

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 (FY2023) and plan

(A) Ultrafast spectroscopy

We have studied chemically and biologically important ultrafast reactions using femtosecond time-resolved spectroscopy. First, we studied schizorhodopsin, which is a newly discovered microbial rhodopsin subfamily with an unusual inward-proton pump functionality. With the aim of clarifying the mechanism for this unusual

functionality, we investigated the excited-state dynamics using femtosecond time-resolved absorption spectroscopy and examined the ground-state structure with impulsive stimulated Raman spectroscopy. The obtained spectroscopic data indicated the existence of unique chromophore-protein interaction in schizorhodopsin. Second, we studied cis-trans photoisomerization, which is of importance in fundamental chemistry and industrial applications. In this reaction, it has been believed that a reaction intermediate appears which has a perpendicular structure around the CC double bond that rotates (Fig. 1). However, such an intermediate has never been experimentally confirmed before, and hence it has been often called the phantom state because of its elusiveness. Applying ultraviolet resonance femtosecond stimulated Raman spectroscopy (UV-FSRS) to methyl-substituted stilbene, we succeeded in detecting and identifying the perpendicular intermediate, providing an indispensable piece of information for completing our understanding of this fundamental chemical reaction.

Future plan, We continue to study the ultrafast dynamics and mechanism of the function of complex molecular systems. Furthermore, we will develop new ultrafast spectroscopy that can yield information on the excited-state potential energy surface that governs ultrafast 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 FY2023, we investigated the vibrational relaxation of OH stretch vibration of water molecules at the air/water interface using TR-HD-VSFG spectroscopy. The results showed that the vibrational relaxation (T_1) times obtained with direct excitations of hydrogen-bonded (HB) OH stretch are 0.2-0.4 ps without noticeable changes with the change in the excitation frequency. This T_1 time is very similar to that of bulk water, which strongly suggests that vibrational relaxation of the interfacial HB OH proceeds predominantly with the intramolecular relaxation to HOH bending overtone, as in the case of bulk water. Moreover, we observed the vibrational dynamics that the excited “free OH” sticking out to the air is first converted to excited HB OH by rotation (~0.9 ps)



Fig. 1. Cis-trans photoisomerization of stilbene and the perpendicular phantom state.

and then it relaxes as excited HB OH. This study provides a complete set of the excitation frequency dependence of T_1 time of the interfacial OH stretch vibration and presents a unified picture of its vibrational relaxation process at the air/water interface (Fig. 2). As for the study of buried interfaces, we investigated the alumina/water interfaces to elucidate the fundamental properties of positively charged interfaces.

Future plan. We will further investigate ultrafast vibrational dynamics of water at the interface using TR-HD-VSFG and 2D HD-VSFG spectroscopies. In particular, we will analyze the 2D-HD-VSFG spectra that we have obtained and measure the 2D-HD-VSFG spectra of isotopically diluted water, to elucidate the ultrafast spectral diffusion of water proceeding at the air/water interface. As for the study of buried material interfaces, we will further study the fundamental properties of oxides/water, polymer/water, and electrode/electrolyte interfaces. Furthermore, we will develop a new time-resolved interface-selective electronic spectroscopy to investigate reaction dynamics at the liquid interface.

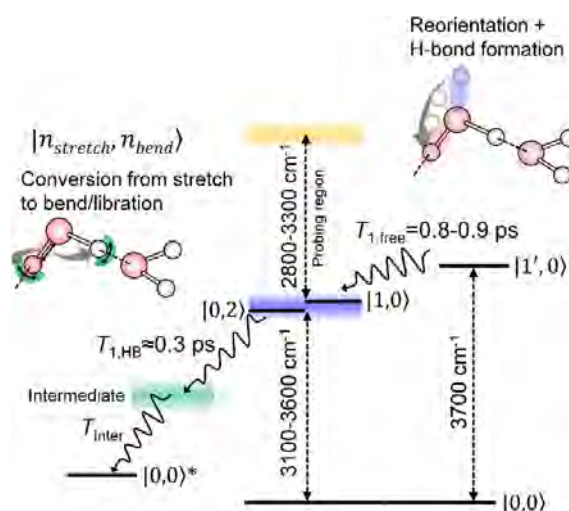


Fig. 2. Vibrational relaxation of OH stretch vibration at the air/water interface. Ref. 5, open access.

(C) Single-molecule spectroscopy

In the single-molecule spectroscopy project, we conducted 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. First, we developed a new method that incorporates the pulsed interleaved excitation (PIE) scheme into 2D FLCS. In a FRET-based 2D FLCS measurement, the target molecule is labeled with two fluorophores, donor and acceptor, and their fluorescence is measured by exciting the donor. In this one-color excitation scheme, however, the coexistence of molecules with incomplete labeling and the spectral cross-talk between the donor and acceptor can prevent accurate determination of the FRET efficiencies. In PIE 2D FLCS, we quantitatively evaluate these possible side effects by introducing a second pulse that directly excites the acceptor. We demonstrated the feasibility of PIE 2D FLCS using photon data obtained by simulation and experiment. Second, we combined single-molecule FRET measurements using a microfluidic device with independent fluorescence component analysis (IFCA), which we developed in the last year, to measure the dynamics of non-equilibrium steady states. We chose the folding process of the DNA i-motif as a target. The folding of the DNA sample was induced by a pH jump in a microfluidic device, and its structural evolution was monitored by using 2D FLCS. IFCA analysis of the resulting 2D maps revealed that an intermediate state was formed within 10 ms after the pH jump, followed by the generation of the i-motif on the millisecond-to-second time scale.

Future plan. We will promote research by applying 2D FLCS to elucidate fundamental properties of biomolecular dynamics such as heterogeneity, hierarchy, and cooperativity, as well as to detect non-equilibrium dynamics of enzymatic reactions. In addition, we will try to study biomolecular dynamics in complex systems such as a living cell. Through these studies, we aim to develop and establish 2D FLCS as a versatile, powerful single-molecule method.

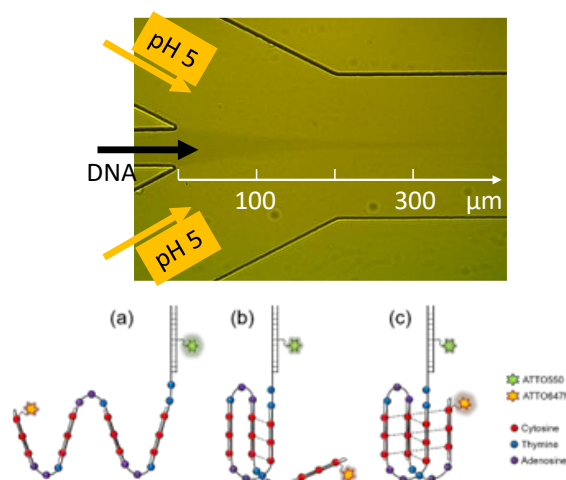


Fig. 3. Measurement of non-equilibrium steady state using a microfluidic device (top) and the folding process of a DNA i-motif (bottom).

(3) Members

(Chief Scientist)

Tahei TAHARA

(Senior Research Scientist)

Kunihiko ISHII, Satoshi NIHONYANAGI

(Research Scientist)

Korenobu MATSUZAKI, Bidyut SARKER,
Woongmo SUNG

(Special Postdoctoral Researcher)

Tsukasa TAKANASHI

(Postdoctoral Researcher)

Subhadip ROY, Tsukasa TOKITA

(JSPS Postdoctoral Researcher)

Tomoaki YAGI

(IPA)

Sandeep YADAV

(Trainee & RIKEN Student Researcher)

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Tatsuya FUJINO, Haruko HOSOI,
Munetaka IWAMURA, Hikaru KURAMOCHI,
Anton MYALITSIN, Ahmed MOHAMMED

(Assistant)

Tomoko Kato

(4) Representative research achievements

1. F. Wei, S. Urashima, S. Nihonyanagi, and T. Tahara, "Elucidation of pH-dependent electric double layer structure at the silica/water interface using heterodyne-detected vibrational sum-frequency generation spectroscopy," **J. Am. Chem. Soc.** 145, 8833-8846 (2023).
2. A. Sayama, S. Nihonyanagi, Y. Ohshima, and T. Tahara, "Origin of solvent stabilization at super-concentrated electrolyte/electrode interfaces revealed by heterodyne-detected vibrational sum frequency generation spectroscopy", **J. Phys. Chem. C** 127, 10524-10531 (2023).
3. A. Kundu, S. Yamaguchi, and T. Tahara, "Local pH at nonionic and zwitterionic lipid/water interfaces revealed by heterodyne-detected electronic sum-frequency generation: A unified view to predict interfacial pH of biomembranes", **J. Phys. Chem. C** 127, 5445-5452 (2023).
4. H. Kuramochi, T. Tsutsumi, K. Saita, Z. Wei, M. Osawa, P. Kumar, L. Liu, S. Takeuchi, T. Taketsugu, and T. Tahara, "Ultrafast Raman observation of the perpendicular intermediate phantom state of stilbene photoisomerization", **Nat. Chem.** 16, 22-27 (2024).
5. W. Sung, K. Inoue, S. Nihonyanagi, and T. Tahara, "Unified picture of vibrational relaxation of OH stretch at the air/water interface obtained by time-resolved, heterodyne-detected vibrational sum frequency generation spectroscopy," **Nat. Commun.** 15, 1258/1 - 1258/11 (2024).

Supplementary

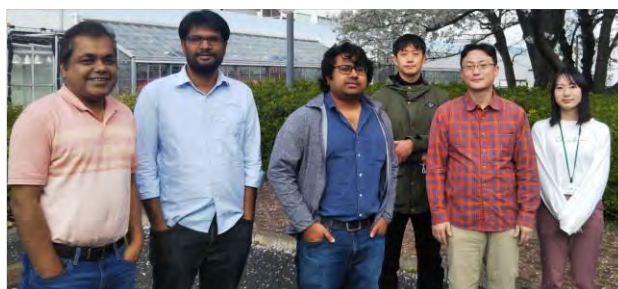
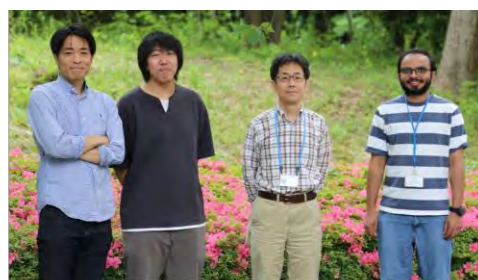
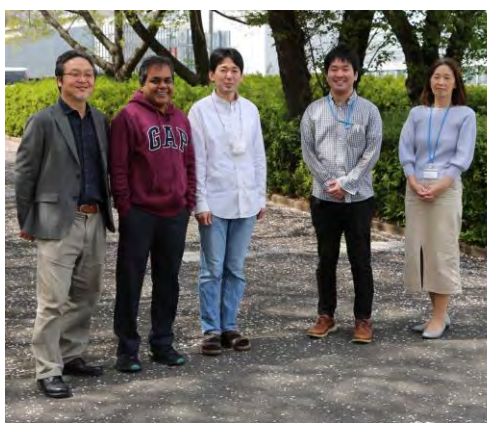


Photo of three research groups in Molecular Spectroscopy Laboratory (MSL)

Laboratory Homepage

https://www.riken.jp/en/research/labs/chief/mol_spectro/index.html

<https://spectroscopy.riken.jp/?lang=en>