

# FY2019 Annual Report

Molecular Cryo-Electron Microscopy Unit  
Associate Professor Matthias Wolf



## Abstract

Fiscal year 2019 was characterized by very fruitful collaborations, including partners at Tokyo University, Osaka University, Hiroshima University, Nagasaki University, and at the University of Otago in New Zealand. Dr. Jaekyung Hyun, who brings extensive experience in cryo-EM, image processing and virology, joined the team. We were able to add several high-impact publications, including in the journals Nature Structural and Molecular Biology, Nature Microbiology and Philosophical Transactions of the Royal Society. The emphasis of the unit's science has further been focused on molecular virology and molecular microbiology.

## 1. Staff

- Dr. Matthias Wolf, Associate Professor
- Dr. Hideyuki Matsunami, Group Leader
- Dr. Satoshi Shibata, Staff Scientist
- Dr. Tae Gyun Kim, Staff Scientist
- Dr. Jaekyung Hyun, Staff Scientist
- Dr. Melissa Matthews, Postdoctoral Researcher
- Eui Kyung Yu, BSc, Technician (externally funded)
- Makoto Tokoro Schreiber, Graduate Student
- Rika Yoshizawa, Research Unit Administrator

## 2. Collaborations

### 2.1 Bacterial locomotion

- Type of collaboration: Joint research
- Researchers:
  - Professor Shin-Ichi Aizawa, Prefectural University of Hiroshima
  - Dr. Satoshi Shibata, Molecular Cryo-EM Unit, OIST

- Dr. Hideyuki Matsunami, Molecular Cryo-EM Unit, OIST

## **2.2 Structure of bacterial adhesive pili**

- Type of collaboration: Joint research
- Researchers:
  - Professor Koji Nakayama, University of Nagasaki
  - Professor Kazumi Imada, Osaka University
  - Assistant Professor Mikio Shoji, University of Nagasaki
  - Dr. Satoshi Shibata, Molecular Cryo-EM Unit, OIST
  - Dr. Hideyuki Matsunami, Molecular Cryo-EM Unit, OIST
  - Dr. Melissa Matthews, Molecular Cryo-EM Unit, OIST

## **2.3 Structure of Nucleosome complexes**

- Type of collaboration: Joint research
- Researchers:
  - Professor Hitoshi Kurumizaka, University of Tokyo
  - Dr. Yoshimasa Takizawa, Molecular Cryo-EM Unit, OIST (now at Tokyo University)

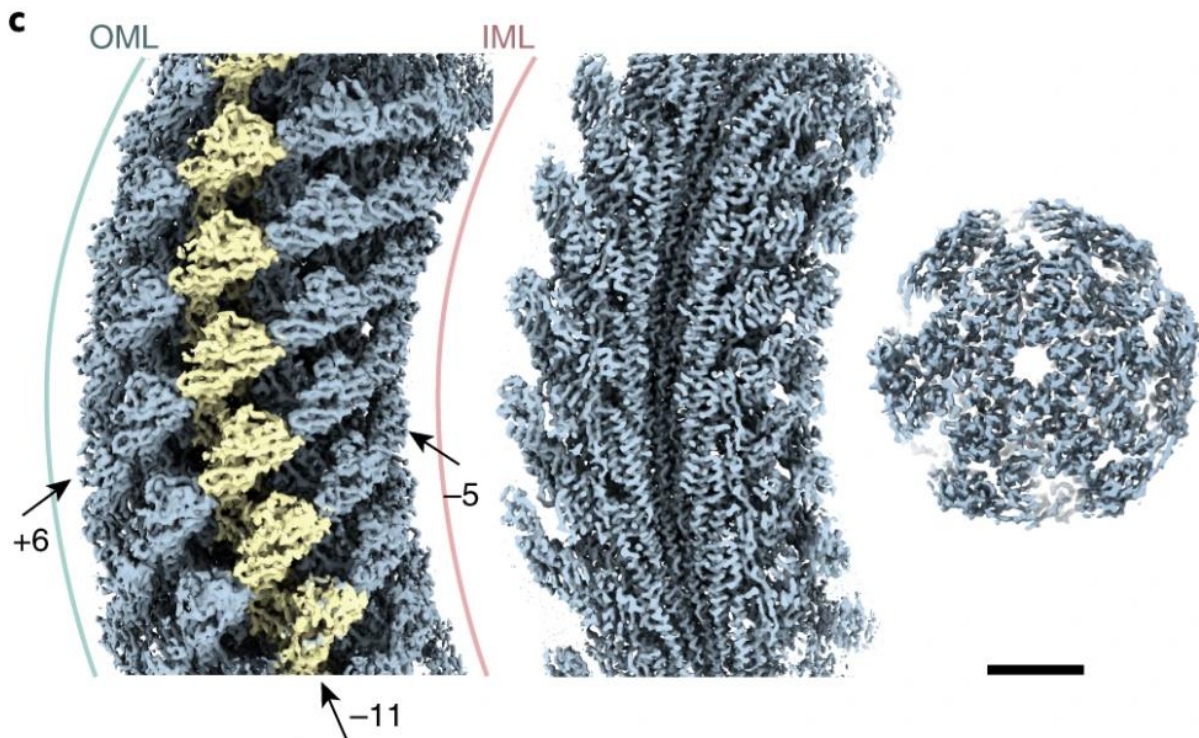
## **2.4 Phage defense by CRISPR-CAS**

- Type of collaboration: Joint research
- Researchers:
  - Professor Mihnea Bostina, University of Otago
  - Professor Peter Fineran, University of Otago

# **3. Activities and Findings**

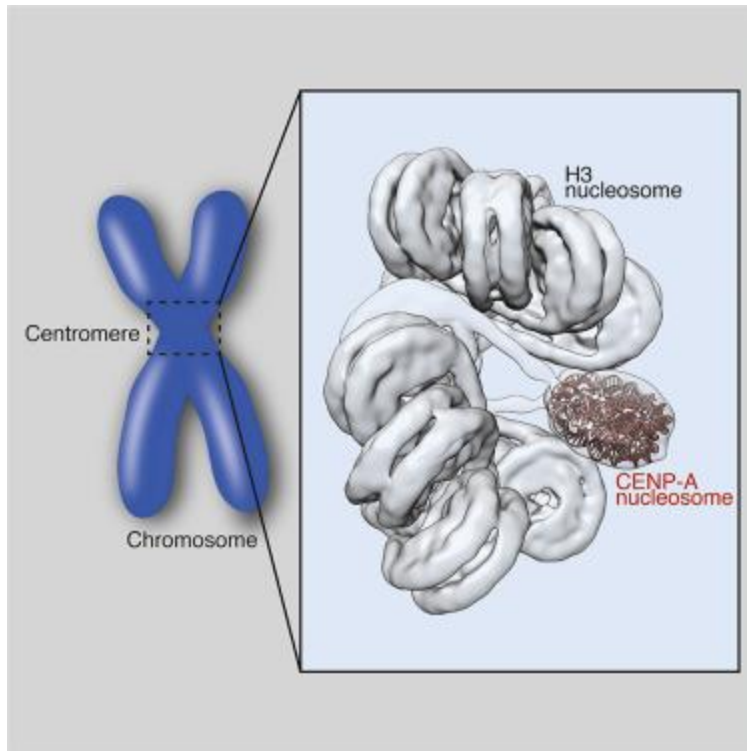
## **3.1 Structure of the supercoiled bacterial flagellar hook**

### 3.1 Structure of the supercoiled bacterial flagellar hook



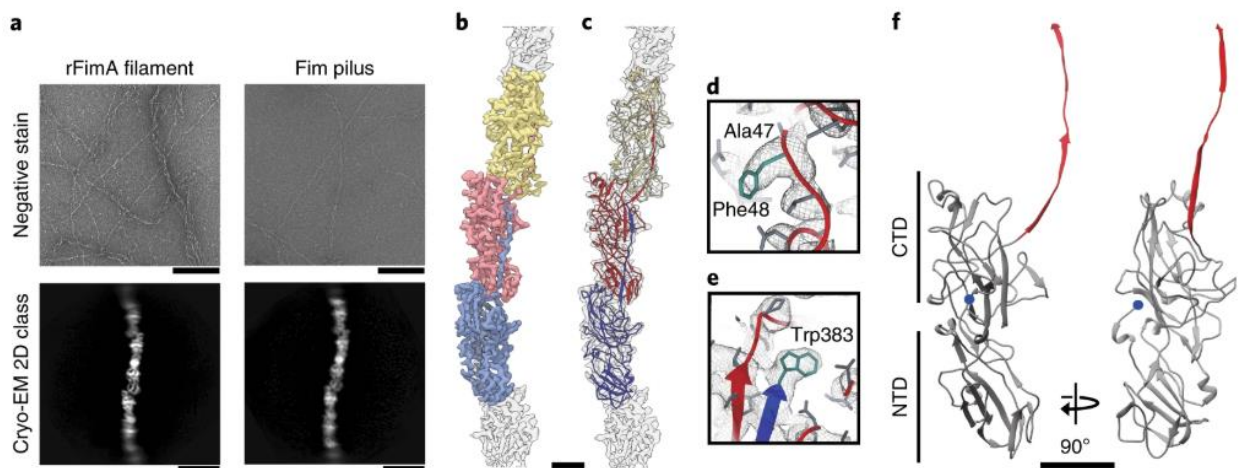
**Figure 1.** This work was a collaboration with Prof. Shin-Ichi Aizawa (Hiroshima University). Dr. Shibata and Prof. Aizawa purified flagellar hooks from a mutant *Salmonella* strain at low pH, resulting in strongly supercoiled polyhooks. We were the first to publish a snapshot of this dynamic structure at 2.8 Angstrom resolution, without imposing helical symmetry. The work was published in **Nature Structural and Molecular Biology** and resolves a series of important questions related to bacterial locomotion. The innovative image processing method inspired several follow-up projects.

### 3.2 CENP-A Trinucleosome



**Figure 2.** In this collaboration with the Kurumizaka Lab, first author Dr. Yoshimasa Takizawa reconstructed several states of in-vitro purified recombinant trinucleosomes from cryo-EM images collected in focus using a phase plate. The work suggests a model for the centromeric attachment site in the kinetochore, which is an essential property of chromosomes in cell division. The paper appeared in the journal **Structure**.

### 3.3 Strand exchange mechanism of major FimA pilus



**Figure 3.** By applying a similar image processing strategy as for the flagellar hook, first author Dr. Satoshi Shibata was able to reconstruct the pilus of the oral pathogen *Porphyromonas gingivalis* at

near-atomic resolution. In collaboration with Prof. Imada at Osaka University, who solved atomic-resolution X-ray structures of the monomeric subunit, and the group of Prof. Nakayama at Nagasaki University, the joint team demonstrated experimentally for the first time the protease-mediated strand exchange mechanism of these adhesive pili. The work was published in **Nature Microbiology**.

## 4. Publications

### 4.1 Journals

1. S Shibata, M Shoji, K Okada, H Matsunami, MM Matthews, K Imada, ...  
Nature Microbiology, 1-8 Structure 28 (1), 44-53. e4  
*Structure of polymerized type V pilin reveals assembly mechanism involving protease-mediated strand exchange*
2. Y Takizawa, CH Ho, H Tachiwana, H Matsunami, W Kobayashi, M Suzuki, ...  
Structure 28 (1), 44-53. e4  
*Cryo-EM structures of centromeric tri-nucleosomes containing a central CENP-A nucleosome*
3. S Shibata, H Matsunami, SI Aizawa, M Wolf  
Nature Structural & Molecular Biology 26 (10), 941-945  
*Torque transmission mechanism of the curved bacterial flagellar hook revealed by cryo-EM*
4. BNJ Watson, RA Easingwood, B Tong, M Wolf, GPC Salmond, RHJ Staals, ...  
Philosophical Transactions of the Royal Society B 374 (1772), 20180090  
*Different genetic and morphological outcomes for phages targeted by single or multiple CRISPR-Cas spacers*
5. VA Meshcheryakov, S Shibata, MT Schreiber, A Villar - Briones, KF Jarrell, ...  
EMBO reports 20 (5)  
*High - resolution archaeellum structure reveals a conserved metal - binding site*

### 4.2 Books and other one-time publications

Nothing to report

### 4.3 Oral and Poster Presentations (invited presentations and lectures)

- 2020.02.27 Chromopalooza, Vienna, Austria, 1-2-3: *mono-, di-, trinucleosomes and their impact on chromatin structure*
- 2020.02.21 Meeting of the Japanese Pharmaceutical Manufacturer's Association, OIST, Japan, *Cryo-EM in Drug Design*
- 2020.02.03 AMED BINDS Cryo-EM Workshop, OIST, Japan, *Asymmetric reconstruction of filamentous proteins by single particle cryo-EM*
- 2019.11.09 60<sup>th</sup> annual meeting of the Japanese Society of Tropical Medicine, Okinawa, Japan, *Structural dynamics of the bacterial flagellar hook*
- 2019.10.25 26th East Asia Joint Symposium on Biomedical Research, Seoul, Korea, *Structural dynamics of the bacterial flagellar hook*

- 2019.10.03 IBC Academia Sinica, Taipei, Taiwan, *New insights into the function of the bacterial flagellar hook*
- 2019.08.08 M&M2019, Portland, Oregon, USA: *Charging Dynamics in Low-Dose Cryo-TEM Imaging*
- 2019.06.10 3DEM GRC, Hong Kong, Short Talk: *Cryo-EM Structure of Supercoiled Flagellar Hook*
- 2019.06.04 RICCEM2019, Moscow, Russia: *Cryo-EM Structure of the Ebolavirus Nucleoprotein RNA complex*
- 2019.05.30 Cryo-EM symposium at Yilan, Taiwan: *Structural Dynamics of the Bacterial Flagellar Hook*
- 2019.05.28 Institute for Biological Chemistry, Academia Sinica, Taipei, Taiwan: *Pilus assembly and Alzheimer's disease - a tale of superhelical reconstruction*
- 2019.04.10 Keystone Symposium Snowbird, UT, USA, Short talk: *3D Imaging of Selectively Labeled Drosophila Neurons with Serial Blockface Electron Microscopy and X-Ray Microscopy Using Genetically Encoded Tags*

## 5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

## 6. Meetings and Events

### 6.1 Controlling Proteins and Organelles with Zapalog and Light

- Date: May 14, 2019
- Venue: OIST Campus Lab1
- Speaker: Dr. Amos Gutnick (FM Kirby Neurobiology Center Boston Children's Hospital / Harvard Medical School)

### 6.2 Cryo-EM reveals the molecular basis for the Plasmodium proteasome specific drug targeting

- Date: Aug 13, 2019
- Venue: OIST Campus Lab1
- Speakers: Dr. Pavel Afanasyev (MRC Laboratory of Molecular Biology)

### 6.3 Structural basis of p62/SQSTM1 polymers by Electron Cryo-Microscopy

- Date: November 1, 2019
- Venue: OIST Campus Lab 3
- Speakers: Dr. Carsten Sachse (Director of Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons ER-C-3: Structural Biology)

## 7. Other

Nothing to report.