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Research Interests

Membrane proteins are important for the function of many biological systems, and are also the major drug targets. My research primarily focuses on the elucidation of the function-related structural dynamics of membrane proteins by nuclear magnetic resonance (NMR) techniques. Currently I am working on following two projects:

(i) Dynamic activation of GPCR signaling revealed by NMR

G protein-coupled receptor (GPCR) family is the largest membrane protein family in eukaryotes. GPCRs induce various cellular responses upon the ligand binding. GPCRs are also a major drug target and one-third of the clinical drugs target GPCRs. The extent of intracellular activation evoked upon ligand binding is called efficacy, however, the molecular mechanism that defines the efficacy of each ligand has not been known.

By analyzing the β2-adrenergic receptor (β2AR) using NMR, we found that the signals from several transmembrane residues shift their positions in an efficacy-dependent manner. The results indicate that the presence of an equilibrium between the active and inactive conformations in the transmembrane region of β2AR, and the population of active conformation defines the drug efficacy (Nat. Commun., 2012).

There are two types of GPCR signaling pathways, one mediated by G proteins and the other by arrestins, and the balance between those two pathways is critical for the side effects of drugs. We showed that the dynamics of the transmembrane region define the balance of these two signaling pathways (Angew. Chem. Int. Ed., 2015). Such an investigation using NMR will lead to rational drug discovery that targets specific GPCRs.

(ii) Sophisticated stable isotope labeling of membrane proteins in eukaryotes

In NMR analyses of proteins, the atoms of interest can be selectively observed by labeling with stable isotopes such as ^{2}H, ^{13}C, and ^{15}N. High-level deuteration of protons surrounding observed atoms

enables sensitive detection of NMR signals in combination with the TROSY methods.

The isotope labeling strategy has been well-established in *E. coli* expression systems, however, it is not that straight forwards in eukaryotic cells, such as insect cells and mammalian cells. Since eukaryotic cells do not grow in heavy water (D_2O), extensive deuteration are thought to be impossible. We have developed a strategy to selectively introduce methyl ^{13}C-labeling with the high-level deuteration of surrounding amino acid residues in insect cells. Using this strategy, we have achieved a five-fold improvement in the sensitivity and succeeded the solution NMR analysis of GPCRs reconstituted in lipid bilayers for the first time (Angew. Chem. Int. Ed., 2014). The application of this technique has also led to the elucidation of the activation mechanism of ligand-dependent ion channels in lipid bilayers (Proc. Natl. Acad. Sci. USA., 2016).

By developing new stable isotope labeling techniques, we will push forward the NMR analysis of high-molecular-weight proteins and membrane proteins, including important drug targets.

Education

2002: B. Sc. (Pharmaceutical Sciences) The University of Tokyo, Japan

2006: M. Sc. (Pharmaceutical Sciences) The University of Tokyo, Japan

2009: Ph. D. (Pharmaceutical Sciences) The University of Tokyo, Japan

Professional Experience

2009/4-2011/3 Researcher, Japan Biological Informatics Consortium

2011/4-2014/4 Project Researcher, Graduate School of Pharmaceutical Sciences, The University of Tokyo

2014/4-2019/5 Project Assistant Professor, Graduate School of Pharmaceutical Sciences, The University of Tokyo

2019/6- Assistant Professor, Graduate School of Pharmaceutical Sciences, The University of Tokyo

Prizes

Young Scientist Poster Award (The Nuclear Magnetic Resonance Society of Japan, 2012)

Teaching experience

Faculty of Pharmaceutical Sciences, The University of Tokyo

- Laboratory Works of Pharmaceutical Sciences III (Biophysical analyses of peptides and proteins)
- First-Year Seminar for Natural Sciences Students (Structural biology and Scientific presentation)
- Pre-study of Practical Field Training in Pharmacy (Supply and management of medicines)

Grantsmanship

Elucidation of GPCR signal regulation mechanism by NMR
JSPS KAKENHI, Grant-in-Aid for Scientific Research (B), ¥17,160,000
Role: Principal Investigator (04/2021-03/2024)

Investigation of membrane proteins as drug targets based on function-related dynamics
JSPS KAKENHI, Grant-in-Aid for Challenging Exploratory Research, ¥6,500,000
Role: Principal Investigator (08/2020-03/2023)

Quantitative investigation of GPCR intracellular signaling based on molecular structures
JSPS KAKENHI, Grant-in-Aid for Scientific Research on Innovative Areas, ¥6,240,000
Role: Principal Investigator (04/2019-03/2021)

Structural basis of signal transduction mediated by various GPCRs
JSPS KAKENHI, Grant-in-Aid for Young Scientists (A), ¥26,130,000
Role: Principal Investigator (04/2017-03/2021)

Structural basis of GPCR signaling regulated by “lipoquality”
JSPS KAKENHI, Grant-in-Aid for Scientific Research on Innovative Areas, ¥9,620,000
Role: Principal Investigator (04/2016-03/2018)

Structural basis of biased signaling mediated by pharmacologically important GPCRs
JSPS KAKENHI, Grant-in-Aid for Young Scientists (B), ¥4,160,000
Role: Principal Investigator (04/2015-03/2017)

Investigation of GPCR signaling regulated by biased ligands
JSPS KAKENHI, Grant-in-Aid for Young Scientists (Start-up), ¥1,430,000
Role: Principal Investigator (04/2014-03/2015)

Publications

1. Biphasic activation of β-arrestin 1 upon interaction with a GPCR revealed by methyl-TROSY NMR.
Shiraishi Y, Kofuku Y, Ueda T, Pandey S, Dwivedi-Agnihotri H, Shukla AK, Shimada I.
Nat Commun. 2021 Dec 9;12(1):7158. doi: 10.1038/s41467-021-27482-3.

2. Function-Related Dynamics in Multi-Spanning Helical Membrane Proteins Revealed by Solution NMR.
Takeuchi K, Kofuku Y, Imai S, Ueda T, Tokunaga Y, Toyama Y, Shiraishi Y, Shimada I.
Membranes (Basel). 2021 Aug 9;11(8):604. doi: 10.3390/membranes11080604.

3. Activation of adenosine A2A receptor by lipids from docosahexaenoic acid revealed by NMR.
Mizumura T, Kondo K, Kurita M, Kofuku Y, Natsume M, Imai S, Shiraishi Y, Ueda T, Shimada I.
Sci Adv. 2020 Mar 18;6(12):eaay8544. doi: 10.1126/sciadv.aay8544.

4. Targeting FROUNT with disulfiram suppresses macrophage accumulation and its tumor-promoting properties.
Terashima Y, Toda E, Itakura M, Otsuji M, Yoshinaga S, Okumura K, Shand FHW, Komohara Y, Takeda M, Kokubo K, Chen MC, Yokoi S, Rokutan H, Kofuku Y, Ohnishi K, Ohira M, Iizasa T, Nakano H, Okabe T, Kojima H, Shimizu A, Kanegasaki S, Zhang MR, Shimada I, Nagase H, Terasawa H, Matsushima K.
Nat Commun. 2020 Jan 30;11(1):609. doi: 10.1038/s41467-020-14338-5.

5. Structural equilibrium underlying ligand-dependent activation of β2-adrenoreceptor.
Imai S, Yokomizo T, Kofuku Y, Shiraishi Y, Ueda T, Shimada I.
Nat Chem Biol. 2020 Apr;16(4):430-439. doi: 10.1038/s41589-019-0457-5.

6. Function-related conformational dynamics of G protein-coupled receptors revealed by NMR.
Ueda T, Kofuku Y, Okude J, Imai S, Shiraishi Y, Shimada I.
Biophys Rev. 2019 Jun;11(3):409-418. doi: 10.1007/s12551-019-00539-w.

7. GPCR drug discovery: integrating solution NMR data with crystal and cryo-EM structures.
Shimada I, Ueda T, Kofuku Y, Eddy MT, Wüthrich K.
Nat Rev Drug Discov. 2019 Jan;18(1):59-82. doi: 10.1038/nrd.2018.180.

8. Deuteration and selective labeling of alanine methyl groups of β2-adrenergic receptor expressed in a baculovirus-insect cell expression system.
Kofuku Y, Yokomizo T, Imai S, Shiraishi Y, Natsume M, Itoh H, Inoue M, Nakata K, Igarashi S, Yamaguchi H, Mizukoshi T, Suzuki EI, Ueda T, Shimada I.
J Biomol NMR. 2018 Jul;71(3):185-192. doi: 10.1007/s10858-018-0174-5.

9. Phosphorylation-induced conformation of β2-adrenoceptor related to arrestin recruitment revealed

by NMR.
Shiraishi Y, Natsume M, Kofuku Y, Imai S, Nakata K, Mizukoshi T, Ueda T, Iwaï H, Shimada I.
Nat Commun. 2018 Jan 15;9(1):194. doi: 10.1038/s41467-017-02632-8.

10. Utilization of paramagnetic relaxation enhancements for structural analysis of actin-binding proteins in complex with actin.
Huang S, Umemoto R, Tamura Y, Kofuku Y, Uyeda TQ, Nishida N, Shimada I.
Sci Rep. 2016 Sep 22;6:33690. doi: 10.1038/srep33690.

11. Conductance of P2X4 purinergic receptor is determined by conformational equilibrium in the transmembrane region.
Minato Y, Suzuki S, Hara T, Kofuku Y, Kasuya G, Fujiwara Y, Igarashi S, Suzuki E, Nureki O, Hattori M, Ueda T, Shimada I.
Proc Natl Acad Sci U S A. 2016 Apr 26;113(17):4741-6. doi: 10.1073/pnas.1600519113.

12. Identification of a Conformational Equilibrium That Determines the Efficacy and Functional Selectivity of the μ-Opioid Receptor.
Okude J, Ueda T, Kofuku Y, Sato M, Nobuyama N, Kondo K, Shiraishi Y, Mizumura T, Onishi K, Natsume M, Maeda M, Tsujishita H, Kuranaga T, Inoue M, Shimada I.
Angew Chem Int Ed Engl. 2015 Dec 21;54(52):15771-6. doi: 10.1002/anie.201508794.

13. Elucidation of the CCR1- and CCR5-binding modes of MIP-1α by application of an NMR spectra reconstruction method to the transferred cross-saturation experiments.
Yoshiura C, Ueda T, Kofuku Y, Matsumoto M, Okude J, Kondo K, Shiraishi Y, Shimada I.
J Biomol NMR. 2015 Dec;63(4):333-340. doi: 10.1007/s10858-015-9992-x.

14. Development of a method for reconstruction of crowded NMR spectra from undersampled time-domain data.
Ueda T, Yoshiura C, Matsumoto M, Kofuku Y, Okude J, Kondo K, Shiraishi Y, Takeuchi K, Shimada I.
J Biomol NMR. 2015 May;62(1):31-41. doi: 10.1007/s10858-015-9908-9.

15. Functional dynamics of deuterated β2 -adrenergic receptor in lipid bilayers revealed by NMR spectroscopy.
Kofuku Y, Ueda T, Okude J, Shiraishi Y, Kondo K, Mizumura T, Suzuki S, Shimada I.
Angew Chem Int Ed Engl. 2014 Dec 1;53(49):13376-9. doi: 10.1002/anie.201406603.

16. Cross-saturation and transferred cross-saturation experiments.
Ueda T, Takeuchi K, Nishida N, Stampoulis P, Kofuku Y, Osawa M, Shimada I.
Q Rev Biophys. 2014 May;47(2):143-87. doi: 10.1017/S0033583514000043.

17. Functional dynamics of cell surface membrane proteins.
Nishida N, Osawa M, Takeuchi K, Imai S, Stampoulis P, Kofuku Y, Ueda T, Shimada I.
J Magn Reson. 2014 Apr;241:86-96. doi: 10.1016/j.jmr.2013.11.007.

18. Identification of a binding element for the cytoplasmic regulator FROUNT in the membrane-proximal C-terminal region of chemokine receptors CCR2 and CCR5.
Toda E, Terashima Y, Esaki K, Yoshinaga S, Sugihara M, Kofuku Y, Shimada I, Suwa M, Kanegasaki S, Terasawa H, Matsushima K.
Biochem J. 2014 Jan 15;457(2):313-22. doi: 10.1042/BJ20130827.

19. Efficacy of the β_2-adrenergic receptor is determined by conformational equilibrium in the transmembrane region.
Kofuku Y, Ueda T, Okude J, Shiraishi Y, Kondo K, Maeda M, Tsujishita H, Shimada I.
Nat Commun. 2012;3:1045. doi: 10.1038/ncomms2046.

20. NMR analyses of the interaction between CCR5 and its ligand using functional reconstitution of CCR5 in lipid bilayers.
Yoshiura C, Kofuku Y, Ueda T, Mase Y, Yokogawa M, Osawa M, Terashima Y, Matsushima K, Shimada I.
J Am Chem Soc. 2010 May 19;132(19):6768-77. doi: 10.1021/ja100830f.

21. Structural basis of the interaction between chemokine stromal cell-derived factor-1/CXCL12 and its G-protein-coupled receptor CXCR4.
Kofuku Y, Yoshiura C, Ueda T, Terasawa H, Hirai T, Tominaga S, Hirose M, Maeda Y, Takahashi H, Terashima Y, Matsushima K, Shimada I.
J Biol Chem. 2009 Dec 11;284(50):35240-50. doi: 10.1074/jbc.M109.024851.

22. High-throughput screening of optimal solution conditions for structural biological studies by fluorescence correlation spectroscopy.
Sugiki T, Yoshiura C, Kofuku Y, Ueda T, Shimada I, Takahashi H.
Protein Sci. 2009 May;18(5):1115-20. doi: 10.1002/pro.92.