

# FY2020 Annual Report

Molecular Cryo-Electron Microscopy Unit  
Professor Matthias Wolf



## Abstract

FY2020 was dominated by COVID-19. Already in spring 2020, we decided to contribute towards addressing the emerging pandemic by establishing a SARS-CoV-2 antibody test in the lab. We followed the first published protocol by Prof. Florian Krammer at Mount Sinai Medical School in New York City, which soon afterwards received emergency use authorization in the USA. For this ELISA, we needed to produce the SARS-CoV-2 spike protein in our lab in significant quantity and high purity. Furthermore, lab automation on a Beckman liquid handling robot needed to get developed for running the assay in 96-well plates for high throughput. This work galvanized and engaged most unit members and attracted collaborators beyond OIST including members of the Okinawan medical community. Over the time span of a year, we went on to test nearly 7,000 people for a study by the Okinawan Prefectural Government testing patients from many hospitals on Okinawa, OIST students and employees, and even veterinarians and fire fighters. We published some of this work in *Scientific Reports*.

## 1. Staff

- 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
- Dr. Chloé Minnai, Postdoctoral Researcher
- Noriko Shibata, BSc, Technician (externally funded)
- Makoto Tokoro Schreiber, Graduate Student
- Keon Young, 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 COVID-19 immunology

- Type of collaboration: Joint research
- Researchers: multiple
  - Wolf unit members
  - Ishikawa Unit members
  - Laurino Unit members
  - Dr. Masashi Narita, Okinawa Prefectural Chubu Hospital
  - Dr. Mary Collins, OIST

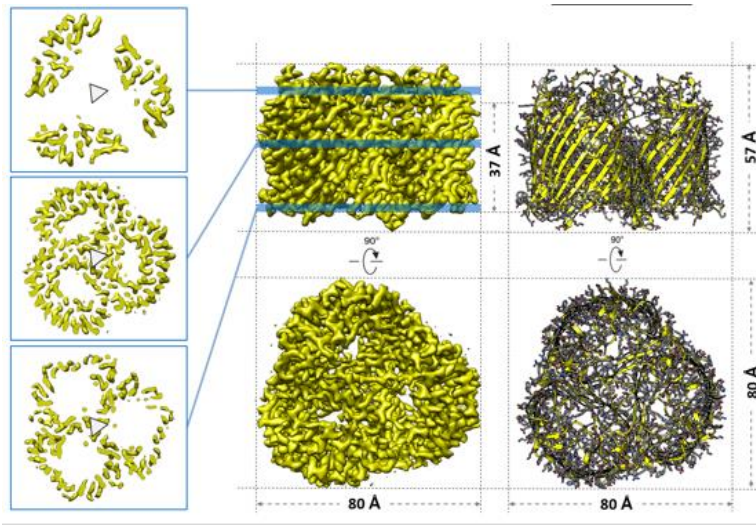
### 2.3 Structure of Nucleosome complexes

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

## 3. Activities and Findings

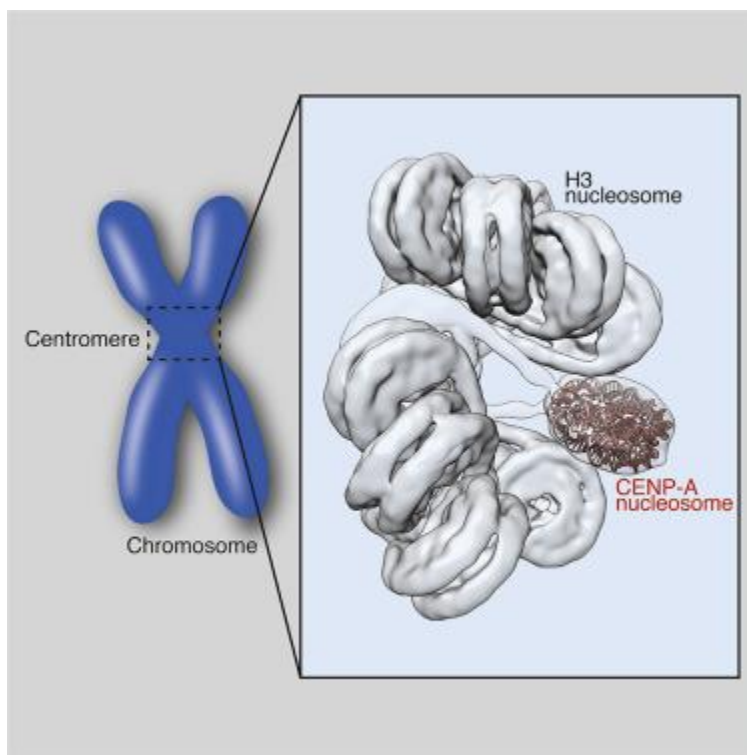
### 3.1 Structure of Bacterial Outer Membrane Porin Lamb

**Figure 1.** This is still work in progress by Dr. Tae Gyun Kim. The outer membrane protein (OMP) is a transmembrane protein that enables import and export of large cargo through the cell membrane.



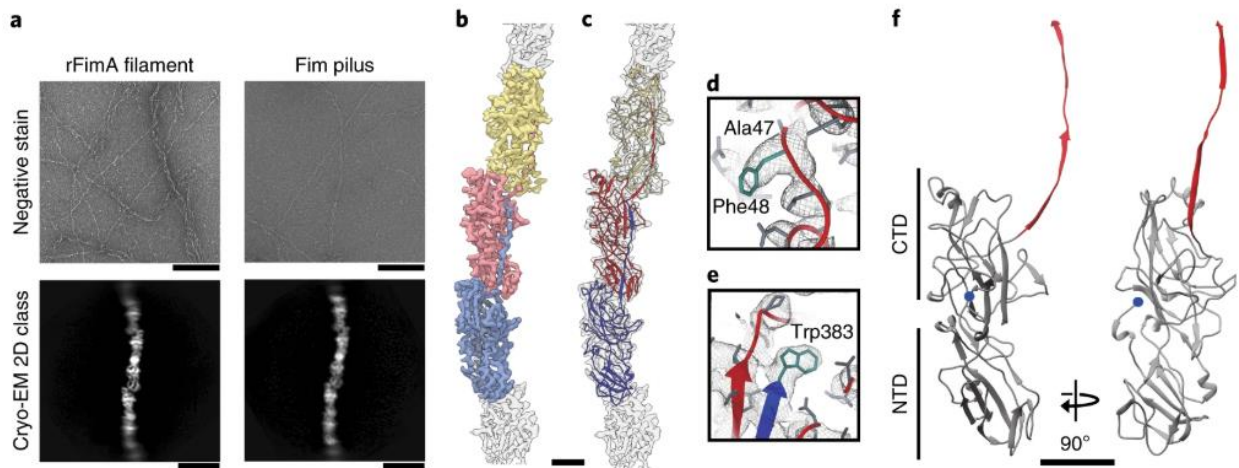
**Figure 1.** Cryo-EM reconstruction (left) and superposed atomic model (right) of a bacterial outer membrane protein complex.

### 3.2 CENP-A Trinucleosome



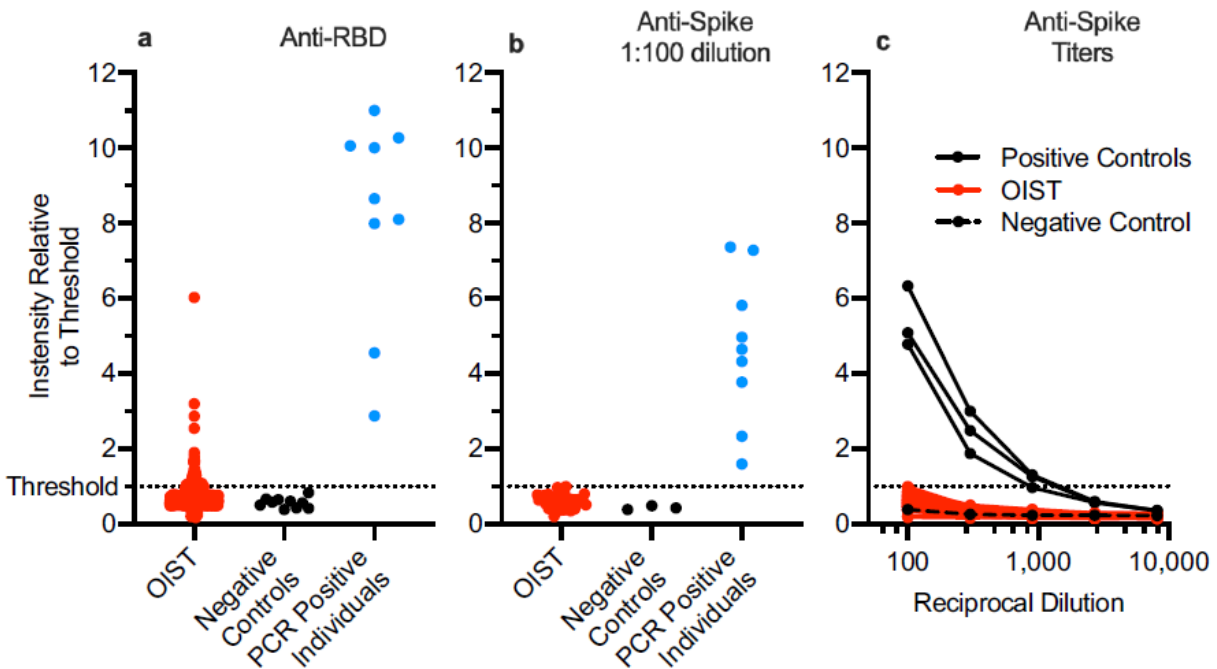
**Figure 2.** In this collaboration with the Kurumizaka Lab, first author Dr. Yoshimasa Takizawa reconstructed several states of in-vitro purified recombinant trinucleosomes from cryo-EM images collected in focus using a phase plate. The work suggests a model for the centromeric attachment site in the 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**.

### 3.4 COVID-19 serological survey using micro blood sampling



**Figure 4.** Results of ELISA detecting antibody levels against SARS-CoV-2 spike protein within the OIST population (N=675). Less than 0.1% tested positive, documenting the low seroprevalence at the onset of the pandemic on Okinawa.

## 4. Publications

### 4.1 Journals

[Structure of polymerized type V pilin reveals assembly mechanism involving protease-mediated strand exchange](#)

S Shibata, M Shoji, K Okada, H Matsunami, MM Matthews, K Imada, ...

Nature Microbiology 5 (6), 830-837

[Measuring Dominant Local Structures in Amorphous Materials Using Nanobeam Electron Diffraction](#)

M Schreiber, M Wolf

Bulletin of the American Physical Society 65

[High-Resolution Architecture of Bacterial Outer Membrane Porin Lamb](#)

TG Kim, J Hyun, E Yu, M Wolf

Biophysical Journal 118 (3), 524a-525a

[Cryo-EM structures of centromeric tri-nucleosomes containing a central CENP-A nucleosome](#)

Y Takizawa, CH Ho, H Tachiwana, H Matsunami, W Kobayashi, M Suzuki, ...

Structure 28 (1), 44-53. e4

### 4.2 Books and other one-time publications

Nothing to report

### 4.3 Oral and Poster Presentations

- 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*
- 2020 Shibata S, Hoji M, Matsunami H, Matthews M, Imada K, Nakayama K, Wolf M, *Cryo-EM Structure of polymerized Type V pilus of P.gingivalis reveals assembly mechanism, 93th Japan Society of Bacteriology General Meeting 2020*



### Conference proceedings:

Schreiber M.T., **Wolf M.\***, Measuring Dominant Local Structures in Amorphous Materials Using Nanobeam Electron Diffraction, Bulletin of the American Physical Society (2020), <http://meetings.aps.org/Meeting/MAR20/Session/D10.7>

Kim TG, Hyun J, Yu E, **Wolf M.\***, High-Resolution Architecture of Bacterial Outer Membrane Porin Lamb, Biophysical Journal 118 (3), 524a-525a, [https://www.cell.com/biophysj/pdf/S0006-3495\(19\)33816-0.pdf](https://www.cell.com/biophysj/pdf/S0006-3495(19)33816-0.pdf)

Schreiber M.T., Maigne A., **Wolf M.\*** (2019). Charging Dynamics in Low-Dose Cryo-TEM Imaging. Microscopy and Microanalysis 25 (S2), 1004-1005, doi: 10.1017/S1431927619005750

## 5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

## 6. Meetings and Events

Schreiber M.T., **Wolf M.\***, Measuring Dominant Local Structures in Amorphous Materials Using Nanobeam Electron Diffraction, Bulletin of the American Physical Society (2020), <http://meetings.aps.org/Meeting/MAR20/Session/D10.7>

Kim TG, Hyun J, Yu E, **Wolf M.\***, High-Resolution Architecture of Bacterial Outer Membrane Porin Lamb, Biophysical Journal 118 (3), 524a-525a, [https://www.cell.com/biophysj/pdf/S0006-3495\(19\)33816-0.pdf](https://www.cell.com/biophysj/pdf/S0006-3495(19)33816-0.pdf)

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## 7. Other

Nothing to report.