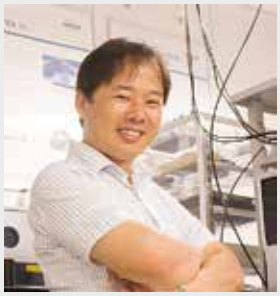
Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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
2004 JSPS Postdoctoral Fellow
2006 Assistant Professor, Nagoya Institute of Technology
2009 Associate Professor, Institute for Molecular Science
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2011 JST-PRESTO Researcher (concurrent post) (–2015)

Awards
2012 Morino Foundation for Molecular Science
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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.

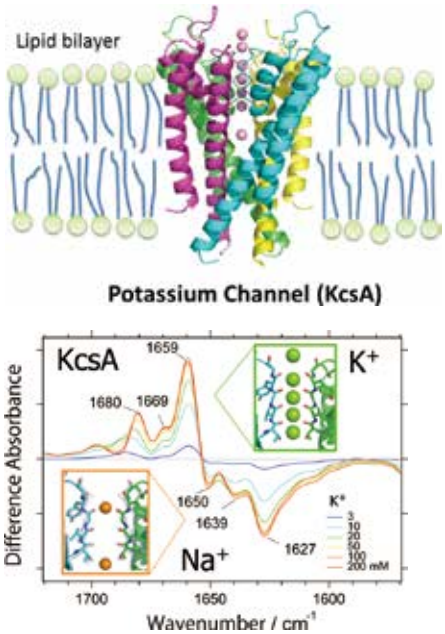

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

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. Ion–Protein Interactions of TWIK1 Potassium Channel with Alkali Metal Cations and Its Implication for the Ion Selectivity[1)]

Potassium channels are selectively permeable to potassium ions (K^+) in cell membrane and function for shaping neuroral signals in nerve cells or maintaining ionic compositions in various cells. The ion selectivity of potassium channels for K^+ over Na^+ is extremely high (typically 1000:1 ratio). The molecular mechanism of the selectivity has been greatly understood by determination of three-dimensional structure of a bacterial potassium channel, KcsA, which stimulates further discussion on the potassium selectivity. As we have shown in previous studies, infrared spectroscopy can detect molecular vibrations and could be a key technique for studying ion–protein interactions in membrane proteins. Indeed, ion-exchange induced difference FTIR spectroscopy successfully discriminated structures of the selectivity filter of the KcsA channel interacting with each kind of alkali metal cations.[2)]

Two-pore domain potassium channel possesses two pore domains in a monomer unit and composes the ion selectivity filter by forming a dimer instead of a conventional tetramer. Among them, TWIK1 shows the peculiar ion selectivity in which sodium ions permeate under low potassium concentration or acidic pH in the extracellular side. The pseudo four-fold rotational symmetry along the pore axis may cause the low potassium selectivity. To understand the molecular mechanism underlying the low potassium selectivity of TWIK1, we applied ion-exchange induced difference FTIR spectroscopy on this protein. Then, we compared the difference spectra of a high potassium selective variant, T118I, and a L228F variant, which is altered on the filter but known to be unaffected to the ion selectivity, with those of the wild-type TWIK-1.

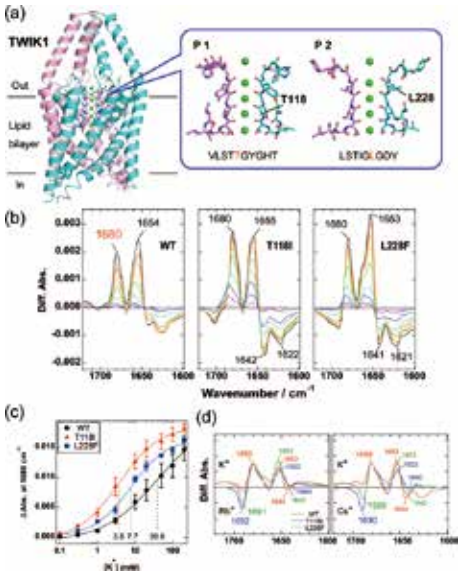

Figure 2. (a) The selectivity filter of TWIK1. (b) The difference IR spectra of WT, T118I and L228F mutants. (c) K^+-concentration dependence for the 1680-cm^{-1} band (d) The difference spectra upon replacement with Rb^+ or Cs^+.

The ion-exchange induced difference spectra in the amide-I region of WT, T118I and L228F are basically similar to the previous spectrum obtained in KcsA. Especially, we found that the 1680-cm^{-1} band would be a general marker band for the selectivity filter interacting with potassium ions. Interestingly, the band at 1680 cm^{-1} shifts to ~1690 cm^{-1} upon replacing K^+ with Rb^+ or Cs^+ in the T118I and L228F variants, but not in WT. The titration experiments provided quantitative information about affinity of the channels with potassium ions and T118I exhibited the highest affinity. Thus, we conclude that the low potassium selectivity of TWIK-1 is well correlated with the structural dynamics of the filter region and its affinity to potassium ions.

2. PDMS-Based Microfluidic Device for Infrared Spectroscopy with an Electro-Chemical Reaction[3)]

Microfluidic technique is a promising method for characterizing chemical and biological reactions with low sample consumption. Polydimethylsiloxane (PDMS) is transparent under visible light and is a soft material, which widely used for making microfluidic devices feasible for various kinds of experimental techniques. A paper applying FTIR microspectroscopy with a PDMS microfluidic device was published as a collaborative work with Dr. M. Srisa-Art in Chulalongkorn University, Thailand. After that, Mr. A. Suea-Ngam stayed in our group as an IMS-IIPA internship student. He tried to fabricate a droplet-based microfluidics coupled with amperometric detection using chip-based carbon paste electrodes (CPEs) for FTIR spectro-electrochemistry. We succeeded to demonstrate infrared spectroscopy of electrochemical reactions of ferrocyanide ($[Fe(CN)_6]^{4-}$) in the microchannel.

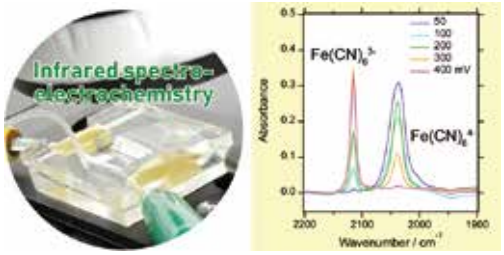

Figure 3. Infrared spectro-electrochemistry in PDMS device.

References

1) H. Tsukamoto, M. Higashi, H. Motoki, H. Watanabe, C. Ganser, K. Nakajo, Y. Kubo, T. Uchihashi and Y. Furutani, *J. Biol. Chem.* **293**, 6969–6984 (2018).

2) Y. Furutani, *Biophys. Rev.* **10**, 235–239 (2018).

3) A. Suea-Ngam, M. Srisa-Art and Y. Furutani, *Bull. Chem. Soc. Jpn.* **91**, 728–734 (2018).