2P169 1 分子蛍光観察によるセロビオヒドロラーゼの結晶性セルロース加水分解反応素過程の解明**Single-molecule imaging analysis of cellobiohydrolase hydrolyzing crystalline cellulose**

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Cellobiohydrolase is a new type of linear molecular motor that hydrolyzes crystalline cellulose into water-soluble disaccharide at solid/liquid interface. Here, with single-molecule fluorescence microscopy and high-speed AFM, we revealed the adsorption-desorption dynamics and linear translocation of *Trichoderma reesei* cellobiohydrolase (*TrCel7A*) hydrolyzing Type I_a crystalline celluloses. *TrCel7A* showed binding rate constant of $9.7 \times 10^6 \text{ M}^{-1} \mu\text{m}^{-2} \text{ s}^{-1}$, including productive and non-productive binding that lasted 8.6 s and 1.2 s respectively. The velocity of movement on cellulose in the productive binding was 5.0 nm/s (5 s^{-1} in turnover rate). Our results shed light on the enzyme reaction mechanism at solid/liquid interface at the single-molecule level for the first time.

**2P170 負荷存在下でのキネシン頭部の運動の高時間分解能観察
High temporal resolution observation of the stepping motion of kinesin-1 under load**

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Kinesin-1 is a motor protein that walks along microtubules to transport cargoes. The velocity of the movement decreases with increasing load, however it is still unclear which conformational transition is the load-dependent. At the last meeting, Isojima in our lab demonstrated that we could distinguish bound and unbound states of the motor head using dark-field microscopy with 50- μs temporal resolution. In this study, we constrained the kinesin movement by fusing a mutant head that cannot detach from the microtubule to the stalk of wild-type dimer. Then we observed the movement of a wild-type head, which showed discrete 16 nm steps and then stalled after several steps. We will discuss the effect of load on the duration of bound and unbound states of a head.

2P171 ジスルフィドクロスリンクを用いたキネシン 1 の二足歩行制御機構の研究**Strain-dependent regulation of the kinesin-1's catalytic activity as studied by disulfide-crosslinking of the neck linker**

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Kinesin-1 walks along microtubules by alternately hydrolyzing ATP and moving two motor domains, although the mechanism of the alternate catalysis remains unknown. Here we focused on the neck linker that connects two motor domains and investigated the effect of the neck linker tension on the motor activity by constraining the neck linker in the forward or backward extended conformation using disulfide-crosslinking. Stopped flow and single molecule measurements showed that the forward-constraint of the neck linker reduced ADP release rate although the backward-constraint suppresses either ATP hydrolysis or Pi release rate. These results suggest that ATP hydrolysis cycle can be differently regulated depending on the direction of the neck linker tension.

2P172 SDSL-ESR により検出したキネシン α -1 ヘリックスのヌクレオチド依存的な動的構造とその変位**Nucleotide-dependent Displacement and Dynamics of α -1 Helix in Motor Protein Kinesin As Revealed by Site Directed Spin Labeling ESR**

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We show the nucleotide-dependent displacement of the α -1 helix of kinesin on microtubule by ESR spectroscopy. Kinesin monomer was doubly spin-labeled at α -1 and α -2. The inter-helix distance distribution was determined by spectral broadening and showed that 40% of spins had a peak at 1.4-1.7 nm, which was close to that from crystal structure, but 60% beyond sensitivity ($>2.5 \text{ nm}$). The fraction of 1.4-1.7 nm was 20 and 25% in the presence of AMPPNP and ADP, respectively. These nucleotide-induced decreases in the fraction of 1.4-1.7 nm were reversely related to those in the docking fraction of neck-linker on motor core, suggesting that shift of spatial equilibrium of α -1 helix from 1.4-1.7 nm toward $>2.5 \text{ nm}$ makes its C-terminal end to be exposed and bind neck-linker.

2P173 微小管上での KIF1A の選択的結合における負に荷電した C 末の役割**The role of negatively-charged C-terminus of tubulin in selective binding of KIF1A on microtubule**

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KIF1A, a well-studied motor protein, functions as a monomer, where the force-generating biased binding to the microtubule (MT) has been observed during the weak-to-strong binding transition. While it is obvious that the weak binding is maintained by the electrostatic interaction between the lysine-rich K-loop of KIF1A and the glutamate-rich C-terminus of tubulin (E-hook), it is less clear why KIF1A selectively binds on the α - β tubulin junction within the tubulin dimer rather than the β - α junction between the tubulin dimers. In this study, by calculating the binding energy between KIF1A and MT, we investigated the molecular mechanism of the selective binding of KIF1A, which also has close relevance to the biased binding, and show that E-hook plays the key role.

2P174 フォトクロミック分子を用いた有糸分裂キネシン Eg5 の光制御型阻害剤**Photo regulated inhibitor composed of photochromic molecules for mitotic kinesin Eg5**

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S-trityl-L-cysteine (STLC) is a well known specific inhibitor for mitotic kinesin Eg5. Trityl group of STLC is a key moiety to inhibit Eg5 ATPase potently. In this study, we designed and synthesized the photo regulated inhibitors to control Eg5 activity. The photochromic inhibitor 4,4'-bis(N-(2-(triphenylmethylamino)acetyl)amino)azobenzene (BTAB) was synthesized. by the coupling reaction of 4,4'-diaminoazobenzene with tritylglycine. And other related inhibitors were also synthesized. BTAB showed cis-trans isomerization upon 380 nm and 480 nm light irradiations. BTAB inhibited reversibly Eg5 ATPase activity in the absence of microtubules by light irradiations. We also examined which step along ATPase kinetic pathway is inhibited by BTAB using stopped flow apparatus.