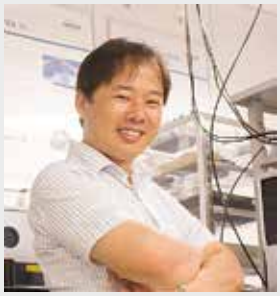
Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Sensing



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1999 B.S. Kyoto University
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Professional Employment
2003 JSPS Research Fellow
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2006 Assistant Professor, Nagoya Institute of Technology
2009 Associate Professor, Institute for Molecular Science
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Awards
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Membrane proteins are important for homeostasis and signaling of living cells, which work as ion channel, ion pump, various types of chemical and biophysical sensors, and so on. These proteins are considered as one of important targets for biophysical studies. Our main goal is to clarify molecular mechanisms underlying functions of the channels, transporters and receptors mainly by using stimulus-induced difference infrared spectroscopy, which is sensitive to the structural and environmental changes of bio-molecules.

We applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to extract ion-binding-induced signals of various kinds of membrane proteins. For example, KcsA is a potassium channel, which is highly selective for K^+ over Na^+, and the selectivity filter binds multiple dehydrated K^+ ions upon permeation. Shifts in the peak of the amide-I signals towards lower vibrational frequencies were observed as K^+ was replaced with Na^+ (Figure 1). These vibrational modes give us precise structural information of the selectivity filter. Moreover, by changing concentrations of K^+ in buffer solutions, we can estimate affinity of the selectivity filter for K^+ ions.

Recently, we have developed a rapid-buffer exchange apparatus for time-resolved ATR-FTIR spectroscopy, which can be utilized for studying dynamics of structural transition in membrane proteins.

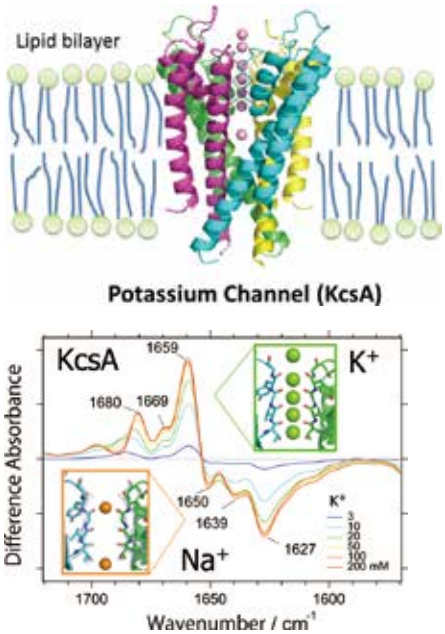

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

Selected Publications

- Y. Furutani *et al.*, "ATR-FTIR Spectroscopy Revealed the Different Vibrational Modes of the Selectivity Filter Interacting with K^+ and Na^+ in the Open and Collapsed Conformations of the KcsA Potassium Channel," *J. Phys. Chem. Lett.* **3**, 3806–3810 (2012).
- Y. Furutani *et al.*, "Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode," *Biophysics* **9**, 123–129 (2013).

1. Light-Induced Structural Changes of Chimeras of Channelrhodopsin-1 and -2 from *Chlamydomonas reinhardtii*[1)]

Optogenetics is a powerful technique for manipulating specific neural activities by light stimulation, which has been rapidly growing up since discovery of light-gated cation channel, channelrhodopsin. There are two kinds of channelrhodopsin called channelrhodopsin-1 and -2 (ChR1 and ChR2) which are expressed in the eyespot of *Chlamydomonas reinhardtii*. Among them, ChR2 and its derivatives have been extensively utilized in optogenetics application. Alteration of channelrhodopsins to achieve a favorable electrophysiological response could be rationally applied when the molecular mechanisms of channelrhodopsin are understood well.

The basic architecture of channelrhodopsin is similar to other microbial rhodopsins which are composed of seven transmembrane helices with an all-*trans* retinal as the chromophore. Photoisomerization of the retinal chromophore upon light absorption causes conformational changes of the protein that result in opening of the channel gate and the influx of cations. The time course of the photocurrent upon continuous illumination of ChR2 shows a peak-and-plateau, while that of ChR1 shows a rectangular shape. The suppression just after the transient maximum photocurrent seen in ChR2 is denoted the "desensitization."

In 2009, several types of ChR1/ChR2 chimeras were characterized using electrophysiological techniques. One of these chimeras consists of the first five transmembrane helices (TM1 to TM5) from ChR1 and the last two transmembrane helices (TM6 and TM7) from ChR2. This chimera is referred to as $ChR_{5/2}$. Another chimera consists of TM1 and TM2 from ChR1 and TM3 to TM7 from ChR2. This chimera is referred to as $ChR_{2/5}$ (for details, see Figure 2). These ChR1/ChR2 chimeras show larger photocurrents than the wild types, and their desensitization is significantly reduced upon continuous illumination. However, the molecular mechanism of suppression of desensitization has remained unknown.

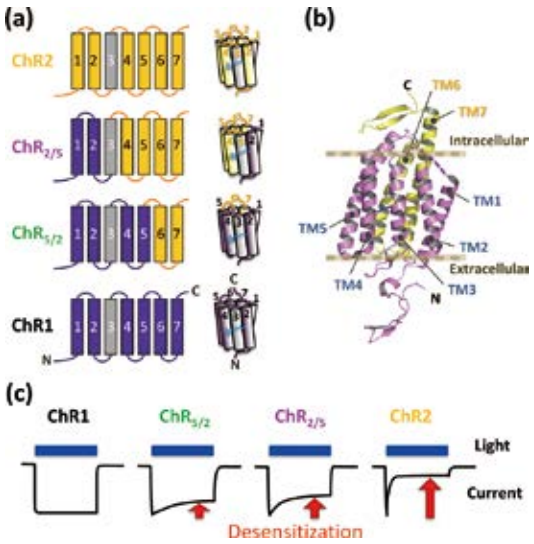

Figure 2. (a) Schematic representation of the chimeric channel rhodopsins. (b) The X-ray crystal structure of a chimeric channelrhodopsin called C1C2, which is nearly identical to $ChR_{5/2}$. (c) The photocurrent profiles of ChR1, ChR2and the chimeras. This figure is adapted from ref. 1.

Fourier-transform infrared (FTIR) spectroscopy has revealed the molecular mechanisms underlying the photo-induced structural dynamics of various microbial rhodopsins, such as bacteriorhodopsin and halorhodopsin.[2)] We applied light-induced difference FTIR spectroscopy on ChR1/ChR2 chimeras and ChR2 with an aim to reveal the molecular basis underlying the differences in electrophysiological properties between them.

As a consequence, we found that ChR1/ChR2 chimeras exhibited structural changes distinct from those in ChR2 upon continuous illumination. In particular, the protonation state of a glutamate residue, Glu129, (Glu90 in ChR2 numbering) in

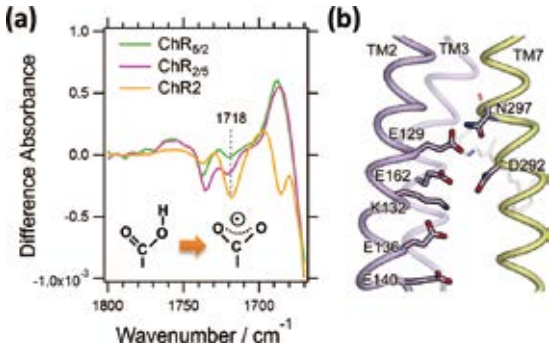

Figure 3. (a) Light-induced difference spectra in the C=O stretching region of carboxylic acid side chains. (c) The X-ray crystal structure of C1C2 shown along TM2 and TM7 helices. This figure is adapted from ref. 1.

the ChR chimeras is not changed as dramatically as seen in ChR2 as a negative band at 1718 cm^{-1} (Figure 3). Moreover, using mutants stabilizing particular photointermediates as well as time-resolved measurements, we identified some differences between the major photointermediates of ChR2 and ChR1/ChR2 chimeras. We couldn't see any substantial change in the protonation state of Glu129 in $ChR_{5/2}$ during the photocycle. Taken together, our data indicate that the gating and desensitizing processes in ChR1/ChR2 chimeras are different from those in ChR2 and that these differences should be considered in the rational design of new optogenetic tools based on channelrhodopsins.

References

1) A. Inaguma, H. Tsukamoto, H. E. Kato, T. Kimura, T. Ishizuka, S. Oishi, H. Yawo, O. Nureki and Y. Furutani*, *J. Biol. Chem.* **290**, 11623–11634 (2015).

2) Y. Furutani*, K. Fujiwara, T. Kimura, T. Kikukawa, M. Demura and H. Kandori, *J. Phys. Chem. Lett.* **3**, 2964–2969 (2012).

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