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Research Interests

My research interest is to elucidate the functions of proteins that are important for biology and drug development, using NMR. Proteins exert their biofunctions with continuously changing their conformations. In relation to this point, solution NMR methods provide quantitative information about the dynamics of proteins over a wide range of frequencies, in aqueous solutions at near-physiological temperatures. I have been utilized solution NMR methods to clarify the function-related ligand-binding mode and conformational dynamics of various membrane proteins, including G protein-coupled receptors (GPCRs), ion channels, and photosynthetic membrane protein complexes, and high molecular weight proteins, such as chemotaxis signaling proteins and UV-damaged DNA repair enzymes.

Currently I primarily focus on the function-related conformational dynamics of GPCRs. GPCRs function as receptors of various chemical messengers, including neurotransmitters, hormones, cytokines (chemokines), and metabolites, and more than 30% of current drugs target GPCRs. For understanding the physiological functions of GPCRs and accelerating the development of drugs that target GPCRs, the following questions should be addressed. (i) What kind of conformation do GPCRs in the full agonist-bound state adopt to interact with both G protein and arrestin? (ii) How do GPCRs induce signaling in a manner dependent on efficacy and functional selectivity? (iii) How does the surrounding lipid bilayer structure and the lipid compositions affect the conformation and activities of GPCRs? Although NMR studies of GPCRs are challenging, because of their large size and the limitations of the stable isotope labeling in eukaryote cell expression systems, which are used for the expression of functional GPCRs, I have been developing NMR methods to overcome these bottlenecks, and utilizing the developed methods to address the aforementioned questions, as follows.

(i) Structure of GPCRs in the full agonist-bound state

We determined the structure of the transmembrane helix of b_2 adrenergic receptor (β_2AR) in the full

agonist-bound state, and found that the structure is favorable for the interaction with G protein (***Nat. Chem. Biol.*** 2020,). In addition, we clarified that the full agonist-bound β_2AR adopts a conformation favorable for the interaction with arrestin, upon phosphorylated by GPCR kinases (***Nat. Commun.*** 2018).

(ii) Efficacy and functional selectivity of GPCRs

We found that β_2AR and m opioid receptor exist in equilibrium between multiple inactive and active conformations, and the populations of the active conformations determine the efficacy and the functional selectivity in each ligand-bound state (***Nat. Commun.*** 2012, ***Angew. Chem. Int. Ed.*** 2015). These findings would be helpful for the development of drugs with desired efficacy and functional selectivity.

(iii) Effect of lipids on the conformation and activity of GPCRs

We utilized nanodiscs to examine the conformation of GPCRs in the lipid bilayer environment. In β_2AR reconstituted into the lipid bilayer of nanodiscs, the exchange rate and the population of the conformational equilibrium were remarkably different from those in micelles, and the population of the active conformation in nanodiscs correlated better with the efficacies than that in micelles (***Angew. Chem. Int. Ed.*** 2015). In addition, our NMR analyses revealed that the lipids containing acyl chains derived from docosahexaenoic acid (DHA) redistribute the equilibrium among multiple active conformations of A_{2A}AR toward conformations preferable for G protein binding (***Sci. Adv.*** 2020). dev

(iv) Development of methods for NMR studies of GPCRs

We developed methods for selective labeling of methionine methyl, alanine methyl, and leucine amide groups in insect cell expression systems (***Nat. Chem. Biol.*** 2020, ***J. Biomol. NMR*** 2018, ***Proc. Natl. Acad. Sci.*** 2016, ***Nat. Commun.*** 2012). We also accomplished the partial deuteration in insect cell systems, leading to >5 time sensitivity enhancement of GPCR signals (***Angew. Chem. Int. Ed.*** 2014). These methods enabled us to observe the conformational equilibria in the transmembrane region of GPCRs. In addition, we established the segmental labeling of the C-terminal region of β_2AR (***Nat. Commun.*** 2018, ***J. Biomol. NMR*** 2012). Furthermore, we developed methods for the sparse sampling and reconstruction of multidimensional NMR data (Co-ANAFOR), leading to > 50% reduction of the measurement time of 2D NMR spectra with retaining the precision and accuracy of the signal intensities (***J. Biomol. NMR*** 2015). This method enabled us to identify the binding interface of chemokine MIP-1α to the chemokine receptors CCR1 and CCR5 (***J. Biomol. NMR*** 2015).

We have been invited to write reviews of these GPCR studies, and the reviews were published in

Nature Reviews Drug Discovery and other journals (***Nat. Rev. Drug Discov.*** 2019, ***J. Magn. Reason.*** 2022, ***Biophs. Rev.*** 2019, ***J. Magn. Reason.*** 2014, ***Curr. Opin. Struct. Biol.*** 2012)

I have recently initiated studies on utilizing the timescales and populations in the function-related conformational equilibria of GPCRs and other proteins, determined by NMR, to integrate multi-scale information, including the protein structures at atomic resolutions and the real-time observations of the cellular responses via GPCR signaling, and construct cellular signaling models that can quantitatively explain real-time cellular responses at atomic resolution. For example, a time-course simulation of G-protein signaling, based on the intracellular signaling reaction rates and the exchange rates between the inactive and active conformations of β_2AR, demonstrated that the β_2AR agonists induce the β_2AR activation, G-protein activation, and the increase in cAMP on timescales of milliseconds, hundreds of milliseconds, and seconds, respectively (***Angew. Chem. Int. Ed.*** 2014). Although the huge size of the parameter spaces of the models is a bottleneck of such studies, I successfully utilized exchange Monte Carlo algorism, which enables us to consider the entire parameter space for arbitrary complicated models and has gained popularity in machine learning fields, to determine the rate constants and their uncertainty of sophisticated models (***Nat. Chem. Biol.*** 2020, ***Sci. Rep.*** 2017). I'm confident that such multi-scale cellular signaling models would be helpful for understanding the mechanism of action of drugs and determining the strategies on the drug development.

Education

2000: B. Sc. (Pharmaceutical Sciences) The University of Tokyo, Japan
2002: M. Sc. (Pharmaceutical Sciences) The University of Tokyo, Japan
2005: Ph.D. (Pharmaceutical Sciences) The University of Tokyo, Japan

Professional Experience

2005/4-2006/12 Researcher, Japan Biological Informatics Consortium
2007/1-2019/5 Research Associate, Graduate School of Pharmaceutical Sciences, The University of Tokyo
2013/10-2017/3 (Additional post) Faculty-Presto, Japan Science and Technology Agency, Tokyo, Japan
2019/6-present Associate Professor, Graduate School of Pharmaceutical Sciences, The University of Tokyo

Honors, Scholarships, and Prizes

Young Scientist Poster Award (The Nuclear Magnetic Resonance Society of Japan, 2002)

Teaching experience

(i) Lectures in Faculty of Pharmaceutical Sciences, The University of Tokyo

- Physical Chemistry (basic theory of quantum chemistry and spectroscopy)
- Molecular Structural Bio-Sciences (basics of NMR)
- Biophysics (biomolecular NMR)
- Laboratory Works of Pharmaceutical Sciences III (Structural analyses of peptides and protein-protein interactions using NMR and other biophysical methods)
- First-Year Seminar for Natural Sciences Students (Presentation)

(ii) Lectures in Graduate School of Pharmaceutical Sciences, The University of Tokyo

- Special Lecture Basic Pharmaceutical Science II (NMR studies on function-related dynamics of proteins)
- Special Lecture Biomolecular Analysis (understanding state-of-the-art NMR methods based on quantum chemistry)

As a research associate and associate professor, I have directly mentored 20 PhD students and several visiting research scientists in pharmaceutical companies.

Grantsmanship

“Elucidation of the residence time-dependent cellular response regulation mechanism of weak GPCR-drug interactions”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥6,000,000
Role: Principal Investigator (04/2020-03/2022)

“Clarification of the GPCR activity regulation mechanism by NMR”
Grant-in-Aid for Scientific Research (B), ¥13,500,000
Role: Principal Investigator (04/2020-03/2023)

“Elucidation of the mechanism underlying GPCR signaling regulation by mathematical analysis based on the conformational equilibria”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥2,400,000
Role: Principal Investigator (04/2020-03/2022)

“Elucidation of the GPCR function under the multimolecular crowding lipid environment”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥4,800,000
Role: Principal Investigator (04/2018-03/2020)

“In situ functional analyses of membrane proteins by NMR”
Grant-in-Aid for Specially Promoted Research, ¥25,000,000
Role: Co-Investigator (04/2017-03/2021)

“Development of multidimensional NMR data analysis method and application to the studies on GPCR functions”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥5,000,000
Role: Principal Investigator (04/2016-03/2018)

“Establishment of methods for studying the structural dynamics of the interactions between ubiquitin and ubiquitin binding proteins by NMR”
Grant-in-Aid for Scientific Research (C), ¥3,800,000
Role: Principal Investigator (04/2016-04/2019)

“Development of method for reconstruction of multidimensional NMR spectra from sparse data and the application to the GPCR studies”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥5,100,000
Role: Principal Investigator (04/2015-03/2017)

“Structural elucidation of the biased signal regulation mechanism of GPCRs”
Grant-in-Aid for Scientific Research (C), ¥3,900,000
Role: Principal Investigator (04/2013-03/2016)

“Structural elucidation of the signaling mechanism of medically important GPCRs”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥3,600,000
Role: Principal Investigator (04/2013-03/2015)

“Structural elucidation of the GPCR signaling mechanism”
Grant-in-Aid for Scientific Research on Innovative Areas, ¥3,600,000
Role: Principal Investigator (04/2011-03/2013)

"Structural clarification of the sequential signal transduction mechanism of the two-component systems"
Grant-in-Aid for Young Scientists (B), ¥3,600,000
Role: Principal Investigator (04/2011-03/2013)

"Elucidation of the efficient electron transport accomplished by interaction between photosynthetic membrane proteins and electron transport proteins"
Grant-in-Aid for Young Scientists (B), ¥3,600,000
Role: Principal Investigator (04/2009-03/2011)

"Elucidation of the function of ferredoxin-quinone reductase by NMR"
Grant-in-Aid for Young Scientists(Start-up), ¥1,300,000
Role: Principal Investigator (04/2007-03/2008)

"Exploration of the methods for analyzing soft protein-protein interactions by NMR"
Grant-in-Aid for Scientific Research on Priority Areas,
Role: Co-Investigator (04/2007-03/2008), ¥2,000,000

"Structural elucidation of the dynamical equilibria of GPCRs under lipid bilayer environment by NMR"
Japan Science and Technology Agency, PRESTO, ¥40,000,000
Role: Principal Investigator (10/2014-03/2018)

Publications

1. Ueda, T., Imai, S., and Shimada*, I. Function-related dynamics of GPCRs. **J. Magn. Reson.** 336: 107164 (2022).

2. Yokomine, M., Morimoto, J., Fukuda, Y., Shiratori, Y., Kuroda, D., Ueda, T., Takeuchi, K., Tsumoto, K., and Sando*, S. Oligo(N-methylalanine) as a peptide-based molecular scaffold with a minimal structure and high density of functionalizable sites. **Angew. Chem. Int. Ed.** E202200119 (2022)

3. Shiraishi, Y., Kofuku, Y., Ueda, T., Pandey, S., Dwivedi-Agnihotri, H., Shukla, AK., and Shimada, I. Biphasic activation of b-arrestin 1 upon interaction with a GPCR revealed by methyl-TROSY NMR. **Nat. Commun.** 12: 7158 (2021).

4. Takeuchi, K., Kofuku, Y., Imai, S., Ueda, T., Tokunaga, Y., Toyama, Y., Shiraishi, Y., and Shimada*, I. Function-related dynamics in multi-spanning helical membrane proteins revealed by solution NMR. **Membranes** 11: 604 (2021).

5. Mizumura, T., Kondo, K., Kurita, M., Kofuku, Y., Natsume, M., Imai, S., Shiraishi, Y., Ueda, T., and Shimada*, I. Activation of adenosine A_{2A} receptor by lipids from docosahexaenoic acid revealed by NMR. **Sci. Adv.** 6: eaay8544 (2020).

6. Imai, S., Yokomizo, T., Kofuku, Y., Shiraishi, Y., Ueda, T., and Shimada*, I. Structural equilibrium underlying ligand-dependent activation of β_2-adrenoreceptor. **Nat. Chem. Biol.** 16: 430-439 (2020).

7. Ueda, T., Kofuku, Y., Okude, J., Imai, S., Shiraishi, Y., and Shimada*, I. Function-related conformational dynamics of G protein-coupled receptors revealed by NMR. **Biphys. Rev.** 11: 409-418 (2019).

8. Shimada* I., Ueda, T., Kofuku, Y., Eddy, M., and Wüthrich*, K. GPCR drug discovery: integrating solution NMR data with crystal and cryo-EM structures. **Nat. Rev. Drug Discov.** 18: 59-82 (2019).

9. Takaoka, Y., Uchinomiya, S., Kobayashi, D., Endo, M., Hayashi, T., Fukuyama, Y., Hayasaka, H., Miyasaka, M., Ueda, T., Shimada, I., Hamachi, I. Endogenous membrane receptor labeling by reactive cytokines and growth factors to chase their dynamics in live cells. **Chem.** 4: 1451-1464 (2018).

10. Kofuku, Y., Yokomizo, T., Imai, S., Shiraishi, Y., Natume, M., Itoh, H., Inoue, M., Nakata, K., Igarashi, S., Yamaguchi, H., Mizukoshi, T., Suzuki, E., Ueda, T., and Shimada*, I. Deuteration and selective labeling of alanine methyl groups of □$_2$-adrenergic receptor expressed in a baculovirus-insect cell expression system. **J. Biomol. NMR.** 71: 185-192 (2018)

11. Shiraishi, Y., Natsume, M., Kofuku, Y., Imai, S., Nakata, K., Mizukoshi, T., Ueda, T., Iwai, H., and Shimada*, I. Phosphorylation-induced conformation of □$_2$-adrenoreceptor related to arrestin recruitment revealed by NMR. **Nat. Commun.** 9: 194 (2018)

12. Minato, Y., Ueda, T., Machiyama, A., Iwai, H., and Shimada*, I. Dynamic domain arrangement of CheA-CheY complex regulates bacterial thermotaxis, as revealed by NMR. **Sci. Rep.** 7: 16462 (2017)

13. Minato, Y., Suzuki, S., Hara, T., Kofuku, Y., Kasuya, G., Fujiwara, Y., Igarashi, S., Suzuki, E., Nureki, O., Hattori, M., Ueda, T., and Shimada*, I. Conductance of $P2X_4$ purinergic receptor is determined by conformational equilibrium in the transmembrane region, **Proc. Natl. Acad. Sci.** 113: 4741-4746 (2016)

14. 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., and Shimada*, I. Conformational equilibrium of □-opioid receptor determines its efficacies and functional selectivities. **Angew. Chem. Int. Ed.** 54: 15771-15776 (2015)

15. Yoshiura, C., Ueda, T., Kofuku, Y., Matsumoto, M., Okude, J., Kondo, K., Shiraishi, Y., Takeuchi, K., and Shimada*, I., Elucidation of the CCR1- and CCR5- binding modes of MIP-1□□by application of an NMR spectra reconstruction method to the transferred cross-saturation experiments. **J. Biomol. NMR**. 63: 333-340 (2015)

16. 12. Ueda, T., Yoshiura, C., Matsumoto, M., Kofuku, Y., Okude, J., Kondo, K., Shiraishi, Y., Takeuchi, K., and Shimada*, I. Development of a method for reconstruction of crowded NMR spectra from undersampled time-domain data. **J. Biomol. NMR** 62: 31-41 (2015)

17. Kofuku, Y., Ueda, T., Okude, J., Shiraishi, Y., Kondo, K., Mizumura, T., Suzuki, S., and Shimada*, I. Functional dynamics of deuterated b_2 -adrenergic receptor in lipid bilayers revealed by NMR Spectroscopy. **Angew. Chem. Int. Ed.** 53: 13376-13379 (2014)

18. Esaki, K., Yoshinaga, S., Tsuji, T., Toda, E., Terashima, Y., Saitoh, T., Kohda, D., Kohno, T., Osawa, M., Ueda, T., Shimada, I., Matsushima, K. and *Terasawa, H. Structural basis for the binding of the membrane-proximal C-terminal region of chemokine receptor CCR2 with the cytosolic regulator FROUNT. **FEBS J.** 281: 5552-5566 (2014)

19. Ueda, T., Takeuchi, K., Nishida, N., Stampoulis, P., Kofuku, Y., Osawa, M., and Shimada*, I. Cross-saturation and transferred cross-saturation experiments. **Q. Rev. Biophys.** 47: 143-187 (2014)

20. Nishida, N., Osawa, M., Takeuchi, K., Imai, S., Stampoulis P., Kofuku, Y., Ueda, T., and Shimada*, I. Functional dynamics of cell surface membrane proteins. **J. Magn. Reson.** 241: 86-96 (2014)

21. Osawa, M., Takeuchi, K., Ueda, T., Nishida, N., and Shimada*, I. Functional dynamics of proteins revealed by NMR. **Curr. Opin. Struct. Biol.** 22: 660-669 (2012)

22. Kofuku, Y., Ueda, T., Okude, J., Shiraishi, Y., Kondo, K., Masahiro Maeda, Hideki Tsujishita, and Shimada*, I. Efficacy of the □$_2$-adrenergic receptor is determined by conformational equilibrium in the transmembrane region. **Nat. Commun.** 3: 1045 (2012)

23. Imai, S., Osawa, M., Mita, K., Toyonaga, S., Machiyama, A., Ueda, T., Takeuchi, K., Oiki, S., and Shimada*, I. Functional equilibrium of the KcsA structure revealed by NMR. **J. Biol. Chem.** 287: 39634-39641 (2012)

24. Ueda, T., Nomoto, N., Koga, M., Ogasa, H., Ogawa, Y., Matsumoto, M., Stampoulis, P., Sode, K., Terasawa, H., and Shimada*, I. Structural basis of efficient electron transport between photosynthetic membrane proteins and plastocyanin in spinach revealed using nuclear magnetic resonance. **Plant Cell** 24, 4173-4186 (2012)

25. Minato Y., Ueda, T., Machiyama, A., Shimada, I., and Iwai*, H. Segmental isotopic labeling of a 140 kDa dimeric multi-domain protein CheA from *Escherichia coli* by expressed protein ligation and protein trans-splicing. **J. Biomol. NMR** 53: 191-207 (2012)

26. Stampoulis, P., Ueda, T., Matsumoto, M., Terasawa, H., and Shimada*, I. Atypical membrane-embedded phosphatidylinositol 3,4-bisphosphate ($PI(3,4)P_2$)-binding site on $p47^{phox}$ Phox homology (PX) domain revealed by NMR. **J. Biol. Chem.** 287: 17848-17859 (2012)

27. Suga, M., Nishiyama, H., Konyuba, Y., Iwamatsu, S., Watanabe, Y., Yoshiura, C., Ueda, T., and *Sato, C. The atmospheric scanning electron microscope with open sample space observes

dynamic phenomena in liquid or gas. **Ultramicroscopy** 111: 1650-1658 (2011)

28. Yoshiura, C., Kofuku, Y., Ueda, T., Mase, Y., Yokogawa, M., Osawa, M., Terashima, Y., Matsushima, K., and Shimada*, I. NMR analyses of the interaction between CCR5 and its ligand using functional reconstitution of CCR5 in lipid bilayers. **J. Am. Chem. Soc.** 132: 6768-6777 (2010)

29. Matsumoto M., Ueda, T., and Shimada*, I. Theoretical analyses of the transferred cross-saturation method. **J. Magn. Reson.** 205: 114-124 (2010)

30. Kofuku, Y., Yoshiura, C., Ueda, T., Terasawa, H., Hirai, T., Tominaga, S., Hirose, M., Maeda, Y., Takahashi, H., Terashima, Y., Matsushima, K., and Shimada*, I. Structural basis of the interaction between chemokine stromal cell-derived factor-1/CXCL12 and its G-protein-coupled receptor CXCR4. **J. Biol. Chem.** (2009) 284, 35240-35250

31. Shimada*, I., Ueda, T., Matsumoto, M., Sakakura, M., Osawa, M., Takeuchi, K., Nishida, N., and Takahashi, H. Cross-saturation and transferred cross-saturation experiments. **Prog. Nuc. Magn. Reson. Spect.** 54: 123-140 (2009)

32. Sugiki, T., Yoshiura, C., Kofuku, Y., Ueda, T., Shimada, I., and Takahashi*, H. High-throughput screening of optimal solution conditions for structural biological studies by fluorescence correlation spectroscopy. **Protein Sci.** 18: 1115-20 (2009)

33. Ueda, T., Kato, A., Kuramitsu, S., Terasawa, H., and Shimada*, I. Identification and characterization of a second chromophore of DNA photolyase from *Thermus thermophilus* HB27. **J. Biol. Chem.** 280, 36237-36243 (2005)

34. Ueda, T., Kato, A., Ogawa, Y., Torizawa, T., Kuramitsu, S., Iwai, S., Terasawa, H., and Shimada*, I. NMR Study of repair mechanism of DNA photolyase by FAD-induced paramagnetic relaxation enhancement. **J. Biol. Chem.** 279: 52574-52579 (2004)

35. Torizawa, T., Ueda, T., Kuramitsu, S., Hitomi, K., Todo, T., Iwai, S., Morikawa, K., and Shimada*, I. Investigation of the cyclobutane pyrimidine dimer (CPD) photolyase DNA recognition mechanism by NMR analyses. **J. Biol. Chem.** 279: 32950-32956 (2004)