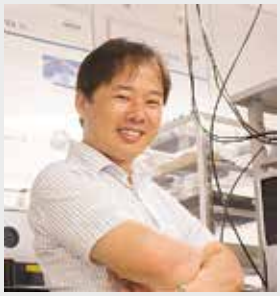
# Investigation of Molecular Mechanisms of Channels, Transporters and Receptors


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



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**Education**
1999 B.S. Kyoto University
2004 Ph.D. Kyoto University

**Professional Employment**
2003 JSPS Research Fellow
2004 JSPS Postdoctoral Fellow
2006 Assistant Professor, Nagoya Institute of Technology
2009 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies
2011 JST-PRESTO Researcher (concurrent post) ( –2015)

**Awards**
2012 Morino Foundation for Molecular Science
2013 The 2013 Young Scientist Awards of the Japan Society for Molecular Science


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**Keywords** Infrared Spectroscopy, Membrane Protein, Ion Channel

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.

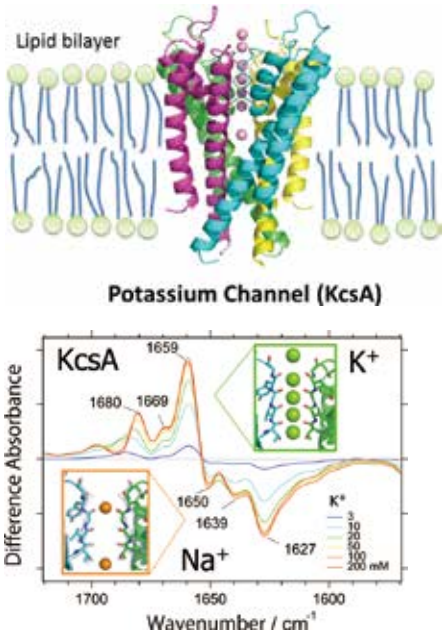


**Figure 1.** (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

## 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. Nucleotide Base Specificity of P2X Receptors[1)]

P2X receptors are cation channels activated by adenosine-5'-triphosphate (ATP), which is a well-known biomolecule utilized for various biological activities. The ATP-gated cation channels sense extracellular ATP concentration and function for muscle contraction, taste signal transduction, nociception and so on. Three monomers form a trimeric structure and three nucleotide binding sites exist in the interfaces between two nearby monomers (Figure 2a).

X-ray crystal structure of P2X associated with cytidine-5'-triphosphate (CTP) was resolved (Figure 2a; the right bottom) and its functionality and nucleotide base specificity was examined by applying electrophysiological and spectroscopic measurements.

As shown in Figure 2b, ATP was tightly bound to WT and most of the bound ATP retained after 15–30 min wash. T189S, which loses a methyl group in the side chain, exhibited similar affinity for ATP, while T189V, which loses a hydroxyl group in the side chain, exhibited very low affinity for ATP. Therefore, the hydroxyl group in the side chain of T189 is very important for the ATP binding to P2X, which is consistent with the X-ray crystal structure (Figure 2a; the right top) showing that the hydroxyl group is located in hydrogen-bonding distance from the adenine ring of ATP. On the other hand, WT exhibited relatively higher affinity for CTP than T189S and T189V mutants, while both mutants exhibited similar affinity for CTP. Therefore, the hydroxyl group of T189 is not so important for the CTP binding to P2X, which is also supported by the X-ray crystal structure (Figure 2a; the right bottom).

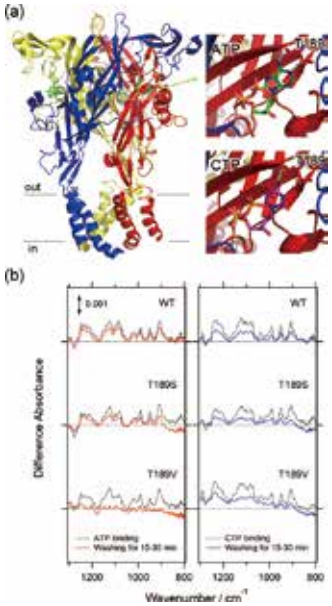


**Figure 2.** (a) X-ray crystal structure of P2X with ATP or CTP molecules. A nucleotide binding site is expanded in the right panel. The dotted lines indicate that the atoms of Thr189 and the nucleotides exist in hydrogen-bonding distance. (b) Ligand-binding-induced difference IR spectra of P2X measured for WT, T189S and T189V mutants with ATP or CTP.

## 2. UV-Sensing Protein in the Brain of a Marine Zooplankton[2)]

Most of animals show some circadian (daily) behaviors. On the earth, two large-scale daily movements of biomass are known. One is human commuting and the other is daily vertical migration of zooplanktons. Zooplanktons move downward in water during daytime and upward at night, in order to avoid predators and UV damage from sunlight. Thus, it is important to understand how zooplankton species sense ambient UV signals.

Larva of the marine ragworm (*Platynereis dumerilii*) has been studied as a zooplankton model, and the larvae possess photoreceptor cells in the brain to control circadian swimming behavior. The brain photoreceptor cells express an opsin (named as c-opsin) that is closely related to visual pigments in our eyes. We expected that *Platynereis* c-opsin is involved in UV detection, and assessed spectral and biochemical properties of the opsin.

We purified the c-opsin protein that was expressed in mammalian cultured cells. The purified opsin showed an absorption maximum at 383-nm in the UV region (Figure 3a). Also, *Xenopus* oocytes expressing the opsin showed electrophysiological responses upon UV irradiation (Figure 3b). These results clearly indicate that the c-opsin is a UV-sensitive pigment. Mutagenesis analyses identified that a single amino acid residue is responsible for UV sensing. Thus, the single residue is essential for the opsin to achieve the ability to receive UV signals. Taken together, the c-opsin would enable the brain of *Platynereis* to sense ambient UV signals.

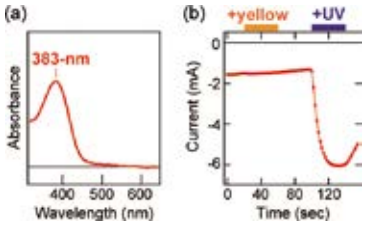


**Figure 3.** UV sensing ability of *Platynereis* c-opsin.
(a) Absorption spectrum of purified *Platynereis* c-opsin. (b) Photoresponses of a *Xenopus* oocyte expressing *Platynereis* c-opsin with a potassium channel GIRK1/GIRK2.

### References

1) G. Kasuya, Y. Fujiwara, H. Tsukamoto, S. Morinaga, S. Ryu, K. Touhara, R. Ishitani, Y. Furutani, M. Hattori and O. Nureki, *Sci. Rep.* **7**, 45208 (2017).
2) H. Tsukamoto, I.-S. Chen, Y. Kubo and Y. Furutani, *J. Biol. Chem.* **292**, 12971–12980 (2017).

### Award

FURUTANI, Yuji; The 3rd Biophysics and Physicobiology Editors' Choice Award (2016).