2P103 哺乳類ミトコンドリア呼吸鎖超複合体の精製**Purification of the respiratory super complex from mammalian mitochondria**

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The mitochondrial respiratory chain consists of 5 large multi-subunit complexes, complex I - V. Complexes I, III and IV catalyze the transfer of electrons from NADH to molecular oxygen and use the energy released in electron transfer to pump protons across the inner membrane. Though the individual complexes have been studied, little is known about how these complexes interact in the membrane to perform their tasks. These complexes assemble into higher level of organization, respiratory supercomplexes, confirmed by BN-PAGE analysis.

Here, we report the purification of respiratory supercomplexes from mammalian mitochondria. The purified complexes stabilized with amphipol show the KCN-sensitive NADH oxidation activity. The absorption spectra of the complexes were obtained.

2P104 定常状態での GPCR シグナルは GPCR ダイマーによって誘起される**Dimers are the key trigger for the GPCR's basic signaling without ligation**

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Monomers and dimers of G-protein coupled receptors (GPCRs) are in dynamic equilibrium, with dimer lifetimes of ~100 ms. Here, we found that trimeric G proteins were recruited to both GPCR monomers and dimers in the steady state, but the addition of the inverse agonist, which blocks the GPCR basal activity, a unique feature of GPCRs, decreased the G-proteins' recruitment to GPCR dimers, using β_2 adrenergic receptor (b2AR) as a paradigm, suggesting that GPCR dimers are responsible for the basic b2AR signaling. A b2AR mutant with lower basal activity formed dimers with a shorter lifetime, comparable to the lifetime for the wild type b2AR bound by the inverse agonist. These results together indicate that GPCR dimers are the key trigger for the GPCR's basic signaling.

2P105 1 分子イメージングによる代謝型グルタミン酸受容体の高次多量体形成と内在化の解析**Single-molecule imaging analysis of higher-order oligomerization and internalization of metabotropic glutamate receptor**

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Metabotropic glutamate receptor (mGluR) is a G protein-coupled receptor involved in the synaptic transmission. It is well-known that mGluR functions as a constitutive homodimer. However, the higher-order oligomerization of mGluR under physiological condition is yet to be cleared. Here we show that deceleration of the diffusion followed by the internalization occurs accompanied with the oligomerization upon mGluR activation. The diffusion and oligomerization of fluorescence-tagged mGluR on HEK293 cell surface were monitored by total internal reflection fluorescence microscopy and analyzed by variational Bayesian hidden Markov model. We will discuss the dynamics of the clathrin-dependent internalization of mGluR from the dual-color single-molecule imaging analysis.

2P106 AFM Probing Opioid Signalosome on Neuroblastoma

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Opiate drugs exert their analgesic actions by binding to mu opioid receptors. However, opioid use is limited by tolerance development. MOR activation results in receptor desensitization and internalization. In this study, dynamic force spectroscopy AFM is used to measure affinity and distribution of MOR on live and fixed SHSY5Y cell treated with agonist by coating the AFM tips with antibody. The agonist-induced internalization is measured in terms of the relative change in the number of binding sites observed during force volume mapping. This approach in receptor localization will facilitate further studies on the correlation of proper receptor function with the dynamic physical properties of the MOR signalosome.

**2P107 プロトン駆動力を細胞外へと捨てる微生物外膜タンパク質
Proton discarded to cell exterior via outer-membrane bound enzyme**

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Certain microbes can produce energy for sustaining life by transporting electrons from cell interior respiratory electron chain to insoluble electron acceptors located outside the cell, a process referred to as extracellular electron transport (EET). This EET process was recently found to occur via semiquinone active species bound to outer-membrane c-type cytochromes. Here, our in-vivo electrochemical analysis shows that the bound flavin cofactor transports not only electrons but also protons to cell exterior from periplasm, implying ATP is produced by substrate-level phosphorylation. Discarding proton motive force would be a unique strategy to slow down microbial growth in a solid surface environment with extremely high cell density.

2P108 ベリプラズム pH 追跡による細胞外電子移動酵素のプロトン移動能検討***In-vivo* periplasmic pH assay for studying proton export by outer membrane cytochromes in extracellular electron transport**

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Microbial extracellular electron transport (EET) is a process to transport electrons to insoluble substrates located outside cell wall. We recently reported that a cell-secreted flavin binds to outer-membrane c-type cytochromes to enhance the rate of EET despite its unfavorable redox potential. Since the redox reaction of flavin couples proton transport, it is, therefore, assumable that the unfavorable flavin potential comes from need for EET to export proton from periplasm to cell exterior. Here, we constructed a periplasmic pH (pH_p) assay based on the membrane permeability of flavin during EET. Our *in-vivo* pH_p assay showed that EET does not accumulate proton in periplasm, suggesting that proton and electron are simultaneously exported to cell exterior by flavin.