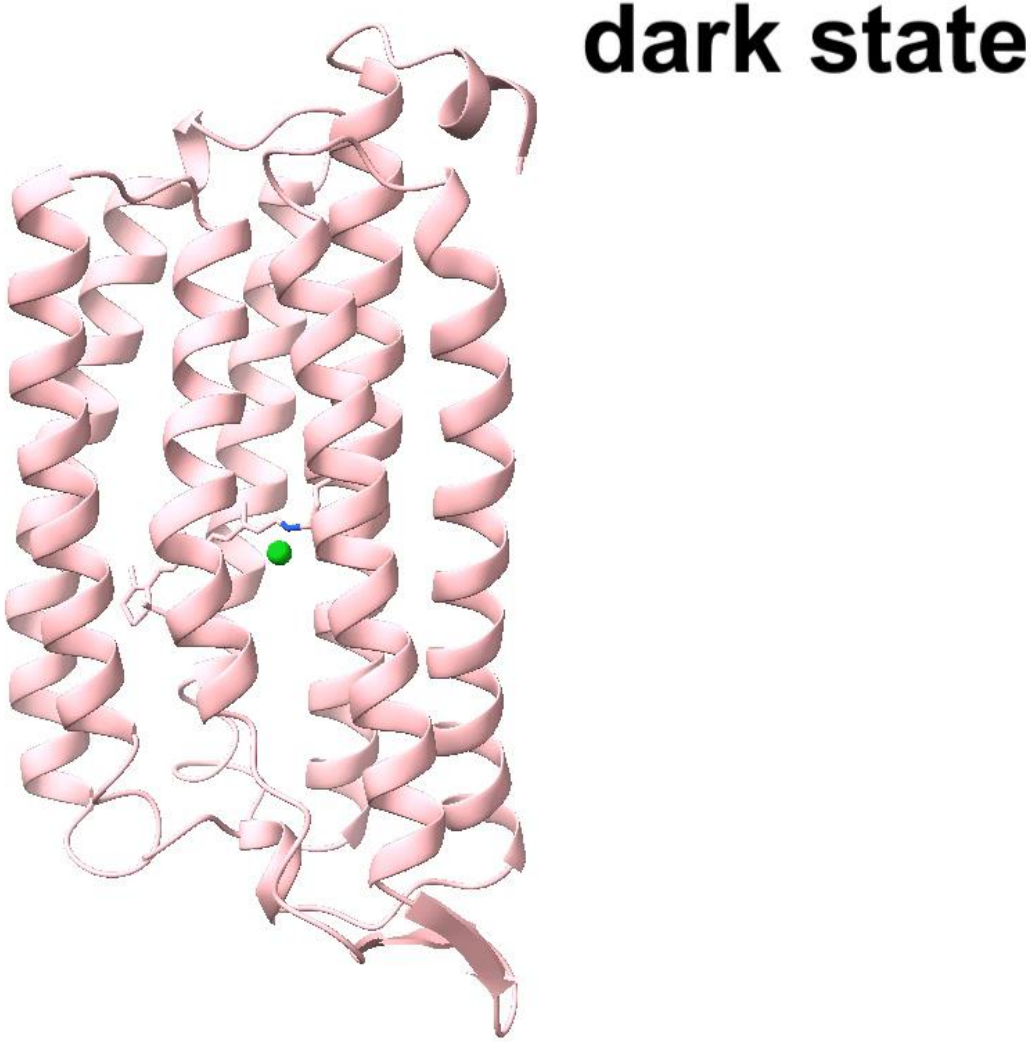




- Crystals were soaked in a bromide-containing precipitant solution
- Measured anomalous dispersion by bromide ions with a 13.7 keV X-ray energy with crystals at room temperature **under continuous illumination** → mixture of states
- Anomalous difference density peaks were the basis for tracing the anion transport pathway

Mous *et al.* (2022) *Science* 375: 845-851.

A molecular movie of *Nm*HR



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Conclusions

- Combined time-resolved crystallography experiments at SwissFEL and SLS to refine *Nm*HR structural intermediates from picoseconds to milliseconds after photoactivation

- Serial crystallography at the synchrotron allows probing of protein dynamics in the millisecond time domain

- Identified the steric and electrostatic molecular gate that controls unidirectional chloride transport

- Four new transient anion binding sites were identified in an MR-SAD experiment, which enabled tracing the anion transport pathway

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