高速AFMで明らかにする真正細菌型イオンポンプロドプシンの多量体構造と機能動態

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Publicly

Project Area	Science on Function of Soft Molecular Systems by Cooperation of Theory and Experiment
Project/Area Number	16H00830
Research Category	Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area)
Allocation Type	Single-year Grants
Review Section	Science and Engineering
Research Institution	Nagoya University (2017) Kanazawa University (2016)
Principal Investigator	**内橋 貴之** 名古屋大学, 理学研究科, 教授 (30326300)
Project Period (FY)	**2016-04-01 – 2018-03-31**
Project Status	Completed (Fiscal Year 2017)
Budget Amount *help	**¥6,890,000 (Direct Cost: ¥5,300,000、Indirect Cost: ¥1,590,000)** Fiscal Year 2017: ¥3,510,000 (Direct Cost: ¥2,700,000、Indirect Cost: ¥810,000) Fiscal Year 2016: ¥3,380,000 (Direct Cost: ¥2,600,000、Indirect Cost: ¥780,000)
Keywords	微生物ロドプシン / 高速原子間力顕微鏡 / 一分子計測 / 多量体構造 / 構造変化 / １分子計測(SMD) / 走査プローブ顕微鏡 / 蛋白質 / 一分子計測(SMD) / １分子イメージング・ナノ計測
Outline of Annual Research Achievements	１．イオンポンプロドプシンの多量体構造の決定: 様々な微生物型ロドプシンを高速AFM法と円二色性(CD)分光法により網羅的に解析し、脂質膜に再構成された状態での多量体構造を決定した。高速AFM観察により、GR、KR2、FR、KrActR、QsActR等の真性細菌で見出された微生物型ロドプシンはリング状の5量体を形成しており、GPRは５量体と６量体が共存していることがわかった。また、センサリーロドプシンであるSRII、ハロロドプシンNpHRなどの古細菌型ロドプシンは三量体であることがわかった。一方、内向きH+ポンプであるPoXeRやセンサリーロドプシンASRは真性細菌で発見された微生物型ロドプシンであるが、アミノ酸残基の相同性からは古細菌型に分類され、実際多量体構造は三量体であった。また、CD分光で観察される三量体と五量体に特徴的なスペクトルは多量体構造に依存したレチナールの配向で説明できることもわかった。これらの結果から、微生物型プロドプシンの多量体構造は進化系統樹と深く関わっている一方、イオンポンプのイオン種や向きと相関がないことがわかった。 2．KR2の光誘起構造変化の観察の観察: 外向きNa+ポンプであるKR2の光誘起による構造変化の観察に向けて、KR2五量体の観察面の決定おより高解像観察に適した試料の調製を行った。野生型に比べて光サイクルが100以上遅いN112A変異体を高解像観察しながら、周期的な光照射を照射したが明瞭な構造変化は観察できなかった。このことから、BRとは異なり、KR2の光サイクル中には大規模な構造変化は起きていない可能性が示唆された。
Research Progress Status	29年度が最終年度であるため、記入しない。
Strategy for Future Research Activity	29年度が最終年度であるため、記入しない。

Report (2 results)

2017 Annual Research Report

2016 Annual Research Report

Research Products (62 results)

All | 2019 | 2018 | 2017 | 2016

All | Journal Article | Presentation | Book | Patent(Industrial Property Rights)

[Journal Article] Structural properties determining low K+ affinity of the selectivity filter in the TWIK1 K+ channel. 2019

[Journal Article] Applications of high-speed atomic force microscopy to real-time visualization of dynamic biomolecular processes 2018

[Journal Article] Mechano-Sensitive Rate constants, processivity, and productive binding ratio of chitinase A revealed by single-molecule analysis. Ion Channels. 2018

[Journal Article] Conversion of functionally undefined homopentameric protein PbaA into a proteasome activator by mutational modification of its C-terminal segment conformation 2018

[Journal Article] Dynamic clustering of dynamin-amphiphysin helices regulates membrane constriction and fission coupled with GTP hydrolysis. 2018

[Journal Article] Negatively charged lipids are essential for functional and structural switch of human 2-Cys peroxiredoxin II 2018

[Journal Article] Sweeping of adsorbed therapeutic proteins on prefillable syringe enhances subvisible particles generation 2018

[Journal Article] Construction of a Triangle-Shaped Trimer and a Tetrahedron Using an α-Helix-Inserted Circular Permutant of Cytochrome c555 2018

[Journal Article] Insight into structural remodeling of the FlhA ring responsible for bacterial flagellar type III protein export 2018

[Journal Article] Translating MOF chemistry into supramolecular chemistry: soluble coordination nanofibers showing efficient photon upconversion 2018

[Journal Article] Quantum-dot antibody conjugation visualized at the single-molecule scale with high-speed atomic force microscopy 2018

[Journal Article] Visualization of Protein Dynamics using High-Speed Atomic Force Microscopy and Image Analysis 2018

[Journal Article] High-Resolution Imaging of a Single Gliding Protofilament of Tubulins by HS-AFM 2017

[Journal Article] Fast Adsorption of Soft Hydrogel Microspheres on Solid Surfaces in Aqueous Solution 2017

[Journal Article] Interdomain flip-flop motion visualized in flavocytochrome cellobiose dehydrogenase using high-speed atomic force microscopy during catalysis 2017

[Journal Article] Visualisation of a flexible modular structure of the ER folding-sensor enzyme UGGT 2017

[Journal Article] Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy 2017

[Journal Article] High-speed atomic force microscopy imaging of live mammalian cells 2017

[Journal Article] Na+-induced structural transition of MotPS for stator assembly of Bacillus flagellar motor. 2017

[Journal Article] Oriented Reconstitution of the Full-Length KcsA Potassium Channel in a Lipid Bilayer for AFM Imaging. 2017

[Journal Article] A natural light-driven inward proton pump. 2016

[Presentation] irect observation of self-assembly process of biological and artificial fibrils using high-speed atomic force microscopy 2018

[Presentation] Dynamic Observationb of Kai Proteins by HS-AFM Reveales a Mechanism of the Robustness in the Cyanobacterial Circadian Oscillator 2018

[Presentation] 生命の構成部品を直接みて理解する ～ 顕微鏡技術で可視化するタンパク質のダイナミクス現象 ～ 2018

[Presentation] Structural Flexibility and Chaperone Activity of TClpB revealed by High-Speed AFM 2017

[Presentation] Direct visualization of dynamic molecular interactions using HS-AFM 2017

[Presentation] Direct visualization of single molecule dynamics by high-speed atomic force microscopy 2017

[Presentation] Oligomeric state and conformational dynamics of eubacterial ion-pumping rhodopsin studied by high-speed AFM 2017

[Presentation] High speed atomic force microscopy for a tool to visualize dynamic events on biological systems from single molecules to living cells 2017

[Presentation] Direct observation of single molecule dynamics at work with high-speed atomic force microscopy 2017

[Presentation] Visualization of Single-Molecule Dynamics Using High-Speed Atomic Force Microscopy 2017

[Presentation] High-speed atomic force microscopy: A tool for visualizing dynamic behavior from proteins to cells 2017

[Presentation] In-line Force Measurements with High-speed AFM 2017

[Presentation] Two-step process for disassembly mechanism of proteasome α7 homo-tetradecamer by α6 revealed by high-speed atomic force microscopy 2017

[Presentation] Development of new mirro-tilter unit for tip-scanning high-speed atomic force microscopy 2017

[Presentation] 高速AFMで明らかにするKaiタンパク質間の動的相互作用 2017

[Presentation] 高速原子間力顕微鏡で可視化するタンパク質の動的秩序 2017

[Presentation] 高速AFMを用いた生体分子のその場観察 2017

[Presentation] 高速原子間力顕微鏡で可視化する生体・人工高分子の動態 2017

[Presentation] 高速原子間力顕微鏡による生体分子のダイナミクス計測 2017

[Presentation] Analysis of Rotational Dynamics of Rotorless Enterococcus hirae V1-ATPase using High-Speed Atomic Force Microscopy 2017

[Presentation] Dynamic remodeling of Dynamin complexes during membrane fission 2017

[Presentation] High-Speed AFM Observation of Domain Flexibility Related to Enzymatic Function of CRISPR-Cas9 2017

[Presentation] 高速原子間力顕微鏡で可視化する Kai タンパク質間相互作用のダイナミクス 2017

[Presentation] 高速原子間力顕微鏡で可視化する生体膜反応ダイナミクス **2017**

[Presentation] Visualization of protein molecules in action by high-speed atomic force microscopy **2016**

[Presentation] Direct Visualization of Single Molecule Dynamics at Work with High-Speed Atomic Force Microscopy **2016**

[Presentation] タンパク質のダイナミクスを可視化する高速原子間力顕微鏡 **2016**

[Presentation] 高速原子間力顕微鏡で可視化する生体分子のダイナミクス **2016**

[Presentation] 高速AFMによる生体分子ダイナミクスのその場観察 **2016**

[Presentation] 高速AFMによる生体試料の動態イメージング **2016**

[Presentation] Dynamic interaction between Kai proteins dependent on phosphorylation states of KaiC revealed by HS-AFM **2016**

[Presentation] Direct Visualization of Single Molecule Dynamics by High-Speed Atomic Force Microscopy **2016**

[Presentation] Visualization of Functional Dynamics of Biological Molecules by High-Speed AFM **2016**

[Presentation] 高速原子間力顕微鏡で調べる回転分子モーターの構造ダイナミクス **2016**

[Presentation] 高速AFMによる膜タンパク質のダイナミクス観察 **2016**

[Presentation] Direct Observation of Single Molecule Dynamics at Work with High-Speed Atomic Force Microscopy **2016**

[Book] "High-Speed Atomic Force Microcopy in "Compendium of Surface and Interface Analysis **2018**

[Book] パリティ，「高速原子間力顕微鏡によるタンパク質の動画撮影」 **2018**

[Patent(Industrial Property Rights)] 走査型プローブ顕微鏡 **2016**

[Patent(Industrial Property Rights)] 昇温ホルダおよびプローブ顕微鏡 **2016**

[Patent(Industrial Property Rights)] チャンバーアレイの製造方法 **2016**

URL: https://kaken.nii.ac.jp/grant/KAKENHI-PUBLICLY-16H00830/

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