BIOGRAPHICAL SKETCH

NAME: Daisuke Yoshioka

POSITION TITLE: Assistant Professor, Department of Physiology, Osaka University, Japan

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Osaka, Osaka, Japan	B.S.	04/2011	03/2015	Biophysics
University of Osaka, Osaka, Japan	Ph.D.	04/2015	03/2020	Biophysics
University of Osaka, Osaka, Japan	Post-doc	04/2020	11/2020	Biophysics
University of Osaka, Osaka, Japan	Assistant Professor	12/2020	---	Physiology

A. Personal Statement

My work has primarily focused on understanding how the spatial patterns of ion channels, such as voltage-gated potassium (Kv) channels and voltage-gated sodium (Nav) channels, are formed and maintained at the axon initial segment (AIS) in neurons.

B. Positions, Scientific Appointments and Honors

Positions

2020 – Present Assistant Professor, Department of Physiology, Graduate School of Medicine, Osaka University, Suita, Japan

Honors and Awards

1. Best Presentation Award, The 15th Basic Biology Forum of Young Scientists, The University of Osaka, Japan, 2024.
2. K-MEDI hub Young Investigator Award, The 10th Federation of the Asian and Oceanian Physiological Societies (FAOPS) Congress, 2023.
3. Best Presentation Award, The 113th Kinki Section Meeting of The Physiological Society of Japan, Japan, 2021.
4. Certificate of Appreciation from the Chairman of RIKEN, Institute of Physical and Chemical Research (RIKEN) , Japan, 2020.
5. Encouragement Prize for article presentations abroad, Association for the Advancement of Manufacturing & Technology, Japan, 2019.

C. Contributions to Science

1. **Single-molecule imaging analysis of PTEN in *D. discoideum*.** PTEN is a type of phosphatase that converts $PI(3,4,5)P_3$ to $PI(4,5)P_2$. We focused on PTEN and its metabolic product, $PI(4,5)P_2$, and analyzed the impact of both molecules on each other's spatiotemporal dynamics. For this purpose, we developed a new *in vitro* single-molecule measurement system that reconstitutes purified PTEN on an artificial lipid membrane. This system can arbitrarily manipulate the lipid membrane composition. Furthermore, we genetically manipulated the $PI(4,5)P_2$ binding affinity of PTEN. By manipulating the

$PI(4,5)P_2$-PTEN interaction from both the lipid and protein sides and analyzing the single-molecule dynamics of both molecules, we revealed that $PI(4,5)P_2$ stabilizes the membrane binding of PTEN and simultaneously restricts lateral diffusion. In conclusion, we demonstrated that the positive feedback that accumulates PTEN on the membrane via $PI(4,5)P_2$ stabilizes the anterior-posterior polarity of eukaryotic cells and enhances the directionality of the cell migration.

2. **Single-molecule imaging analysis of M-channels in neurons.** The M-channel (Kv7.2/7.3) is one of the most crucial voltage-gated potassium channels. In neurons, M-channels are primarily localized on the axon initial segment (AIS), playing a key role in generating action potentials. The spatial pattern of the M-channel is directly linked to neuronal excitability. Consequently, pathological defects in M-channel trafficking can lead to various neurological diseases, such as epilepsy. Despite its importance, the detailed mechanism of M-channel trafficking remains elusive. To address this, we established a live imaging system specifically for the M-channel. Our findings revealed a new correlation between functionality and trafficking of M-channels, where the AIS selectivity of M-channels is regulated in a manner dependent on channel functionality. Furthermore, single-molecule imaging of M-channels showed that all aspects of 3D dynamics, such as lateral diffusion and exo/endocytosis, are influenced by the channel functionality.

3. **Imaging analysis of Nav1.6 channel turnover in neurons.** In collaboration with Dr. Sakimura from Niigata University, we developed a genetically modified mouse that can switch the fluorescent tag attached to Nav1.6 at any time using the Cre/loxP system. Utilizing this mouse, we were able to visualize the turnover of Nav1.6 in neurons and discovered that the process of appearance and disappearance varies spatially along the axon. The lifetime of Nav1.6 was found to be approximately 5 days, which is roughly equivalent to the lifetime of ankyrin-G.

Bibliography:

Original Papers

1. **Yoshioka D**, Fukushima S, Koteishi H, Okuno D, Ide T, Matsuoka S, Ueda M. Single-molecule imaging of $PI(4,5)P_2$ and PTEN in vitro reveals a positive feedback mechanism for PTEN membrane binding. Commun Biol. 2020 Feb 28;3(1):92. doi: 10.1038/s42003-020-0818-3. PMID: 32111929; PMCID: PMC7048775.
2. Okamura Y, **Yoshioka D**. What voltage-sensing phosphatases can reveal about the mechanisms of ion channel regulation by phosphoinositides. Biochem Soc Trans. 2023 Apr 26;51(2):827-839. doi: 10.1042/BST20221065. PMID: 37052219.
3. **Yoshioka D**, Okamura Y. Coupling Between Functionality and Trafficking to the Axon Initial Segment in KCNQ2/3 K^+ Channels. bioRxiv. 2024.10.17.617761; doi: https://doi.org/10.1101/2024.10.17.617761.
4. Zhou J, Andriani RT, Mizutani N, Yamamoto K, **Yoshioka D**, Kawanabe A, Kawai T, Okochi Y, Okamura Y. Optical control of PI(4,5)P2 sensitivity of ion channels by manipulation of single lysine residue. J Gen Physiol. 2025. doi: 10.1085/jgp.202513811.
5. **Yoshioka D**, Okamura Y. Coupling of functionality to trafficking of KCNQ2/3 potassium channels at the axon initial segment. Proc Natl Acad Sci U S A. 2026 Mar 10;123(10):e2527749123. doi: 10.1073/pnas.2527749123. Epub 2026 Mar 2. PMID: 41770922.