3P140 自己組織化生体システムの機能創発機構解明に向けたメソスケール反応場のデザインと単分子分解能計測

Designing of self-assembled biomolecular system and the detection at the single molecule resolution

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Biological system is unique in that component biomolecules are self-organized and emerge adaptable, stable and energy-saving functions. To understand the essential condition for reproducing the properties and constructing stable system from unstable flexible components, we are designing artificial muscle using myosin and DNA nanostructure. System size, spatial positioning, degree of intrinsic noise and the mechanical communication in the synthesized muscle can be modulated and we'll observe the internal dynamics and the system behavior at the single molecule resolution. We have developed several novel tools to modulate the system and monitor the internal dynamics with high precision.

3P141 ナノスリット基板を用いたアクチンの重合の観察 Observation of actin polymerization in linear zero-mode waveguide

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Actin dynamics contribute to many cellular processes, while the detail mechanism of actin polymerization is unknown. Thus, a single molecule imaging of actin polymerization should be performed. As critical concentration of actin is >100 nM, it is not possible to observe actin polymerization at a single molecule level by TIRFM. Hence, we applied a linear zero-mode waveguide (ZMW), which has a 100-nm wide slit structure, allowing single molecule imaging at higher concentrations. We found that single BODIPY-FL-labelled actin was observed in a linear ZMW at 100 nM. F-actin was immobilized in a linear ZMW. Actin elongated in a linear ZMW at a similar elongation rate on a cover glass. Actin polymerization at a single molecule level in a linear ZMW is now under investigation.

3P142 中性環境における好アルカリ性 *Bacillus* 細菌が持つ Na⁺駆動型べん毛モーター固定子の遊泳低下に関するアミノ酸残基の探索 Critical amino acid residues for motility of the Na⁺-driven flagellar motor stator in alkaliphilic *Bacillus* decrease at neutral pH

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Alkaliphilic *Bacillus pseudofirmus* OF4 has a MotPS complex as a stator and the flagellum is driven by a Na⁺-motive force. Previous studies showed that strain OF4 swimming is dependent on Na⁺ at pH 8-10, but exhibited poor motility at neutral pH even in the presence of Na⁺. It was hypothesized there could be competitive inhibition by H⁺ of the Na⁺translocation by the stator-force generator MotPS. In contrast, *B. subtilis* has a MotPS complex similar to strain OF4, but it was reported that *B. subtilis* can swim dependent on Na⁺ concentrations at neutral pH. We investigated important amino acid residues for the motility decrease at neutral pH of the alkaliphilic *Bacillus*. Critical amino acid residues for reduced motility at neutral pH were identified in the MotP subunit.

3P143 アクトミオシン複合体におけるミオシン・サブフラグメント1の首振り運動の分子動力学シミュレーション

Molecular dynamics simulation for the swinging lever-arm motion of a myosin subfragment-1 in an actomyosin complex

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Molecular dynamics simulation was conducted for an actomyosin complex consisting of a myosin subfragment-1 and seven actin monomers solved in water. External force was applied to the end of the neck domain in the direction opposite to the power stroke by using the "pull code" in the GROMACS software.

The myosin neck domain showed a swinging lever-arm motion from the post-power stroke position to the pre-power stroke position, while the myosin head did not detach from the actin filament. This motion was in accordance with the myosin power stroke mechanism named "Driven by Detachment (DbD)" theory, which assumes that the power stroke is caused by the elasticity at the joint between the head and the neck domains and is not directly related to ATP hydrolysis.

3P144 Nonequilibrium dissipation-free transport of F1-ATPase and the thermodynamic role of asymmetric allostereism

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Recent experiments on F1-ATPase have clarified that the dissipative heat inside the motor is very small, irrespective of the velocity of rotation and energy transport. In this presentation, we focus on the problem that the amount of internal dissipation is not simply determined by the sequence of equilibrium pictures, but also relies on the truly nonequilibrium aspect. We show that the totally asymmetric affinity model, where ATP binding to F1 is assumed to have low dependence on the angle of the rotating shaft, produces results that are most consistent with experiment. The principle adopted in the model is simple enough to be considered generic in molecular motors, and may help providing a blueprint for artificial nanomachines.

3P145 エフェクター分泌機構解明を目指した細菌Ⅲ型分泌装置の回転-分泌相関の解析

Correlation analysis between rotation and secretion of bacterial type III secretion system for elucidate of effector secretion mechanism

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Bacterial type III secretion apparatus (T3SA) is a secretion-machinery for effector proteins, which involved in infection or toxicity. The secretion mechanism is still unknown. Based on the high similarities between T3SA and flagellum, we hypothesized that T3SA would secrete effectors via the proton-motive force-dependent rotation of T3SA needle like flagellum. Thus, we attempted to observe T3SA rotation, and evaluate the relationship between rotation and effector secretion. As the result, we succeeded in observation of T3SA rotation in situ on *Pseudomonas aeruginosa*, and found out its proton motive force dependence and its correlation with effector secretion. These results suggested that T3SA secretes effectors via the proton-motive force-dependent rotation of T3SA.