1P163 TF1 β E190D 変異体の外部トルク依存性
The response of TF1 β E190D mutant to the external torque

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F1-ATPase is a motor protein driven by ATP hydrolysis. It converts chemical free energy change into the kinetic energy of the rotation. Toyabe examined the external torque dependency of the rotation rate of wild type F1-ATPase (PANS 2011). He found the maximum work estimated from stall torque is nearly equal to the free energy change of ATP hydrolysis, reached conclusion of high energy efficiency. Thus, interest issue is to find out the mechanism of this high efficiency. In the previous annual meeting, we reported that β E190D mutant exhibited response to external torque different from WT. Furthermore, we found that the stall torque was smaller than that of WT. Here, we discuss origin of the characteristic response of β E190D mutant to the external torque.

1P164 軸系外腕ダイニンによるトルク発生
Torque generation by axonemal outer-arm dynein

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Outer-arm dynein is the main engine providing the motive force in cilia. *Tetrahymena* outer-arm dynein consists of three different heavy chains, termed α , β and γ , and is decomposed into two-headed $\beta\gamma$ subparticle and one-headed α subparticle by chymotryptic digestion. Using three-dimensional tracking microscopy, we found that contrary to previous reports the outer-arm dynein and its subparticles rotated sliding microtubules around their longitudinal axis in an *in vitro* microtubule gliding assay. Furthermore microtubule corkscrewing motion driven by the dynein and its subparticles showed different ATP concentration dependencies. Our results suggest that the three-headed outer-arm dynein integrates three dynein motor domains with distinct mechanical properties.

1P165 演題取り消し

1P166 偏光変調 TIRFM とデフォーカスイメージングによる単一蛍光色素の角度と回転方向の検出
Detection of 3-D orientation and rotation of single fluorophores by combination of polarization-modulation TIRFM and defocused imaging

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Determination of rotating conformational changes in a protein at the single molecular level is crucial to uncover mechanochemical characteristics of working enzymes. Under TIRF illumination with *p*-polarized beam, the intensity of the fluorophore becomes maximal when its dipole directs perpendicular to the sample plane. Sequential defocused images of sparsely labelled microtubules, which slid on the lawns of single-headed kinesin-1, were captured under the illumination, and applied for the verification of our system. The left-handed rotation was detected as in 3-D tracking method (Yajima, Mizutani & Nishizaka, 2008), and the dipole angle against the microtubule axis was successfully estimated as $\sim 30^\circ$.

1P167 ビブリオ菌べん毛の MS リングを構成し大量発現で可溶性となる膜タンパク質 FliF の生化学的解析
Biochemical analysis of the membrane protein FliF, a MS-ring component of *Vibrio* flagellar motor with being soluble when overproduced

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The MS-ring is a part of the bacterial flagellar basal structure embedded in the inner membrane and composed of membrane protein FliF. It has two transmembrane segments and a large periplasmic region. When *Vibrio alginolyticus* *fliF* is overexpressed in *Escherichia coli*, FliF is equally detected from soluble and membrane fractions. Size exclusion chromatography showed that soluble FliF behaves as an oligomer. Here we examined several conditions for *Vibrio* FliF purification and found that it was eluted at smaller molecular size from a gel filtration chromatography, when solubilized by detergent at an alkaline condition (pH 11). We will optimize the purification procedures to purify FliF as a monomer and crystallize.

1P168 クライオ電子線トモグラフィー法を用いたフィロポディア内構造解析
The ultrastructure of filopodia were observed with cryo-ET

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In the last decade, the *in vivo* biophysical research techniques have been developed. However most methods for structural biology have been limited to the *in vitro* level. Hence, we revealed the structure of proteins in cells with cryo-ET.

We focus on the intracellular structure of filopodia, and we successfully obtained the 3D volumes of filopodia with cryo-ET. In filopodia, actin filaments were bundled tightly and parallelly. They were bundled by actin bundling protein, presumably assigned as fascia. It bound to actin filaments in 36 nm period, which is equal to the half pitch of actin filaments. In the next step, we are introducing 3D subtomogram averaging techniques to improve the resolution of intracellular protein structures.