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 the various phenomena occurring in complex molecular systems, we need to clarify their electronic and vibrational states, the response of the surroundings, and the fluctuations 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 (FY2024) and plan

(A) Ultrafast spectroscopy

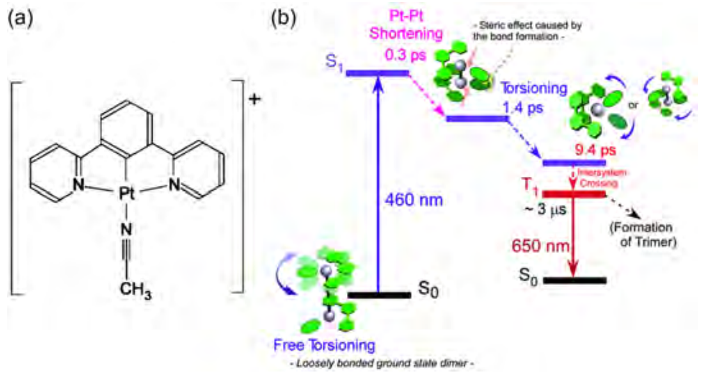

Fig. 1. (a) Structure of $[Pt(NCN)MeCN]^+$. (b) The relaxation process of the dimer of $[Pt(NCN)MeCN]^+$ following its photoexcitation. (From Ref. 1 with permission)

We study chemically and biologically important ultrafast reactions using femtosecond time-resolved spectroscopy. This year, the dynamics of the dimer of a platinum complex $[Pt(NCN)MeCN]^+$ following photoexcitation was investigated using femtosecond time-resolved absorption spectroscopy. In this system, a chemical bond formation between the two Pt atoms occurs upon photoexcitation, and it therefore allows us to study the chemical bond formation process experimentally. The obtained experimental result showed an excited-state absorption signal in the 500-700 nm range, and the signal intensity increased with the time constants of 0.3 ps, 1.4 ps, and 9.4 ps. With the help of time-resolved fluorescence measurement and quantum chemical calculation, the 0.3 ps component was assigned to the shortening of the Pt-Pt distance due to the chemical bond formation, the 1.4 ps component to the torsional angle change around the Pt-Pt bond due to the steric hindrance among the ligands, and the 9.4 ps component to the intersystem crossing. The torsional angle change around the metal-metal bond was observed for the first time in this study as the structural relaxation mechanism of metal complex oligomers following photoexcitation.

Future plan**,** We continue to study the ultrafast dynamics and mechanism of the function of complex molecular systems. Furthermore, we will develop and utilize new ultrafast spectroscopy that can yield information on the excited-state potential energy surface that governs ultrafast reactions.

(B) Interface-selective nonlinear spectroscopy

We have been developing various interface-selective nonlinear spectroscopic methods and applying them to the molecular science of liquid interfaces. In fiscal year 2024, we first employed time-resolved heterodyne-detected vibrational sum-frequency generation (TR-HD-VSFG) spectroscopy to determine the vibrational relaxation time (T_1) of the OH stretching mode of water at the air/isotopically diluted water interface. Water molecules at interfaces are expected to exhibit structures and dynamics distinct from those in bulk water, and TR-HD-VSFG spectroscopy provides a powerful means to probe such interfacial dynamics selectively. In this study, we measured the T_1 of the OH stretching vibration at the air/isotopically diluted water interface (H_2O: HOD: D_2O = 1:8:16) using the excitation in the frequency region of 3200–

$3500\ \mathrm{cm}^{-1}$. The results revealed that the T_1 of the OH stretch in isotopically diluted water is ca. 0.3 ps in the $3200–3400\ \mathrm{cm}^{-1}$ range, whereas it increases to about 0.6 ps at $3500\ \mathrm{cm}^{-1}$. This finding supports the conclusion of our previous study that the relaxation of the hydrogen-bonded OH stretch proceeds primarily via Fermi resonance (See Figure 2). Second, we developed a new spectroscopic method: time-resolved heterodyne-detected electronic sum-frequency generation (TR-HD-ESFG). Previously, interfacial molecular dynamics had been investigated using TR-HD-VSFG and time-resolved Electronic SFG (TR-ESFG) spectroscopy. TR-ESFG is a powerful technique for tracking photoexcitation processes of solute molecules at interfaces. However, heterodyne detection, which provides direct and quantitative information, had not yet been achieved. In this work, we realized TR-HD-ESFG measurements for the first time. Using malachite green, a prototypical dye, at the air/water interface, we demonstrated the measurement of time-resolved electronic $\Delta\mathrm{Im}\chi^{(2)}$ spectra (changes in the imaginary part of the second-order nonlinear susceptibility induced by pump excitation). The spectra clearly showed not only ground-state bleach but also the appearance of new bands originating from the excited state. These results show that TR-HD-ESFG spectroscopy has the potential to become a powerful new tool for elucidating ultrafast reaction dynamics at interfaces.

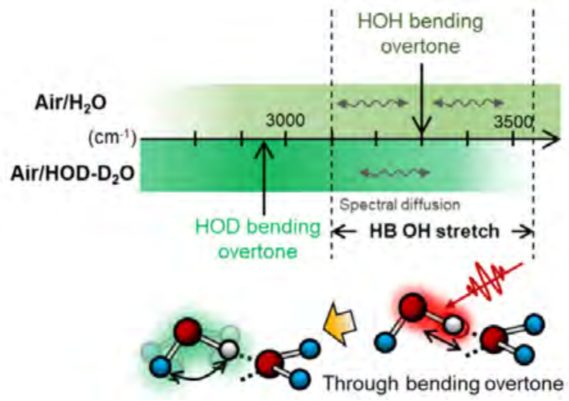

Fig. 2. Relaxation model of the OH stretch vibration at the air/water interface. Reprinted from Ref. 2 with permission.

Future plan, We will study buried interfaces such as oxide/water, polymer thin film/solution, and electrode/electrolyte interfaces using HD-VSFG spectroscopy. Simultaneously, we will continue investigations of ultrafast vibrational dynamics at water interfaces using TR-HD-VSFG, and further develop new interface-selective time-resolved spectroscopic methods for tracking chemical reactions at the interface.

(C) Single-molecule spectroscopy

In the single-molecule spectroscopy project, 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 this year, we first developed a new method to probe protein-DNA interactions utilizing protein-induced fluorescence enhancement (Fig. 3). By using 2D FLCS, we investigated the binding of a restriction enzyme BamHI to a Cy3-labeled DNA. As a result, three fluorescence lifetime components were identified and assigned to unbound DNA, BamHI-bound DNA, and binding-insensitive species. From the relative population of the bound and unbound species and their diffusion times, the dissociation constant and the diffusion coefficients were determined. This method has the potential to be a powerful method for studying protein-DNA interactions in complex biological environments. Second, we tried to apply 2D FLCS to study intracellular dynamics. We analyzed the autofluorescence signal from HeLa cells upon 530-540 nm irradiation and detected three fluorescence lifetime components. These components showed different emission wavelengths and diffusion times, indicating that multiple emissive molecular species in the cell were detected. Since intracellular diffusion dynamics is a useful measure of cellular conditions, in-cell 2D FLCS is expected to have a broad range of biomedical applications.

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 apply a newly developed method, IFCA, to study heterogeneity in biomolecular systems. Through these studies, we aim to develop and establish 2D FLCS/IFCA as versatile, powerful single-molecule methods.

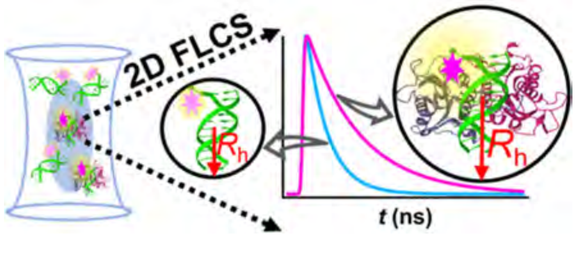

Fig. 3. Concept of PIFE-2D FLCS. Reprinted from Ref. 3 with permission.

(3) Members

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Tomoko Kato

(4) Representative research achievements

1. H. Watanabe, M. Iwamura, K. Nozaki, T. Takanashi, H. Kuramochi, and T. Tahara, “Torsional structural relaxation caused by Pt-Pt bond formation in the photoexcited dimer of Pt(II) N^C^N complex in solution,” **J. Phys. Chem. Lett.** 16, 406-414 (2025).
2. E. Kinoshita, W. Sung, S. Nihonyanagi, H. Okuyama, and T. Tahara, “Frequency-dependent vibrational relaxation time of OH stretch at the air/isotopically diluted water interface,” **J. Phys. Chem. Lett.** 16, 1088-1094 (2025).
3. S. Yadav, K. Ishii, and T. Tahara, “Probing protein-DNA interactions with two-dimensional fluorescence lifetime correlation spectroscopy utilizing protein-induced fluorescence enhancement,” **J. Phys. Chem. B** 129, 11729-11736 (2025).
4. S. Roy, M. Ahmed, S. Nihonyanagi, and T. Tahara, “Time-resolved heterodyne-detected electronic sum frequency generation (TR-HD-ESFG) spectroscopy: A new approach to explore interfacial dynamics,” **J. Chem. Phys.** 161, 174202/1-9 (2024).
5. B. Sarkar, K. Ishii, and T. Tahara, “Pulsed-interleaved-excitation two-dimensional fluorescence lifetime correlation spectroscopy,” **J. Phys. Chem. B** 128, 4685-4695 (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