Molecular Spectroscopy Laboratory
Chief Scientist: Tahei Tahara (D.Sci.)

(0) Research field

CPR Subcommittee**:** Chemistry
Keywords: Ultrafast spectroscopy, Interface-selective nonlinear spectroscopy, Single molecule spectroscopy

(1) Long-term goal of laboratory and research background

Spectroscopy is the “eyes” of modern science, and hence it plays essential roles in a variety of fields covering physics, chemistry, engineering, and biology. We develop and utilize the most advanced spectroscopy for studying complex molecular systems. To elucidate a variety of phenomena occurring in complex molecular systems, we need to clarify their electronic and vibrational states, the response of surroundings, and the fluctuation and dissipation of energy behind them. Based on this view, we carry out fundamental research of molecular science using the most advanced linear/nonlinear spectroscopic methods with the most suitable time- and space-resolution for the problems. Currently, we are carrying out the following projects: (1) Study of ultrafast dynamics using advanced ultrafast spectroscopy; (2) Study of soft interfaces using novel interface-selective nonlinear spectroscopy; (3) Study of structural dynamics of biomolecules by developing new single molecule spectroscopy.

(2) Current research activities (FY2021) and plan (until Mar. 2025)

(A) Ultrafast spectroscopy

We applied the advanced femtosecond time-resolved spectroscopy techniques to the studies on molecular systems of scientific importance. First, we used UV femtosecond stimulated Raman spectroscopy and femtosecond time-resolved impulsive stimulated Raman spectroscopy to study the ultrafast dynamics of a photoreceptor protein PYP and a fluorescence protein LSSmOrange. The structural information provided by the time-resolved Raman data revealed that the PYP chromophore does not undergo large structural changes in the excited state in the protein and suggested that the complex excited-state relaxation dynamics of LSSmOrange arises from the coexistence of chromophores having cis and trans conformations. Furthermore, we conducted femtosecond time-resolved absorption studies on a molecular shuttle based on a rotaxane, and the photoreceptor proteins BLUF and rhodopsin. Particularly for rhodopsin, we revealed that varied excited-state relaxation dynamics observed so far for different rhodopsins can be largely rationalized in a unified manner in terms of the difference in the protonation state of an amino acid residue in the vicinity of the chromophore (Fig. 1). In addition to the studies on ultrafast dynamics, we developed a new method to realize ultra-sensitive absorption measurements using quantum entangled light as a light source.

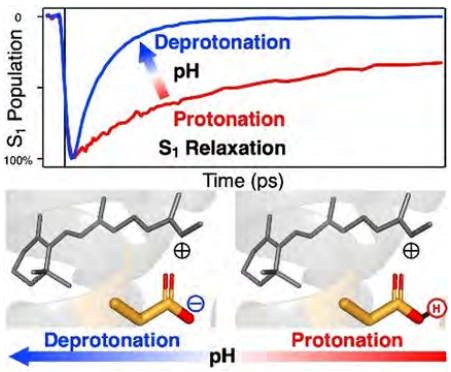

Fig. 1. Change in the excited-state dynamics of rhodopsins, reflecting the change of the protonation/ deprotnation state of an amino acid residues in the vicinity of the chromophore. Reprinted with permission from Ref. 2.

***Future plan*,** We will develop new ultrafast spectroscopy that can yield information on excited-state potential energy surfaces to advance our understanding on the ultrafast dynamics of complex molecular systems as well as the transition state in chemical reactions.

(B) Interface-selective nonlinear spectroscopy

We study the structure and dynamics of molecules at interfaces using heterodyne-detected vibrational sum-frequency generation (HD-VSFG), time-resolved (TR-) HD-VSFG, and two-dimensional (2D-) HD-VSFG spectroscopies developed in RIKEN. In FY2021, we obtained new insights into the DNA adsorption onto lipid monolayer/solution interfaces by monitoring the water orientation at the lipid interfaces using HD-VSFG spectroscopy (Fig. 2). We found that the adsorption of DNA at a cationic lipid interface drastically decreases the orientation of interfacial water, reflecting the neutralization of the positively charged interface. On the other hand, the adsorption of DNA at a zwitterionic lipid interface makes interfacial water more “H-up,” indicating the enhancement of the negative charge at the interface due to the DNA adsorption. The molecular-level information about the DNA−lipid complexation

obtained in this study is expected to help the strategic designing of appropriate nonviral vectors for targeted delivery. As for the study of the dynamics, we have achieved 2D HD-VSFG measurements of the air/water interface with the best time resolution ever (~90 fs) using the interferometric excitation scheme. This made it possible to observe the initial ultrafast spectral diffusion dynamics of the interfacial water. In addition, we succeeded in accurate HD-VSFG measurements of a buried silica/water interface while changing pH, enabling us to discuss the change of the water in the electric double layer and water in the very vicinity of the interface, which are induced by the change of the silica surface.

Future plan, Utilizing the high time resolution of interferometric 2D HD-VSFG, we will elucidate the ultrafast vibrational dynamics of water at the air/water interface. We will also investigate the structure of excess protons at the interface, for which Zundel/Eigen structures have intensively discussed in the bulk solution. Furthermore, we will study the properties of buried material interfaces such as polymer/water and electrode/electrolyte interfaces to clarify fundamental molecular processes occurring at material interfaces.

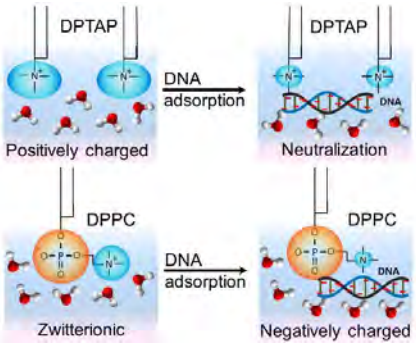

Fig. 2. Sketch of the DNA-adsorbed lipid interfaces. The adsorption of DNA at a cationic lipid interface (top) drastically decreases the orientation of interfacial water, reflecting the neutralization of the positive charge at the interface, whereas the adsorption of DNA at a zwitterionic lipid interface (bottom) makes interfacial water become more "H-up", indicating enhanced negative charge due to the DNA adsorption. Reprinted with permission from Ref. 3.

(C) Single molecule spectroscopy

We conduct research aiming at elucidating conformational heterogeneity and dynamics of biomolecules based on a novel single-molecule fluorescence method, two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS), which was originally developed in RIKEN. In FY2021, we carried out two subprojects. First, we studied the ligand-binding mechanism of a riboswitch using 2D FLCS. The data clearly showed that the conformational change was accelerated by the ligand binding through the induced-fit mechanism. Furthermore, we found that the conformational transition accompanying the ligand binding proceeds in two steps, including the fast process occurring on the microsecond time scale. We proposed a molecular mechanism for how the riboswitch controls the gene expression with this microsecond structural change. This work is the first application of 2D FLCS to the study of the functional mechanism of biomolecular systems. Second, we developed the scanning 2D FLCS method for extending the temporal dynamic range of 2D FLCS. This new method allowed us to observe molecules immobilized on a substrate, thereby extending the observation time window of 2D FLCS from ~1 millisecond to several hundred milliseconds. Using this method, we studied the conformational isomerization dynamics of a DNA Holliday junction (HJ). The obtained results indicate heterogeneous dynamics that includes a portion of the HJ molecules trapped in a long-lifetime state, the fluctuation of the inter-duplex angle of HJ, as well as the change of inter-duplex angle and regidfication of the junction part depending on the Mg^{2+} concentration.

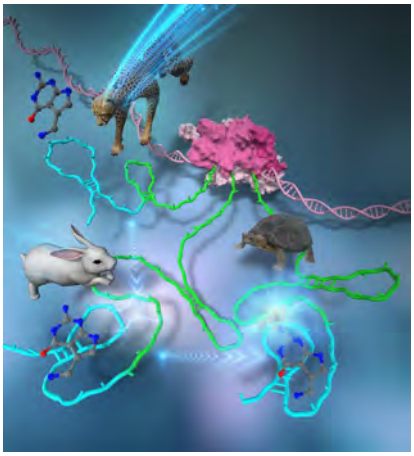

Fig.3 Molecular mechanism for the microseconds structural dynamics detected by 2D FLCS to control the gene expression has been proposed.

Future plan, We will elucidate fundamental properties of biomolecular dynamics such as heterogeneity, hierarchy, and cooperativity, as well as non-equilibrium dynamics of enzymatic reactions using 2D FLCS. We will also develop new methods of single-molecule fluorescence spectroscopy based on 2D FLCS by incorporating approaches of multivariate data analysis. Through these researches, we will develop 2D FLCS as a versatile, powerful single-molecule spectroscopic method.

(3) Members as of March, 2022

(Chief Scientist)
Tahei TAHARA

(Senior Research Scientist)
Kunihiko ISHII, Satoshi NIHONYANAGI

(Research Scientist)
Korenobu MATSUZAKI
Ahmed MOHAMMED, Bidyut SARKAR

(Contract Researcher)
Feng WEI

(Special Postdoctoral Researcher)
Tsukasa TAKANASHI

(Postdoctoral Researcher)
Pardeep KUMAR, Woongmo SUNG, Li LIU, Wooseok HEO, Chun-Fu CHANG

(Technical Staff I)
Subhadip ROY

(IPA)
Sandeep YADAV

(Assistant)
Tomoko Kato

(4) Representative research achievements

1. "Excited-state proton transfer dynamics in LSSmOrange studied by time-resolved impulsive stimulated Raman spectroscopy", P. Kumar, E. Fron, H. Hosoi, H. Kuramochi, S. Takeuchi, H. Mizuno, T. Tahara, **J. Phys. Chem. Lett.** 12, 7466-7473 (2021).

2. "A unified view on varied ultrafast dynamics of the primary process in microbial rhodopsin", C.-F. Chang, H. Kuramochi, M. Singh, R. Abe-Yoshizumi, T. Tsukuda, H. Kandori, T. Tahara, **Angew. Chem. Int. Ed.** 61, e202111930/1-9 (2021).

3. "DNA-Induced reorganization of water at model membrane interfaces investigated by heterodyne-detected vibrational sum frequency generation spectroscopy", P. C. Singh, M. Ahmed, S. Nihonyanagi, S. Yamaguchi, T. Tahara, **J. Phys. Chem. B**, **126**, 840−846 (2022).

4. "Microsecond folding of preQ$_1$ riboswitch and its biological significance revealed by two-dimensional fluorescence lifetime correlatin spectroscopy", B. Sarkar, K. Ishii, T. Tahara, **J. Am. Chem. Soc.** 143, 7968-7978 (2021).

5. "Scanning tow-dimensional fluorescence lifetime correlation spectroscopy: Conformational dynamics of DNA Holliday-junction from microsecond to sub-second", W. Heo, K. Hasegawa, K. Okamoto, Y. Sako, K. Ishii, T. Tahara, **J. Phys. Chem. Lett.** 13, 1249-1257 (2022).

Supplementary

Group photo of RIKEN Molecular Spectroscopy Laboratory (MSL)

Laboratory Homepage
https://www.riken.jp/en/research/labs/chief/mol_spectro/index.html
https://spectroscopy.riken.jp/?lang=en