- S05I1 赤外分光法によるタンパク質間相互作用の解析—ロドプシンをモデルとして—
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FT-IR Study of Protein-Protein Interaction -Rhodopsin as a Model System-
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The cells communicate with each other in many kinds of organisms. To understand the molecular mechanism of such signal transduction, it is important to reveal structural changes of receptors upon interaction with their transducer proteins. Fourier-transform infrared (FTIR) spectroscopy is a powerful tool to investigate molecular structures. We established the measuring system for the light-induced difference spectra of rhodopsins in the whole mid-infrared region. Because of light-induced activation, the molecular mechanism of the signal transduction in rhodopsins can be studied much more precisely than other receptors.

As an example, I will talk about the protein-protein interaction between pharaonis phoborhodopsin (ppR) and its transducer protein (pHtrII) that works for negative phototaxis in haloarchaea. We observed a hydrogen-bonding alteration of Thr204 in ppR [1, 2], which was later revealed to be functionally important [3, 4]. This study demonstrates a spectroscopic technique opening a new road to biological sciences. I will also present a recent study on another light sensor in haloarchaea, sensory rhodopsin I, and a trial for detecting protein-protein interaction in buffer suspension.

[1] Y. Furutani et al., *Biochemistry* (2003). [2] Y. Sudo, Y. Furutani et al., *Biochemistry* (2003). [3] Y. Sudo, Y. Furutani et al., *J. Biol. Chem.* (2006). [4] Y. Sudo, Y. Furutani et al., *J. Biol. Chem.* (2007).

- S05I3 超短パルスを用いた新しい非線形分光で拓くソフトな界面の科学
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Development of novel nonlinear spectroscopy and its application to soft interfaces
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Interfaces buried in condensed media play very important roles in a number of scientific fields, ranging from industrial chemistry to molecular biology. In biological systems, for example, we can see rich interfacial phenomena such as selective permeability and molecular recognition of cell membranes. The IR-visible vibrational sum-frequency generation (VSFG) spectroscopy takes advantage of the interface selectivity of the second-order nonlinear optical process, and it has been widely used to investigate interfacial phenomena. Practically, VSFG has been only single spectroscopy that can provide vibrational spectra of liquid interfaces. For buried interfaces, however, the applicability of VSFG is limited, because an IR input beam cannot reach interfaces sandwiched by most common liquids and solids. Therefore, the experimental research for buried interfaces has been left unexplored. Recently, we developed several new even-order nonlinear electronic and vibrational spectroscopy. Especially, novel interface-selective fourth-order Raman spectroscopy can provide vibrational spectra of interfacial molecules for the whole fingerprint region [1,2]. These new methods can be applied to buried interfaces because they utilize only visible or near IR light. They have a potential to disclose new phenomena at buried interfaces, including those at membranes in biological systems. Ref. [1] S. Yamaguchi and T. Tahara, *J. Phys. Chem. B*, 109 (2005) 24211. [2] S. Yamaguchi and T. Tahara, *Angew. Chem. Int. Ed.*, in press.

- S05I2 全反射赤外分光法：生理条件下で酵素反応がラクラクはかれる（かもしれない）
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ATR-FTIR: Probing enzymatic reactions under physiological conditions
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ATR-FTIR (attenuated total reflection-Fourier transform infrared) techniques allow proteins to be manipulated between different states under physiological conditions. The ATR methods provide increased flexibility of the types of changes that can be induced in proteins in comparison to transmission methods. Quantitative measurements can be made of vibrational changes associated with conversion between catalytic intermediates, ligand binding and oxidation-reduction. The sample proteins can be deposited as either hydrophobic thin layers or as solution on the ATR prism. To induce the chemical changes in proteins, one can use photochemistry, electrochemistry or perfusion buffer exchange. Particularly useful is the buffer exchange, which is practically impossible in transmission methods. Specially designed ATR cells enable us to monitor UV/visible changes simultaneously while running ATR-FTIR measurements to confirm the chemical transitions. The ability to induce transitions repetitively allows IR difference spectra to be acquired at a signal/noise sufficient to resolve changes due to specific cofactors or amino acids. Such spectra can often be interpreted at the atomic level by comparisons with model compounds, by isotope and mutation effects and, increasingly, by ab initio simulations. Combination of such analyses with atomic 3D structural studies can lead to a deeper understanding of molecular mechanisms of enzymatic reactions.

(Ref) P. R. Rich and M. Iwaki, *Molecular Biosystems*, 2007, 3, 398.

- S05I4 時間分解共鳴ラマン分光法で観たタンパク質ダイナミクス：ピコ秒からミリ秒まで、可視領域から紫外領域まで
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Protein Dynamics Probed by Time-resolved Resonance Raman Spectroscopy
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Protein dynamics spans over a wide range of time scales. To answer questions on protein dynamics, we need the concatenation of experimental results recorded over many orders of magnitude of time. In this regard it is important that a single experimental technique can examine protein structures evolving from the earliest moments, such as the picosecond regime, toward time scales that are highly relevant to biological functions, such as the microsecond or millisecond regimes. One method for studying the dynamic motions of proteins is to pursue the ensuing relaxation after shifting a protein from equilibrium. If we use short pulses to initiate a very rapid photoreaction at an active site, immediately after the start of a reaction, the result is a metastable structure whose active site is different but the tertiary and quaternary structures of the protein are similar to the original state. A short probe pulse following the initiating pulse provides direct information on the relaxation of this metastable form into the equilibrium protein structure. Some of the faster processes in protein motions involve relatively small alterations in the overall structure. Therefore, the probes used to examine them must be sensitive to subtle changes in the protein structure. For this reason we adopted resonance Raman spectroscopy, which is sufficiently structure sensitive at chemical-bond resolution, so as to allow identification of any ultrafast steps in protein dynamics. In this talk, we present our recent Raman work on some allosteric proteins.