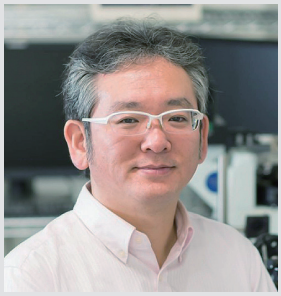
Operation and Design Principles of Biological Molecular Machines

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Activity of life is supported by various molecular machines made of proteins. Protein molecular machines are tiny, but show very high performance, and are superior to man-made machines in many aspects. One of the representatives of protein molecular machines is linear and rotary molecular motors (Figure 1). Molecular motors generate mechanical forces and torques that drive their unidirectional motions from the energy of chemical reaction or the electrochemical potential across the cell membrane.

We unveil operation principles of molecular motors with advanced single-molecule functional analysis. With the help of site-saturation mutagenesis and robot-based automation, we also engineer non-natural molecular motors to understand their design principles.

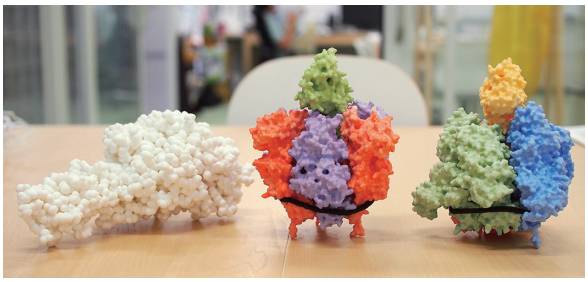

Figure 1. Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors F_1-ATPase and V_1-ATPase, respectively.

Selected Publications

- A. Visootsat, A. Nakamura, P. Vignon, H. Watanabe, T. Uchihashi and R. Iino, "Single-Molecule Imaging Analysis Reveals the Mechanism of a High-Catalytic-Activity Mutant of Chitinase A from *Serratia marcescens*," *J. Biol. Chem.* **295**, 1915–1925 (2020).
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- A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, "Processive Chitinase is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin," *Nat. Commun.* **9**, 3814 (2018).
- A. Nakamura, T. Tasaki, Y. Okuni, C. Song, K. Murata, T. Kozai, M. Hara, H. Sugimoto, K. Suzuki, T. Watanabe, T. Uchihashi, H. Noji and R. Iino, "Rate Constants, Processivity, and Productive Binding Ratio of Chitinase A Revealed by Single-Molecule Analysis," *Phys. Chem. Chem. Phys.* **20**, 3010–3018 (2018).

1. Domain Architecture Divergence Leads to Functional Divergence in Binding and Catalytic Domains of Bacterial and Fungal Cellobiohydrolases[1)]

Cellobiohydrolases directly convert crystalline cellulose into cellobiose and are of biotechnological interest to achieve efficient biomass utilization. As a result, much research in the field has focused on identifying cellobiohydrolases that are very fast. Cellobiohydrolase A from the bacterium *Cellulomonas fimi* (CfCel6B) and cellobiohydrolase II from the fungus *Trichoderma reesei* (TrCel6A) have similar catalytic domains (CDs) and show similar hydrolytic activity. However, TrCel6A and CfCel6B have different cellulose-binding domains (CBDs) and linkers: TrCel6A has a glycosylated peptide linker, whereas CfCel6B's linker consists of three fibronectin type 3 domains. We previously found that TrCel6A's linker plays an important role in increasing the binding rate constant to crystalline cellulose. However, it was not clear whether CfCel6B's linker has similar function. Here we analyze kinetic parameters of CfCel6B using single-molecule fluorescence imaging to compare CfCel6B and TrCel6A. We find that CBD is important for initial binding of CfCel6B, but the contribution of the linker to the binding rate constant or to the dissociation rate constant is minor. The crystal structure of the CfCel6B CD showed longer loops at the entrance and exit of the substrate-binding tunnel compared with TrCel6A CD, which results in higher processivity. Furthermore, CfCel6B CD showed not only fast surface diffusion but also slow processive movement, which is not observed in TrCel6A CD. Combined with the results of a phylogenetic tree analysis, we propose that bacterial cellobiohydrolases are designed to degrade crystalline cellulose using high-affinity CBD and high-processivity CD.

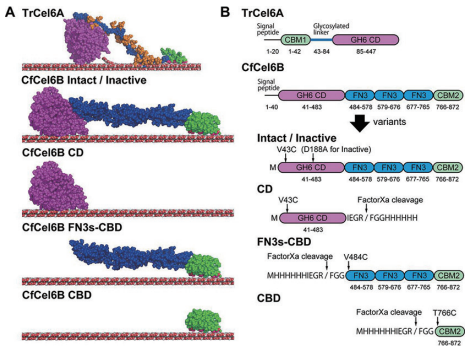

Figure 2. Structures of TrCel6 and CfCel6B. A, model structures of Intact TrCel6A, CfCel6B, and CfCel6B domain constructs used in this study. TrCel6A structure is the same as in the previous report. For CfCel6B, structure of CD is X-ray crystal structure (PDB code 7CBD), and FN3s and CBD are modeled by SWISS-MODEL server. Figures were prepared by PyMOL. B, detailed descriptions of domain compositions for each construct. Positions of mutation sites, histidine tags, and FaXa cleavage sites and estimated amino acid numbers for each domain are shown.

2. Combined Approach to Engineer a Highly Active Mutant of Processive Chitinase Hydrolyzing Crystalline Chitin[2)]

Serratia marcescens chitinase A (SmChiA) processively hydrolyzes recalcitrant biomass crystalline chitin under mild conditions. Here, we combined multiple sequence alignment, site-saturation mutagenesis, and automated protein purification and activity measurement with liquid-handling robot to reduce the number of mutation trials and shorten the screening time for hydrolytic activity improvement of SmChiA. The amino acid residues, which are not conserved in the alignment and are close to the aromatic residues along the substrate-binding sites in the crystal structure, were selected for site-saturation mutagenesis. Using the previously identified highly active F232W/F396W mutant as a template, we identified the F232W/F396W/S538V mutant, which shows further improved hydrolytic activity just by trying eight different sites. Importantly, valine was not found in the multiple sequence alignment at Ser538 site of SmChiA. Our combined approach allows engineering of highly active enzyme mutants, which cannot be identified only by the introduction of predominant amino acid residues in the multiple sequence alignment.

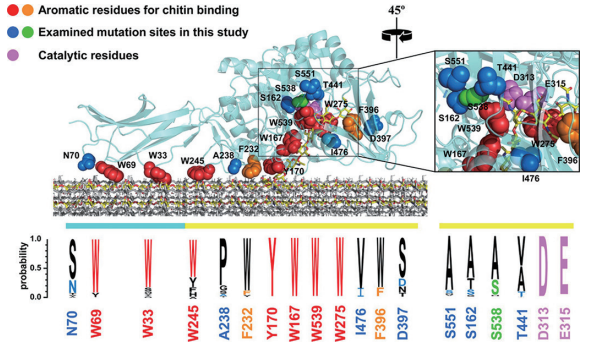

Figure 3. Model structure of SmChiA bound to crystalline chitin, important residues for binding and catalysis, and residues mutated in this study. (Top) A crystal structure of SmChiA (PDB entry 1CTN; ribbon model) aligned with the crystalline chitin chains (stick model). Amino acid residues responsible for binding to chitin (red and orange), catalytic residues (pink), and examined mutation sites (blue and green) are highlighted with sphere models. An expanded image around the catalytic site (transparent box in the left structure) is also shown in the right-hand-side box with 45° turn from the left structure. The cyan and yellow bars under the structure indicate the binding and catalytic domains, respectively. (Bottom) Result of multiple sequence alignment for amino acid residues highlighted in the top. The residues of SmChiA are shown in the same color as the top.

References

1) A. Nakamura *et al.*, *J Biol. Chem.* **295**, 14606–14617 (2020).
2) A, Visootsat *et al.*, *ACS Omega* **5**, 26807–26816 (2020).

Award

OTOMO, Akihiro; Best Presentation Award, 2020 Annual Meeting of the Biophysical Society of Japan Chubu Branch Meeting (2020).