Research Center for
MOLECULAR MECHANISMS
of Aging and Age-Related Diseases

MIPT
MOSCOW INSTITUTE OF PHYSICS AND TECHNOLOGY

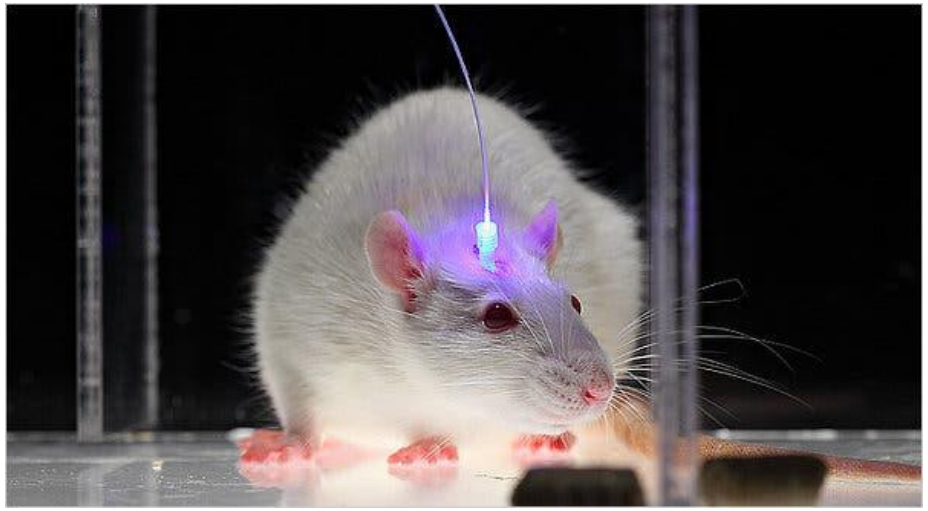
SEMI-RATIONAL OPTIMIZATION OF VIRCHR1, A VIRAL CHANNELRHODOPSIN, VIA SITE-DIRECTED MUTAGENESIS

Grigory Matveev
Center for Molecular Mechanisms of Aging at MIPT

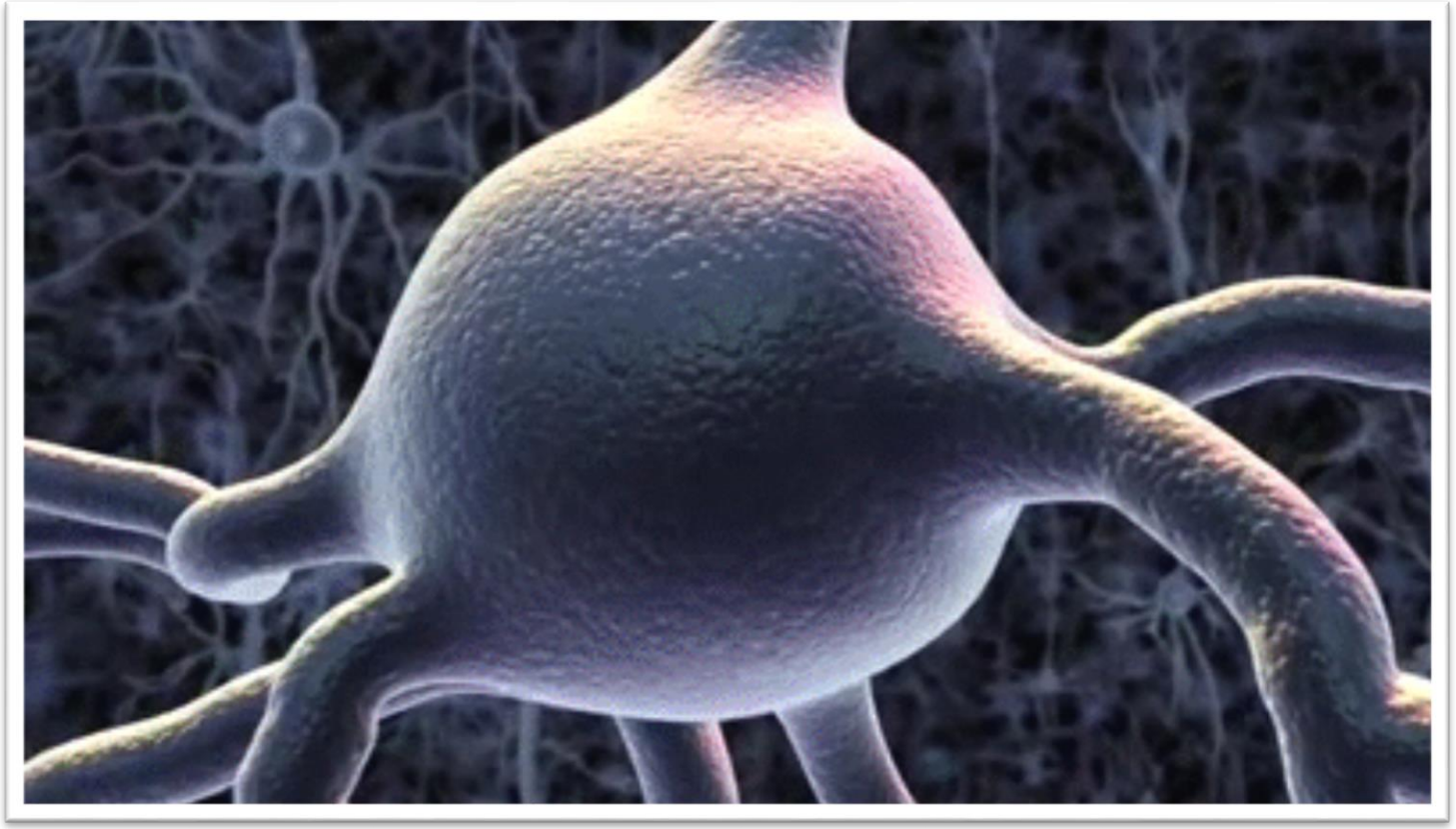
Co-authors:
E.Y. Podolyak, F.M. Tsybrov, D. V. Zabelskii, K.V. Kovalev, A.A. Alekseev

Optogenetics in a Nutshell

- *Optogenetics* – biotechnology to manipulate electrically sensitive cells with light



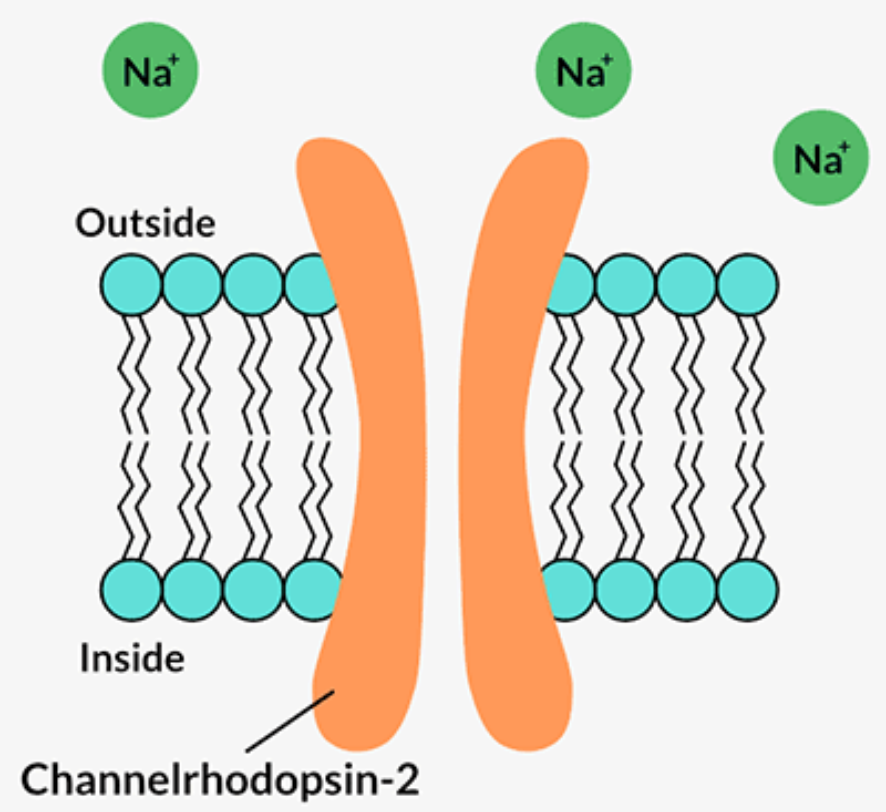
Optogenetics in a Nutshell



- *Optogenetics* – biotechnology to manipulate electrically sensitive cells with light

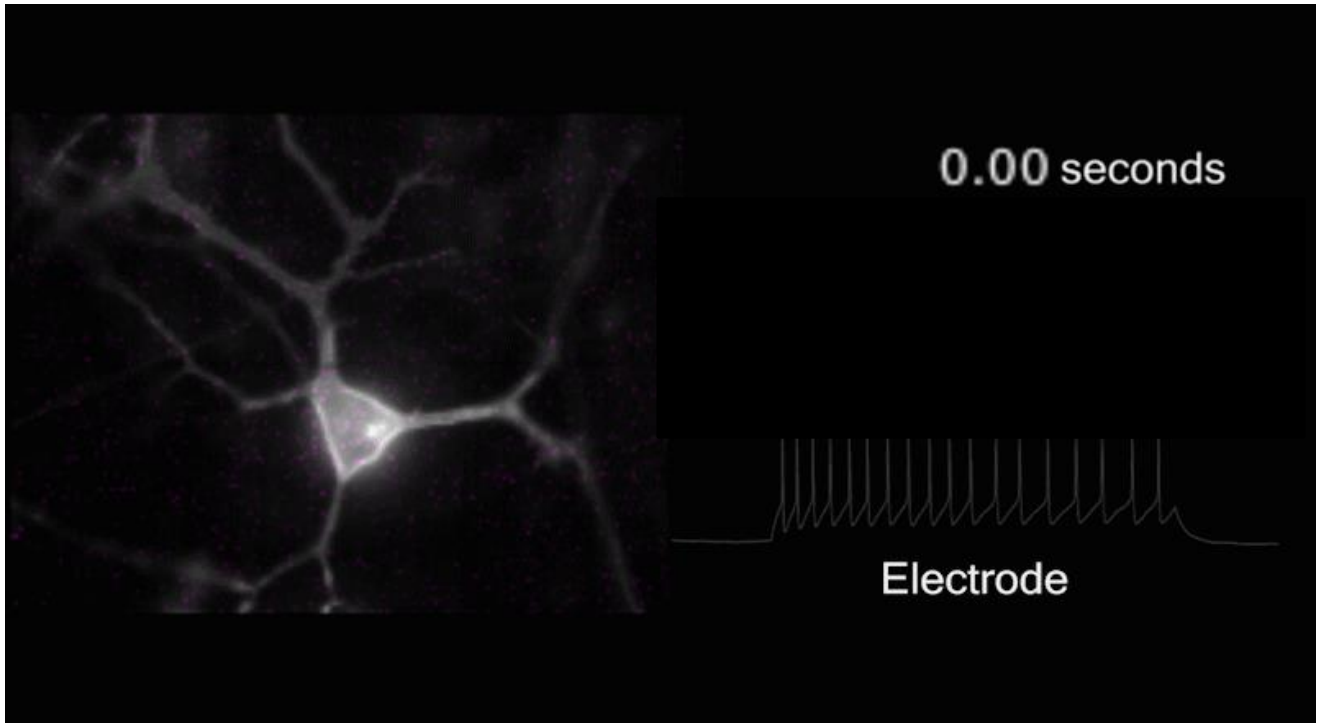
- Optogenetic Stimulation: neuronal signaling activation

Optogenetics in a Nutshell



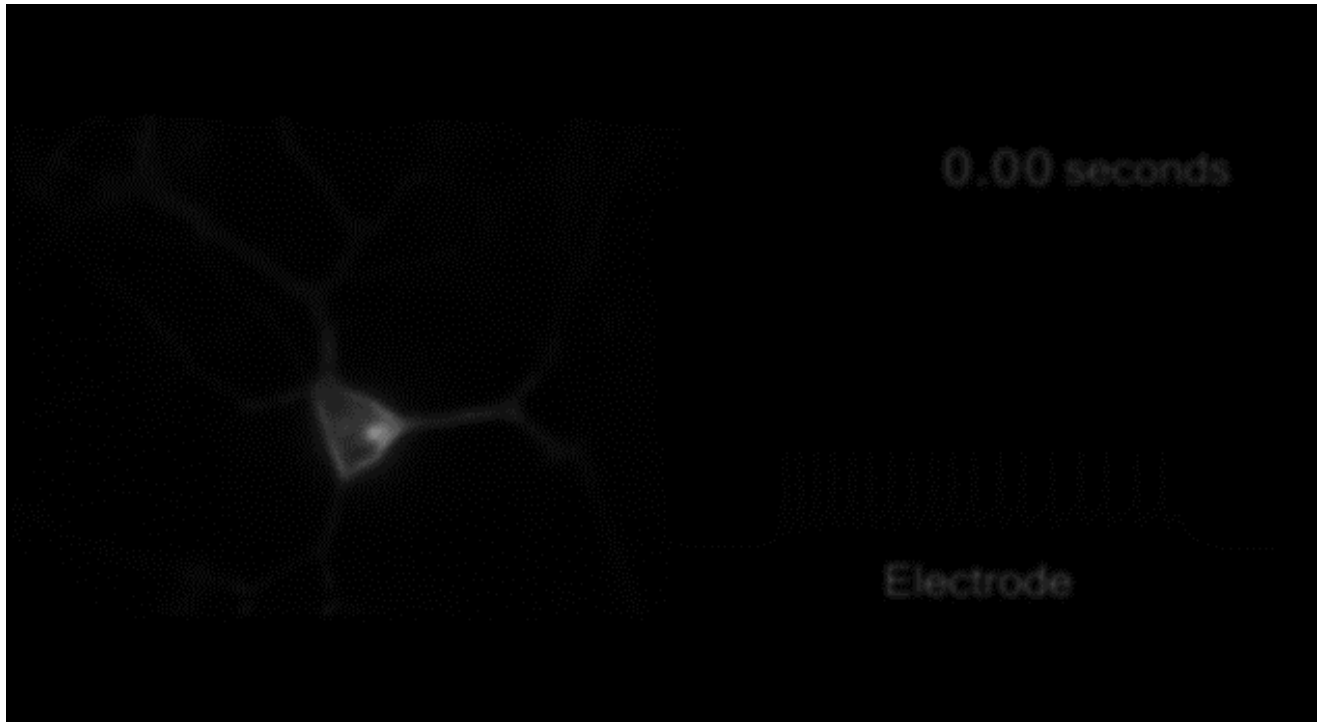
- Rhodopsin ion transport photoactivation

Optogenetics in a Nutshell



- Optogenetics: driving neural activity with light

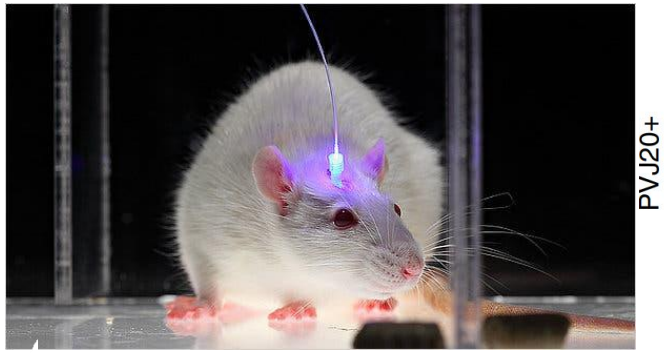
Optogenetics in a Nutshell

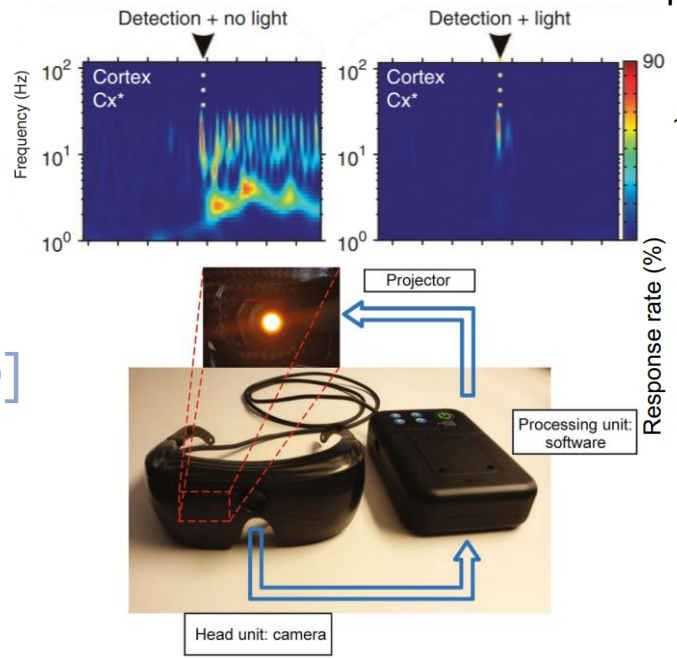


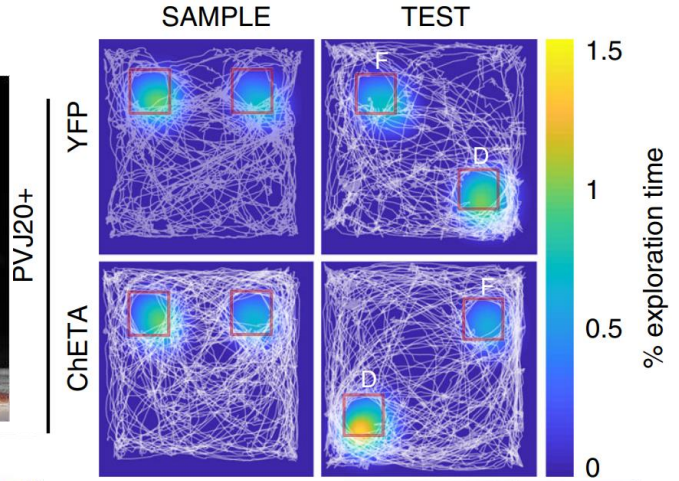
- Optogenetics: driving neural activity with light

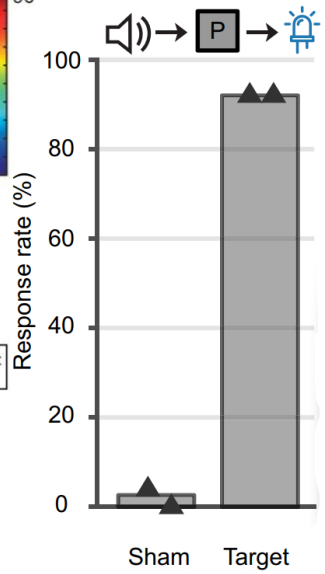
Applications of Optogenetics

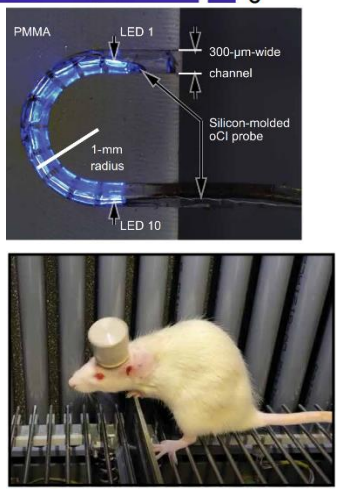
- Neuroanatomy research
  [*Namburi et al., Nat, 2019*]
- Memory restoration
  [*Etter et al., NatComm, 2019*]
- Alzheimer's, epilepsy
  [*Paz et al., NatNeurosci, 2013*]
- Restoration of hearing & eyesight
  [*Sahel et al., NatMed, 2021*]
  [*Keppeller et al., SciTranslMed, 2020*]



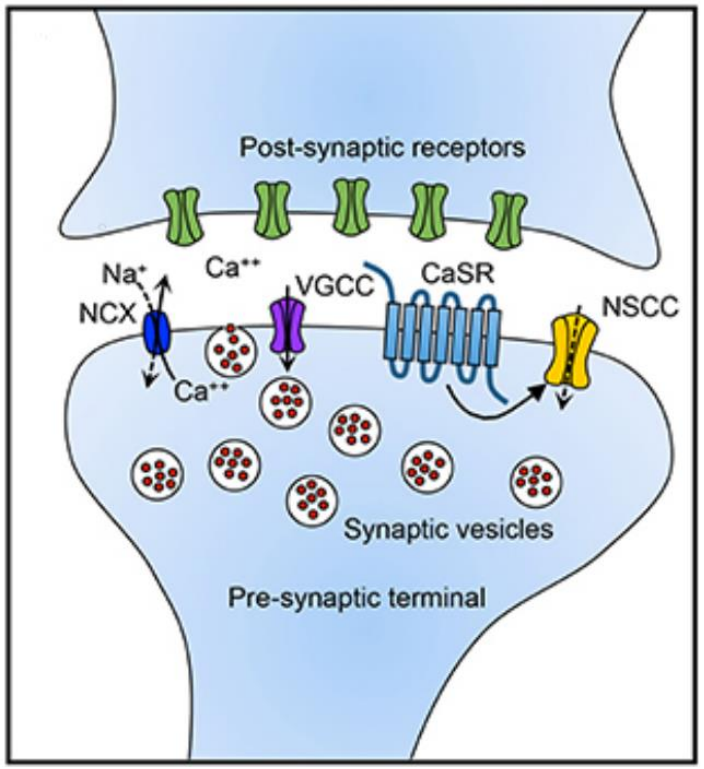




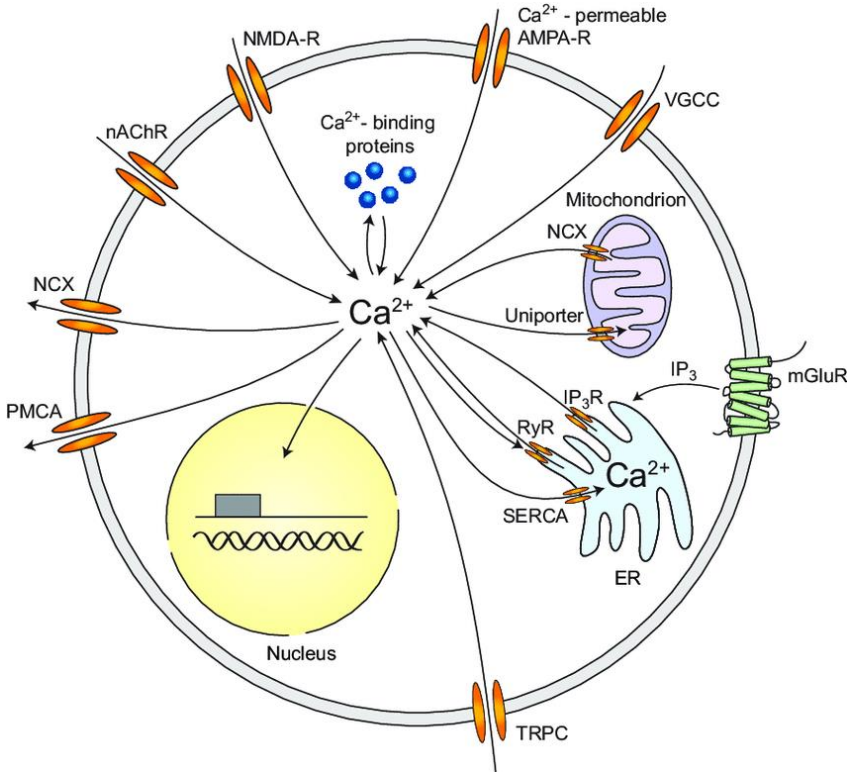




Limitations: calcium influx

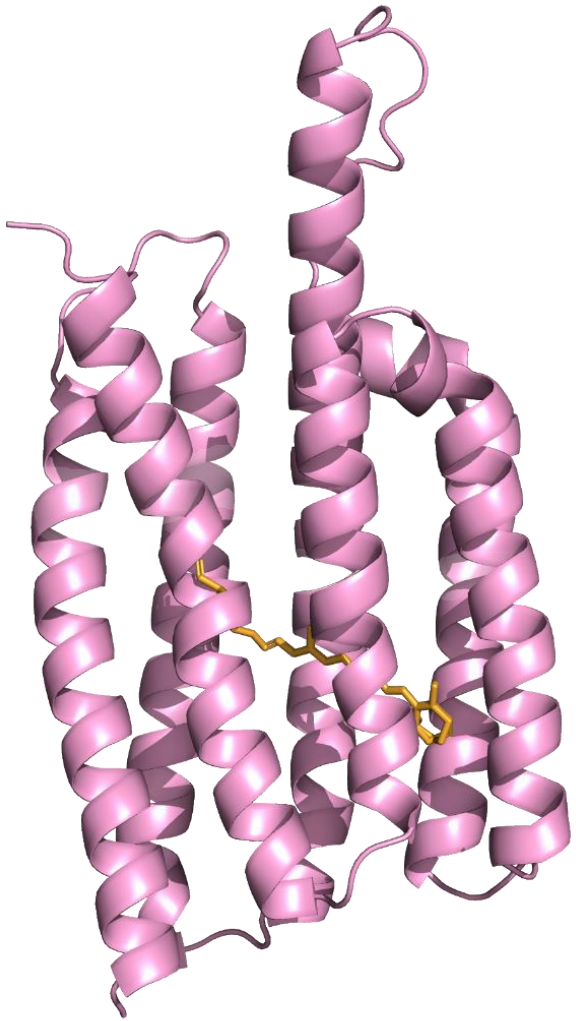


Jones et al., FrontPhys, 2016

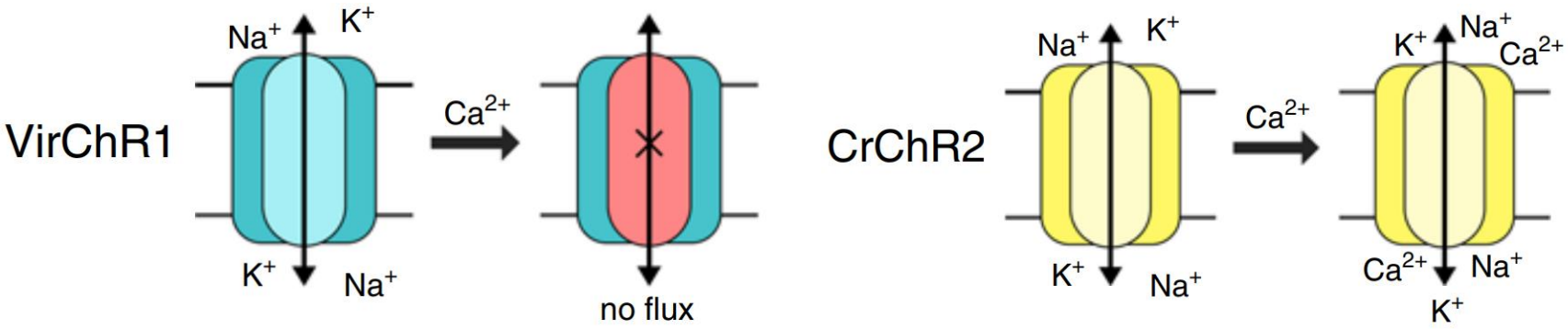


Grienberger et al., Neu, 2012

VirChR1: a Viral Channelrhodopsin



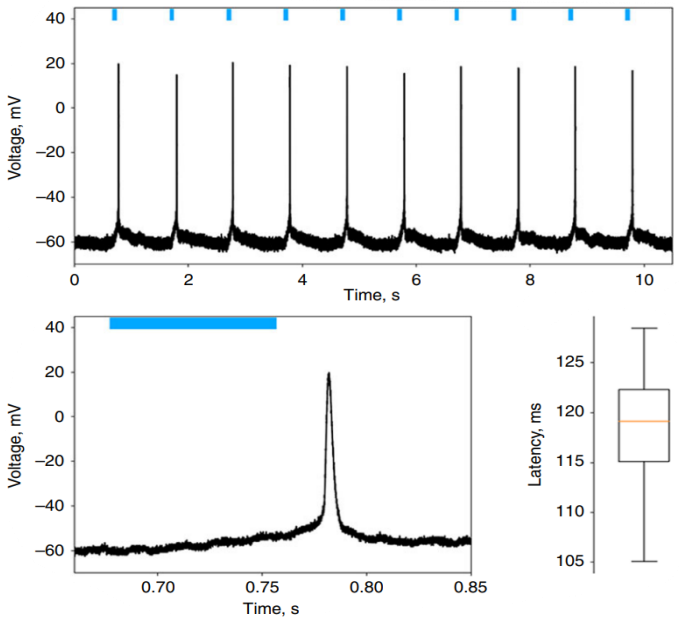
- Impermeable to Ca2+
- Viral rhodopsin 1 (VR1) family
- Expressible in human cells
- Characterized at CMM (2020)



Zabelskii et al., NatComm, 2020

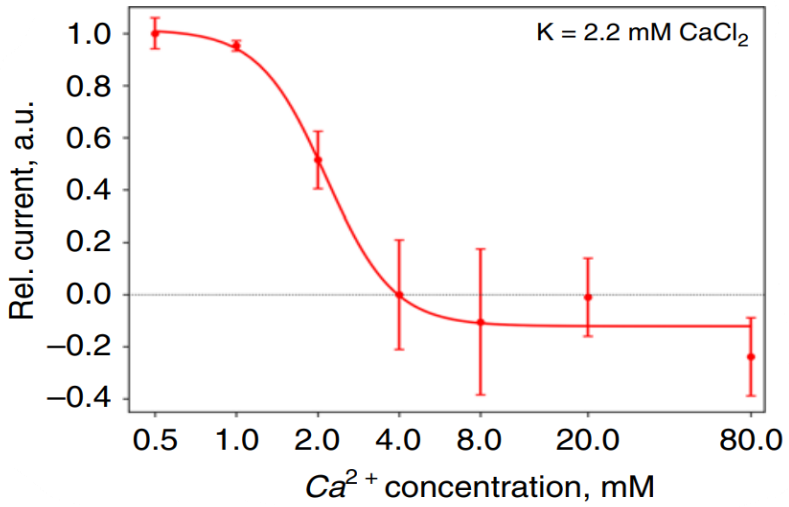
VirChR1 Features: Previous Research

Neural Firing Control



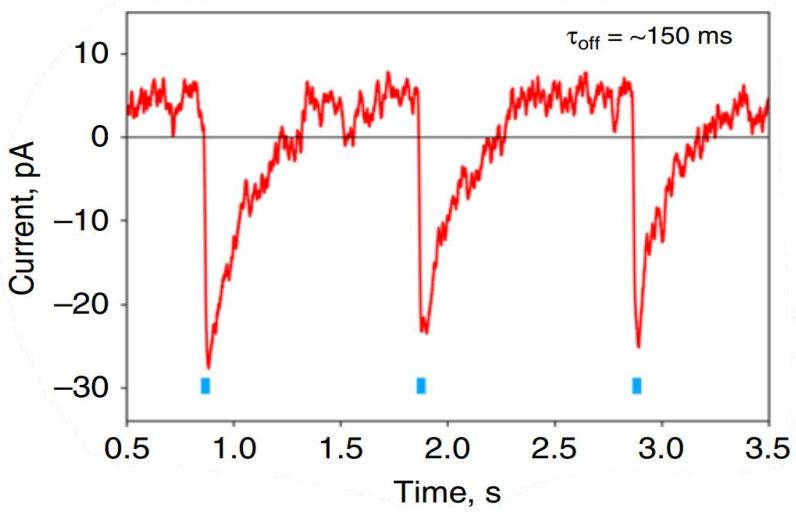
- Driving neural potential spikes ✓

Ion Selectivity



- Impermeability to Ca2+ ✓

Off-Kinetics



Zabelskii et al., NatComm, 2020

- Temporal precision: $\tau_{\text{off}} = 150$ ms ✓

VirChR1 Features: Previous Research

Neural Firing Control

- Driving neural potential spikes ✓

- **Stronger photocurrents**

Ion Selectivity

- Impermeability to Ca2+ ✓

- **Conserved selectivity for Ca2+**

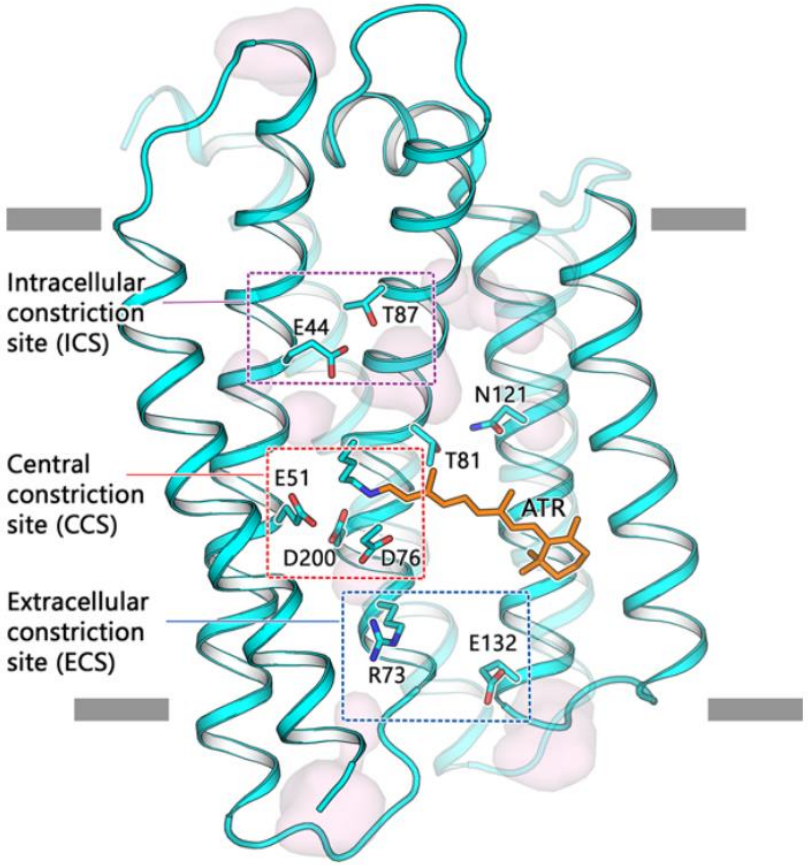
Off-Kinetics

- Temporal precision: $\tau_{\text{off}} = 150\ \text{ms}$ ✓

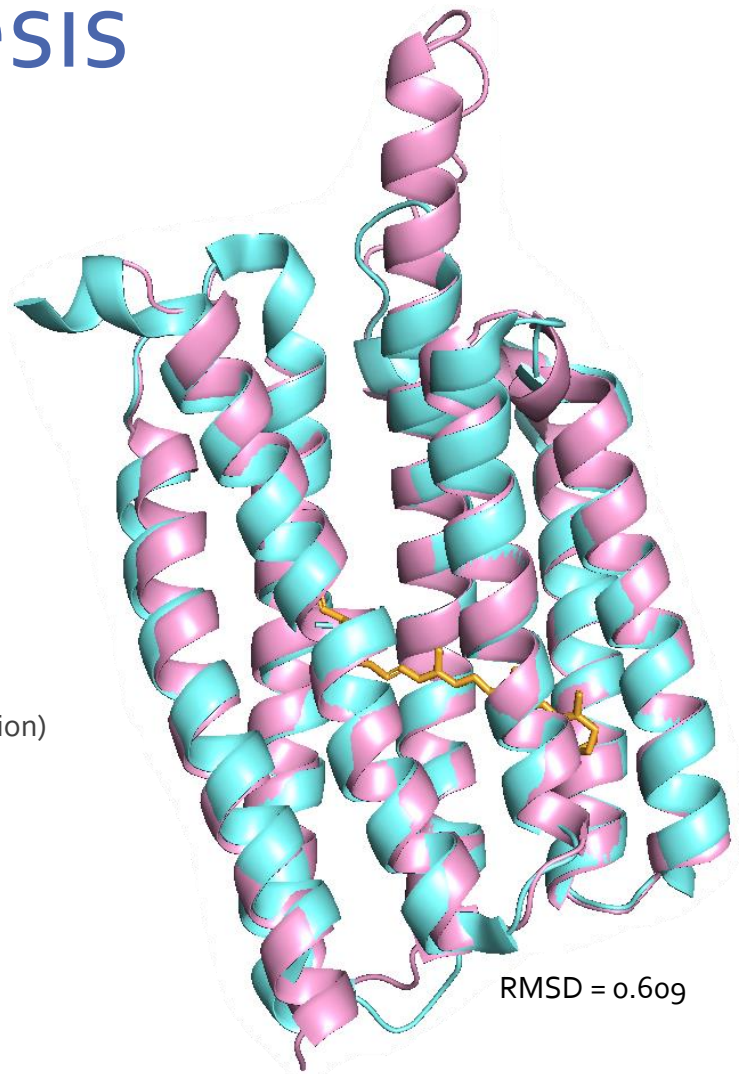
- **Enhanced kinetics**

METHODS

Semi-rational Mutagenesis

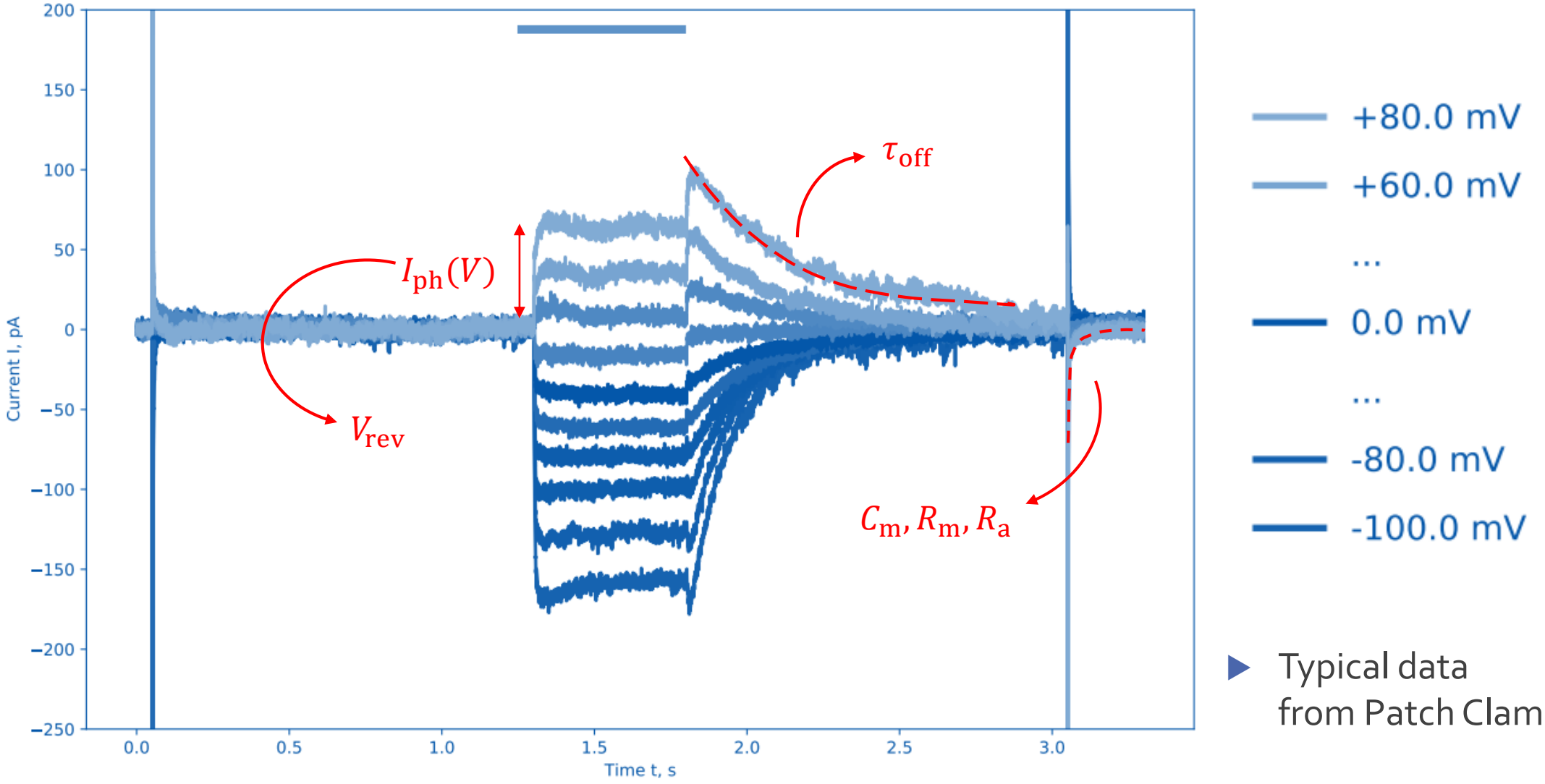


- OLPVRI
- Retinal
- VirChR1
  (AlphaFold2 prediction)



- Structural insights
- Rhodopsins' motifs
- Rhodopsin mutations lore

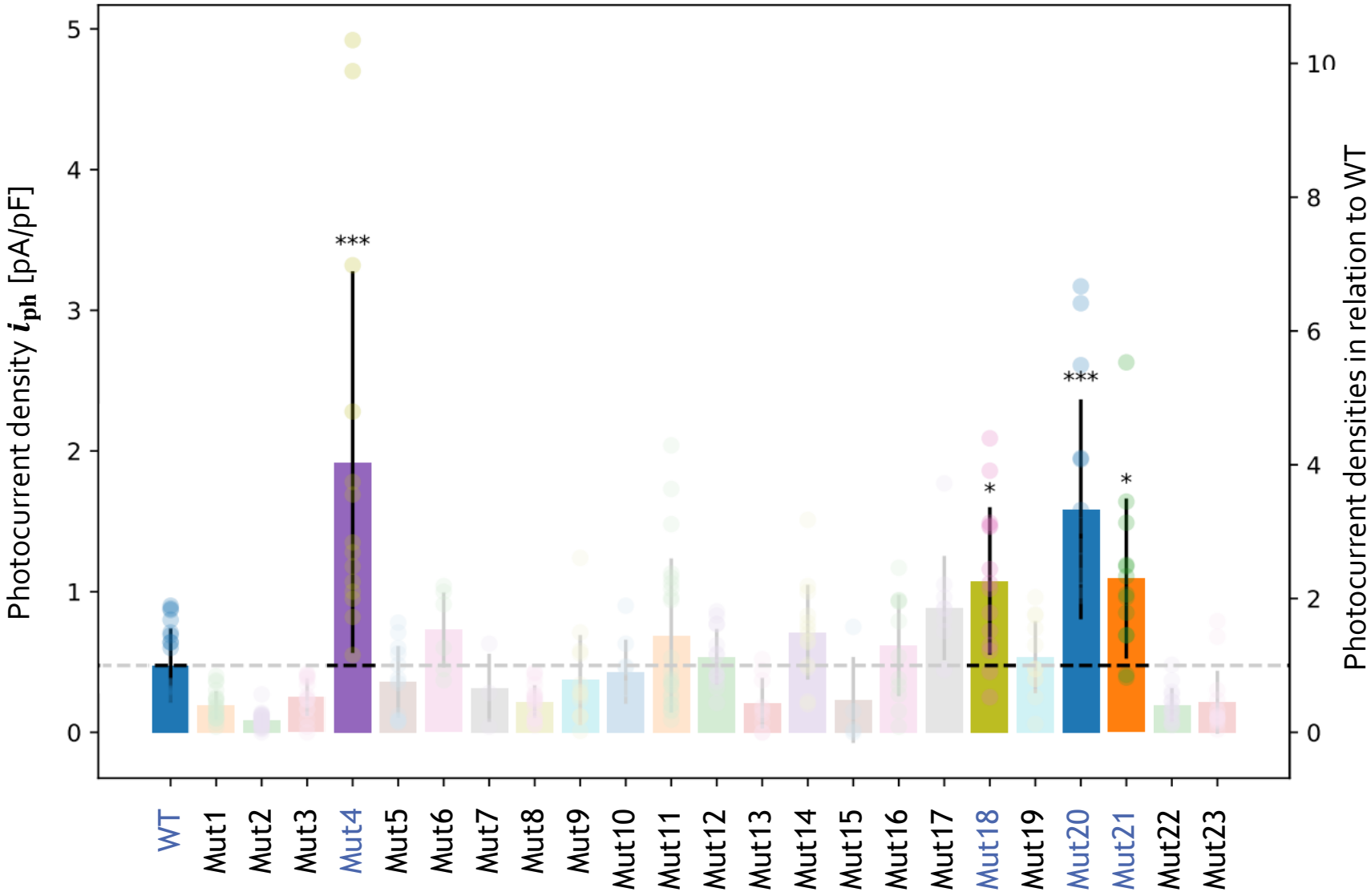
Patch Clamp Technique



- Typical data from Patch Clamp

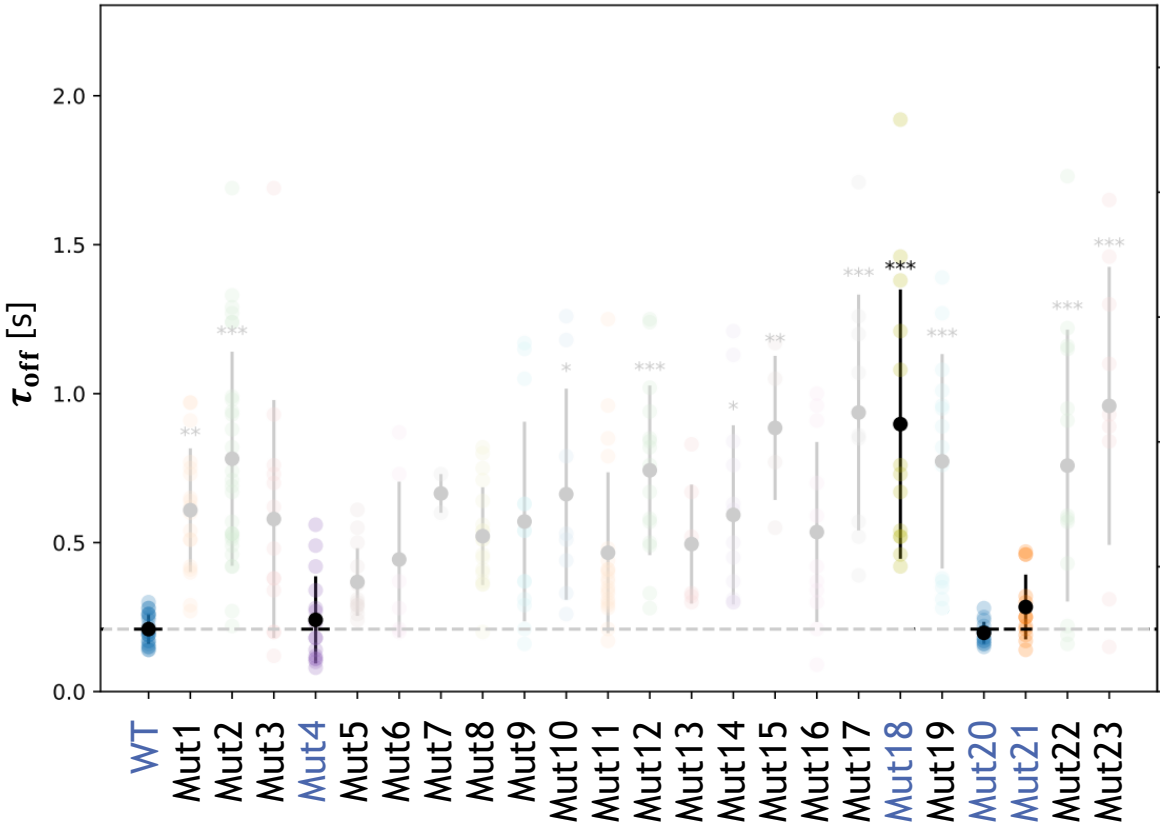
RESULTS

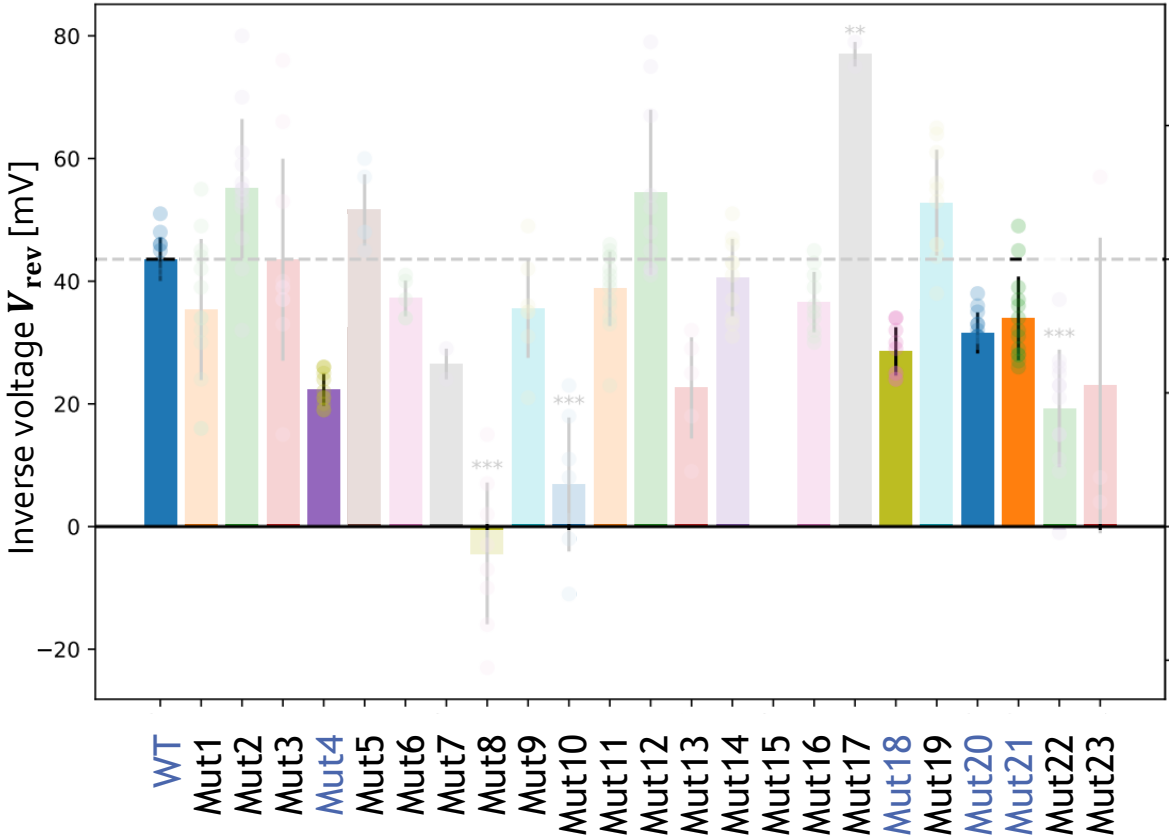
Results: Photocurrent



- Mut4
- Mut18
- Mut20
- Mut21

Results: Kinetics & Selectivity





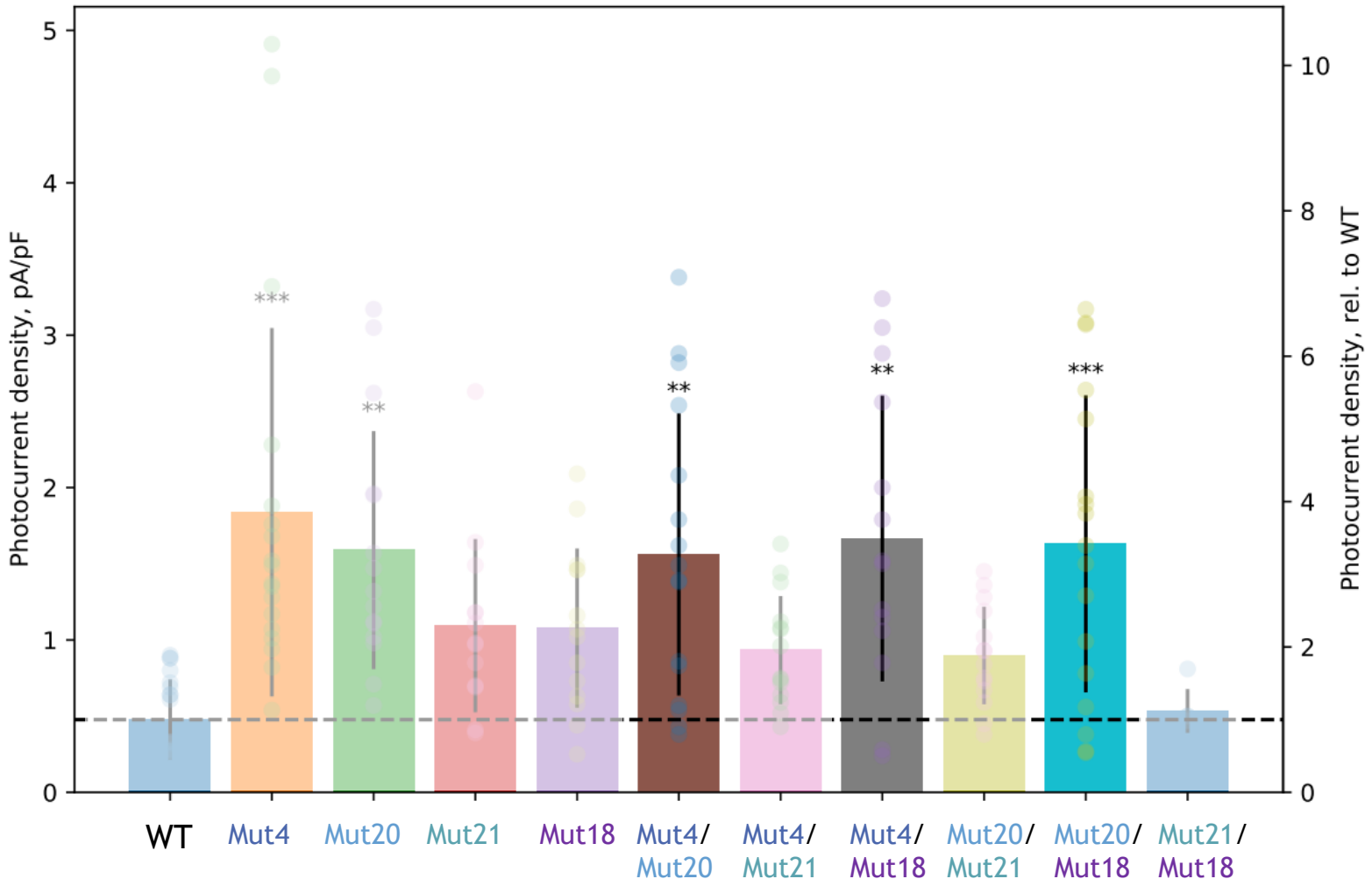
Combining mutations

Mutation that have significantly optimized VirChR1:

- Mut4
- Mut18
- Mut20
- Mut21

- Mut4/Mut18
- Mut4/Mut20
- Mut4/Mut21
- Mut18/Mut20
- Mut18/Mut21
- Mut20/Mut21

Results: Photocurrent in Double Mutants



- Mut4/Mut18
- Mut4/Mut20
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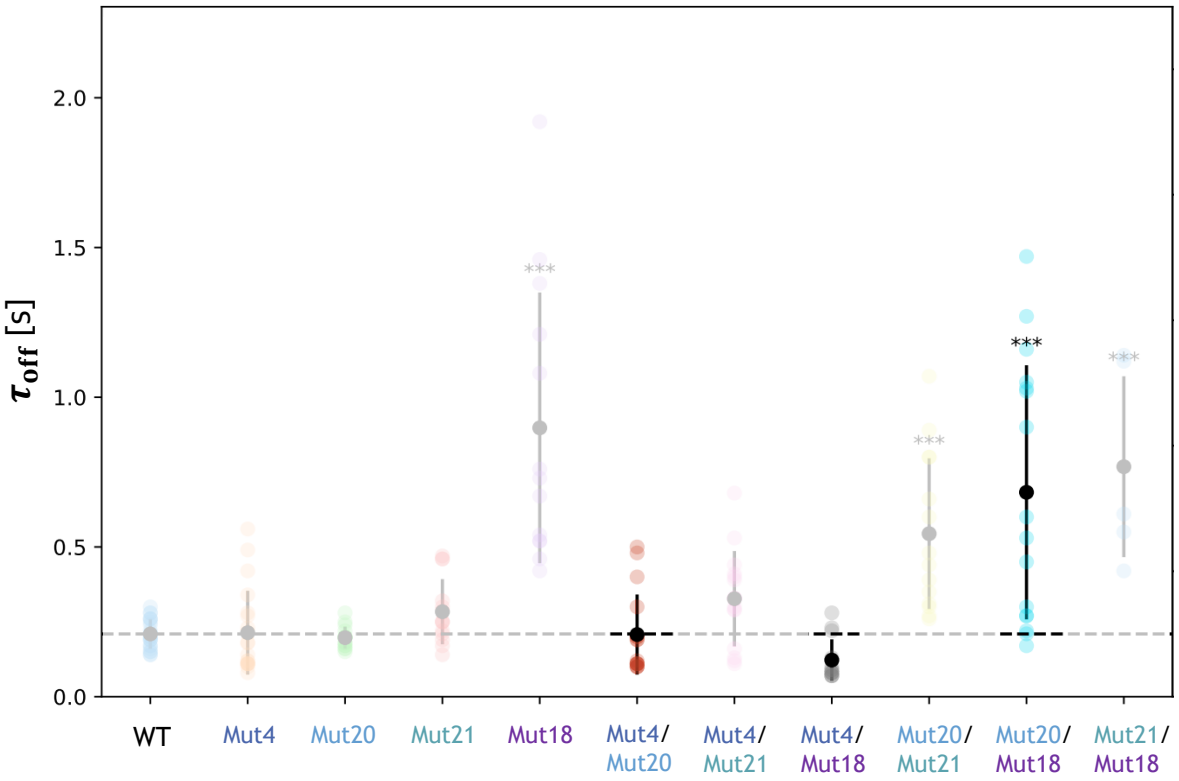
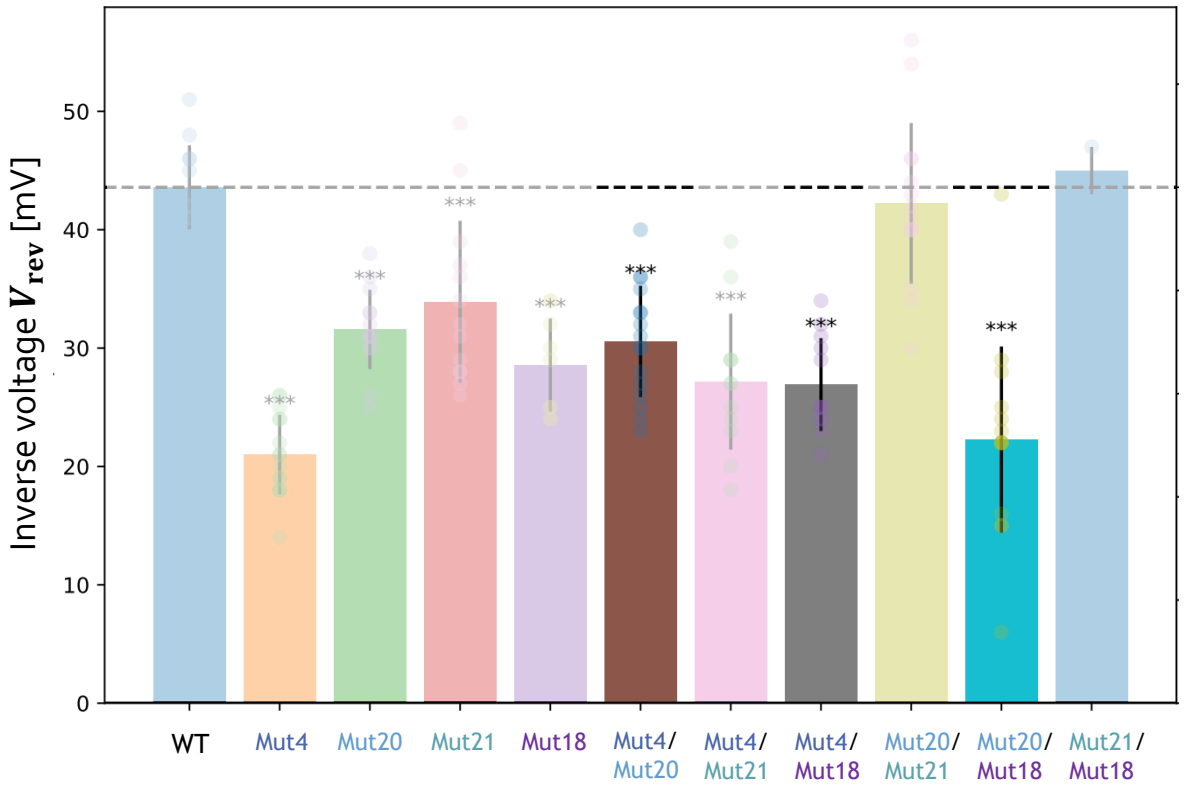
Result: Kinetics & Selectivity in Double Mutants





Results: Combining mutations

Double Mutations that optimized VirChR1:

- Mut4/Mut18
- Mut4/Mut20
- Mut4/Mut21
- Mut18/Mut20
- Mut18/Mut21
- Mut20/Mut21

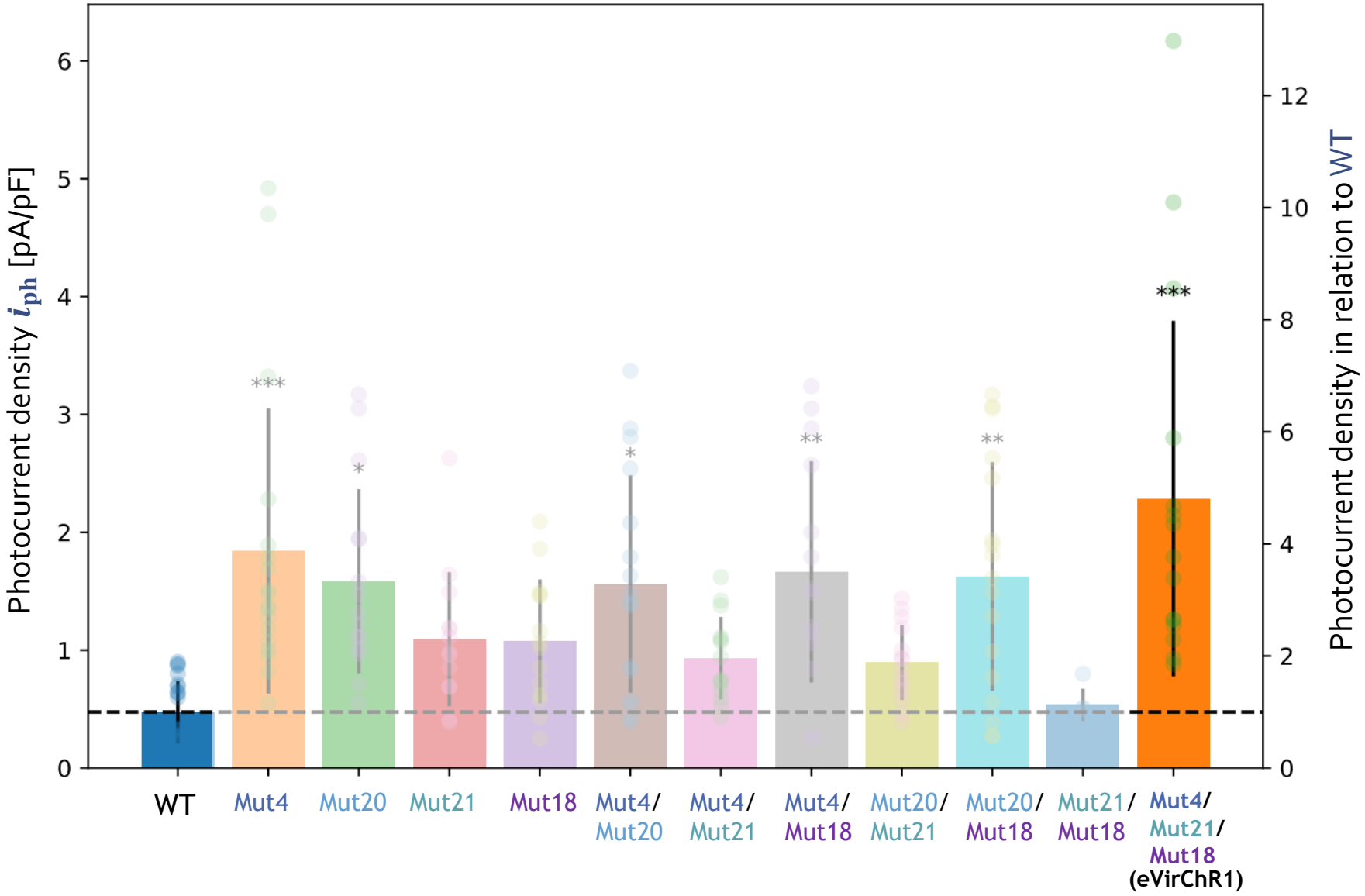
Mutations that further optimize VirChR1's features when paired:

- Mut4
- Mut18
- Mut20
- Mut21

Candidate Triple Mutant:

Mut4/Mut18/Mut21

Result: Photocurrent in Triple Mutant



- eVirChR1: 5x increase in photocurrent densities

Roadmap to Optimized VirChR1

- Screening characterization of 20+ single amino-acid mutants ✓
- "Successful" mutations combined ✓
- Design of an optimized protein variant: eVirChR1 ✓

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- Improve localization of eVirChR1 in plasma membrane
- Ensure functioning in neurons

Acknowledgements

Special thanks to Egor Marin, Aisha Idiatullina, Polina Arzhevikina, Yakov Kayumov, Demid Doroginin, Daria Kuklina for valuable comments and suggestions along the way.

Thank you for attention!

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