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Research Interests

I am interested in how proteins form their unique and complicated steric structures, recognize and interact with other biomolecules, transfer and regulate cellular information, and orchestrate life. In addition, improving and diversifying the way we develop therapeutics, which would progress when the former questions were addressed, are also in my scope. To these ends, I have been investigated the mechanisms of regulating enzymatic activity and interaction of proteins by structural analyses using nuclear magnetic resonance (NMR) spectroscopy in solution.

Summary of representative achievements by me are described below:

(i) Modulation of hydrogen bond networks in a protein by sub-THz radiation (*Biophys J.*, 2021)

Revealing the roles of hydration or hydrogen bond networks in structure and function of aqueous proteins is a fundamental but difficult issue to address. By performing hydrogen-deuterium exchange NMR experiments for a protein, ubiquitin, which was irradiated with the electromagnetic wave at a 0.1 terahertz (sub-THz) frequency, we found that the sub-THz radiation can perturb the hydrogen bond networks both within ubiquitin and between ubiquitin and solvent water molecules. The sub-THz effects were qualitatively opposite to those induced by temperature raise, indicating that the sub-THz radiation non-thermally affected the hydrogen bond networks in ubiquitin.

Further understanding of the mechanism of structural change of ubiquitin (or other proteins) induced by sub-THz irradiation and utilization of such effects for functional regulation of proteins is our present interests.

(ii) Robust kinase activity of a stress-activated kinase p38 α（*Nat. Struct. Mol. Biol.*, 2014）

Mitogen-activated protein kinases (MAPKs) comprise only kinase domains, in which there is a conserved allosteric site, so called docking site, responsible for specific and high affinity binding to

their substrates. We found that the substrate-binding at the docking site of a stress-activated MAPK p38α enhances its kinase activity by enhancing both ATP- and substrate phosphoacceptor site-bindings and accelerating phosphotransfer kinetics. Docking interaction shifted the conformational equilibrium of p38α between two states with distinct affinity for ATP toward the higher one. This seems to contribute to robust signaling via p38α under stress conditions, where otherwise p38α's activity would be severely compromised due to ATP depletion.

We are now investigating the effects of changes in the cellular environment upon stress stimuli on the conformational equilibrium and obtaining exciting results. Furthermore, we are also interested in the behavior of signaling modules, i.e., the strength of activation of p38α in the presence of other proteins comprising the signaling pathway, for deeper understanding of the roles of structural dynamics in the context of cellular information transfer.

(iii) Development of NMR techniques (*J. Med. Chem.*, 2020 ; *Molecules*, 2017)

J. Med. Chem., 2020 : NMR needs to be kept improved to be fully valuable for resolving essential biological questions and addressing medical problems. As especially for solution NMR, high molecular weight (HMW) limitation is one of the largest problems. Isotope labeling such as perdeuteration and methyl-selective labeling on a perdeuteration background combined with transverse relaxation-optimized spectroscopic schemes (TROSY-type experiments) have conquered HMW proteins by making the observed resonances sharper and stronger. However, many disease-associated mammalian proteins are prepared as non-deuterated samples, because recombinant expression of these proteins are possible only in mammalian and/or insect cells, which cannot survive perdeuterated media. For these proteins, conventional TROSY schemes depending on the perdeuteration were not fully applicable. We developed a novel approach based on the ^{15}N direct detection TROSY (Takeuchi et al, *J. Biomol. NMR*, 2015). The transverse relaxation of ^{15}N TROSY components are less sensitive to external protons than ^{1}H components and thus produce sharper resonances. This feature is fully exploited by ^{15}N direct detection as the direct dimension generally gives higher digital resolution with negligible time cost. We extended the HMW limit of the ^{15}N direct detection by introducing the CRINEPT scheme (Riek et al, *PNAS*, 1999) and by truncating/concatenating the pulse scheme. As a result, we for the first time achieved in observation of the backbone amide resonances of an analogue of an intact therapeutic monoclonal antibody (150 kDa) dissolved in different types of clinical formulation at a storage temperature of 4^{o}C (effective MW ~300 kDa, as estimated from the temperature- and solute-dependent increase in viscosity). Structural variations according to the difference in the glycosylation pattern were revealed.

Molecules, 2017: One of the unique contributions from NMR in structural biology is experimentally

obtained, site-resolved, quantitative dynamics information of proteins in solution in mostly universal time range (picoseconds – seconds) related to biological functions. Among these, fast dynamics in the ps – ns time range, which can be obtained by a forbidden coherent transfer (FCT) measurements in the case of methyl side chains, reflects the local dynamics of a chemical moiety. We have shown that this information is useful in ligand optimization toward higher target selectivity (Mizukoshi, Tokunaga et al, *Angew. Chem. Int. Ed.*, 2016). We extended the FCT experiment to the trifluoromethyl (CF_3) groups that are frequently contained in drug molecules. By optimizing the experimental conditions for the CF_3 group by considering their relaxation propensity such as large chemical shift anisotropy, we succeeded in determining a quantitative indicative of CF_3 dynamics that would help lead optimization processes.

Education

2007: B. Sc. (Pharmaceutical Sciences) The University of Tokyo, Japan
2009: M. Sc. (Pharmaceutical Sciences) The University of Tokyo, Japan
2012: Ph. D. (Pharmaceutical Sciences) The University of Tokyo, Japan

Professional Experience

2012/4-2013/3	Postdoctoral fellow, Japan Biological Informatics Consortium
2013/4-2013/7	Postdoctoral fellow, National Institute of Institute of Advanced Industrial Science and Technology, Japan
2013/8-2016/3	Postdoctoral fellow, Japan Biological Informatics Consortium
2016/4-2020/3	Research scientist, Molecular Profiling Research Center for Drug Discovery, National Institute of Institute of Advanced Industrial Science and Technology, Japan
2021/4-2021/10	Research scientist, Cellular and Molecular Biotechnology Institute, National Institute of Advanced Industrial Science and Technology, Japan
2021/11-present	Assistant Professor, Graduate School of Pharmaceutical Sciences, The University of Tokyo

Honors, Scholarships, and Prizes

Young Scientist Encouragement Award (Division of Physical Sciences, the Pharmaceutical Society of Japan, 2014)
Young Scientist Poster Award (The Nuclear Magnetic Resonance Society of Japan, 2014)

Grantsmanship

“Development of methods for evaluating and improving the membrane permeability of middle-sized molecules”
Grant-in-Aid for Challenging Research (Exploratory), **¥1,000,000**
Role: Co-Investigator (07/2020-03/2022)

“Development of dynamic Structure based drug development for lead optimization and improvement of druggability of human proteome”
Grant-in-Aid for Scientific Research (B), **¥1,500,000**
Role: Co-Investigator (04/2020-03/2023)

“Redefining genetic information: collective motion of DNA and water encoded in the repetitive sequences”
Grant-in-Aid for Scientific Research (B), **¥1,500,000**
Role: Co-Investigator (04/2020-03/2023)

“Structural basis of redox sensing via cysteine residues in the transmembrane region of membrane proteins”
Grant-in-Aid for Scientific Research (C), **¥4,290,000**
Role: Principal Investigator (04/2019-03/2022)

“NMR method to obtain structural fingerprints of an intact monoclonal antibody acquired under formulated storage conditions”
Grant-in-Aid for Challenging Research (Exploratory), **¥1,000,000**
Role: Co-Investigator (06/2018-03/2020)

“Structural basis for the selection mechanism of specific kinase signaling pathways via stimuli-dependent modulations of the functional equilibrium”
Grant-in-Aid for Young Scientists (B), **¥4,420,000**
Role: Principal Investigator (04/2017-03/2019)

“Elucidation of the mechanism of stimulus-dependent selection of kinase signaling pathways by NMR analyses of the functional equilibrium”
Grant-in-Aid for Research Activity Start-up, **¥2,990,000**

Role: Principal Investigator (08/2016-03/2018; declined for 04/2017-03/2018 for duplicated issues with another grant)

Publications

1. Takeuchi K, Kofuku Y, Imai S, Ueda T, Tokunaga Y, Toyama Y, Shiraishi Y, Shimada I, Function-Related Dynamics in Multi-Spanning Helical Membrane Proteins Revealed by Solution NMR, *Membranes (Basel)* (2021), **11**, 604. doi: 10.3390/membranes11080604.
2. Tokunaga Y, Tanaka M, Iida H, Kinoshita M, Tojima Y, Takeuchi K, Imashimizu M, Nonthermal excitation effects mediated by sub-terahertz radiation on hydrogen exchange in ubiquitin *Biophys J* (2021), **120**, 2386. doi: 10.1016/j.bpj.2021.04.013.
3. Takeuchi K, Misaki I, Tokunaga Y, Fujisaki M, Kamoshida H, Takizawa T, Hanzawa H, Shimada I, Conformational Plasticity of Cyclic Ras-Inhibitor Peptides Defines Cell Permeabilization Activity, *Angew Chem Int Ed* (2021), **60**, 6567. doi: 10.1002/anie.202016647.
4. Tokunaga Y, Takeuchi K, Role of NMR in High Ordered Structure Characterization of Monoclonal Antibodies, *Int J Mol Sci* (2020), **22**, 46. doi: 10.3390/ijms22010046.
5. Mizukoshi Y, Takeuchi K, Tokunaga Y, Matsuo H, Imai M, Fujisaki M, Kamoshida H, Takizawa T, Hanzawa H, Shimada I, Targeting the cryptic sites: NMR-based strategy to improve protein druggability by controlling the conformational equilibrium, *Sci Adv* (2020), **6**, eabd0480. doi: 10.1126/sciadv.abd0480.
6. Imashimizu M, Tokunaga Y, Afek A, Takahashi H, Shimamoto N, Lukatsky DB, Control of Transcription, Initiation by Biased Thermal Fluctuations on Repetitive Genomic Sequences, *Biomolecules* (2020), **10**, 1299. doi: 10.3390/biom10091299.
7. Tokunaga Y, Takeuchi K, Okude J, Ori K, Torizawa T, Shimada I, Structural Fingerprints of an Intact Monoclonal Antibody Acquired under Formulated Storage Conditions via (15)N Direct Detection Nuclear Magnetic Resonance, *J Med Chem* (2020), **63**, 5360. doi: 0.1021/acs.jmedchem.0c00231.
8. Tokunaga Y, Viennet T, Arthanari H, Takeuchi K, Spotlight on the Ballet of Proteins: The Structural Dynamic Properties of Proteins Illuminated by Solution NMR, *Int J Mol Sci* (2020), **21**, 1829. doi: 10.3390/ijms21051829.
9. Tatebayashi K, Yamamoto K, Tomida T, Nishimura A, Takayama T, Oyama H, Hata Y, Akabane S, Tokunaga Y, Saito H, Osmostress enhances activating phosphorylation of Hog1 MAP kinase by mono-phosphorylated Pbs2 MAP2K, *EMBO J* (2020), **39**, e103444. doi: 10.15252/embj2019103444.
10. Tokunaga Y, Takeuchi K, Shimada I, Forbidden Coherence Transfer of (19)F Nuclei to Quantitatively Measure the Dynamics of a CF_3-Containing Ligand in Receptor-Bound States,

Molecules (2017), **22**, 1492. doi: 10.3390/molecules22091492.

11. Mizukoshi Y, Takeuchi K, Arutaki M, Tokunaga Y, Takizawa T, Hanzawa H, Shimada I, Improvement of Ligand Affinity and Thermodynamic Properties by NMR-Based Evaluation of Local Dynamics and Surface Complementarity in the Receptor-Bound State, *Angew Chem Int Ed* (2016), **55**, 14606. doi: 10.1002/anie.201607474.
12. Tokunaga Y, Takeuchi K, Takahashi H, Shimada I, Allosteric enhancement of MAP kinase p38α's activity and substrate selectivity by docking interactions, *Nat Struct Mol Biol* (2014), **21**, 704. doi: 10.1038/nsmb.2861. Epub 2014 Jul 20.
13. Sugiki T, Takeuchi K, Yamaji T, Takano T, Tokunaga Y, Kumagai K, Hanada K, Takahashi H, Shimada I, Structural basis for the Golgi association by the pleckstrin homology domain of the ceramide trafficking protein (CERT), *J Biol Chem* (2012), **287**, 33706. doi: 10.1074/jbc.M112.367730.
14. Moriya J, Sakakura M, Tokunaga Y, Prosser RS, Shimada I, An NMR method for the determination of protein binding interfaces using TEMPOL-induced chemical shift perturbations, *Biochim Biophys Acta* (2009), **1790**, 1368. doi: 10.1016/j.bbagen.2009.06.001.