3P097 完全酸化型チトクロム *c* 酸化酵素の酸化還元活性金属中心とアザイドの相互作用の分光学的研究
Spectroscopic characterization of the interaction of azide with the redox-active metal sites of fully oxidized cytochrome *c* oxidase

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Cytochrome *c* oxidase (CcO) is the terminal oxidase in the mitochondrial respiratory chain. It catalyzes the reduction reaction of the molecular oxygen, which is coupled with the proton-pumping. Azide is a potent inhibitor of CcO, and considered to bind to heme *a*₃ in the oxidized state. Thus far, there is no resonance Raman spectrum of azide-bound CcO. Here we report spectroscopic characterization of the interaction of azide with fully oxidized CcO. Addition of azide to CcO shifts the Soret peak and changes the intensity of the band, showing two azide-binding sites with *K*_d values of 59 μM and 44 mM at pH 7.4. Resonance Raman analysis using 441.6 nm as the exciting laser shows very small spectral changes. The results suggest the absence of direct azide binding to heme *a*₃.

3P098 一酸化炭素・シアン化物結合混合原子価型ウシ心筋チトクロム酸化酵素の構造解析
Structural analysis of bovine heart cytochrome *c* oxidase in the CO- and cyanide-bound mixed valence states

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Cytochrome *c* oxidase (CcO) catalyzes the O₂ reduction coupled to proton pump. CcO contains four redox active metal sites, Cu_A, Fe_a, Fe_{a3} and Cu_B. Carbon monoxide (CO) and cyanide ion (CN⁻) are bound to the Fe_{a3}-Cu_B site and stabilize oxidized (Fe³⁺-Cu²⁺) and reduced (Fe²⁺-Cu¹⁺) states, respectively. Previously, structures in the CN⁻-bound fully oxidized and CO-bound fully reduced states were analyzed.

To investigate redox dependent effect of Cu_A and Fe_a on the proton transfer pathways, we analyzed X-ray crystal structures of bovine heart CcO in the CO- and CN⁻-bound mixed valence states. The refined structures indicate that conformations in H-pathway, a proposed proton pump pathway, are regulated by redox states of these metal sites.

3P099 人工設計膜貫通ペプチドを用いたシグナル伝達モデル系の構築
Design of transmembrane peptide for constructing a signaling model

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Transmembrane(TM) signaling proteins such as receptor tyrosine kinases and T-cell receptors control many fundamental cellular processes. Signaling of TM proteins is frequently activated by ligand-mediated dimerization of TM domains in membrane. The interaction between TM domains is very important for control of signaling. However, due to the large molecular weight and hydrophobicity of TM proteins, it is quite difficult to analyze the interaction in aqueous solution. We thus tried to constitute a simple and small model of the signaling by using designed peptides based on the TM domain, EphA1. It was shown that the peptides associated in membrane-mimetic environment and its association state could be controlled by external stimulus.

3P100 好熱菌 F₀F₁-ATP 合成酵素 *c* サブユニットリングの活性部位の構造
The Active-Site Structure of Thermophilic F₀F₁-ATP Synthase *c*-Subunit Rings in Membranes

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In F₀F₁-ATP synthase, the electrochemical potential across membranes or ATP hydrolysis is used to rotate the F₀*c*-subunit ring. To facilitate elucidation of the chemical mechanism, we have developed a solid-state NMR method for analysis of a focused structure of the *c*-subunits from thermophilic *Bacillus* (TF₀*c*) in membranes. Structural information on its active site was obtained, using stereo-array-isotope-labeling (SAIL) with a cell-free system. The membrane-reconstituted TF₀*c*-oligomer was confirmed by the H⁺-translocation activity and high-speed atomic force microscopic images. The Cδ chemical shift of Glu56 essential for H⁺-translocation revealed that its carboxyl group is protonated in membranes, forming the H⁺-locked conformation with Asn23.

3P101 Analysis of Structure and Function of Synaptotagmin 4
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Synaptotagmin-4 (Syt4) regulates exocytosis of brain-derived neurotrophic factor (BDNF). Despite sharing the universal molecular design with other members of Syt family, having tandem C2 domains with a flexible linker of conserved length in between, mammalian Syt4 appears not to function as a Ca ion sensor. A preliminary molecular dynamics simulation also indicates that Syt4 does not bind Ca²⁺ ions as well as Synaptotagmin-1(Syt1) in the presence of anionic phospholipid such as Phosphatidylserine (PS) or phosphatidylinositol 4,5-bisphosphate (PIP₂). We shall report the MD results to discuss the molecular mechanism behind the different behavior as a result of minor structural differences. We shall also speculate on the role of Syt4 in LTP and BDNF regulation.

3P102 擬環状リン脂質リボソーム中のバクテリオロドプシンの構造と機能
A Biophysical Study of Bacteriorhodopsin in Pseudocyclic Phosphatidylcholine Liposome

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It is well known that in Archaea, macrocyclic tetraether phospholipids are a major component of biomembrane and contribute to its high stability against extreme environments. In the present study, a novel pseudo-cyclic phosphatidylcholine (PC), an acyclic analog of cyclic phospholipids bearing the two PC groups as the head groups, was synthesized and the reconstitution of bacteriorhodopsin (bR) was performed. Laser flash photolysis experiments on successfully reconstituted bR demonstrated that bR in the pseudo-cyclic PC has a photocycle very similar to native purple membrane, which is significantly different from that of bR in DMPC. Effects of pseudo-cyclization of the phospholipid on structure and function of bR and physical properties of membrane will be discussed.