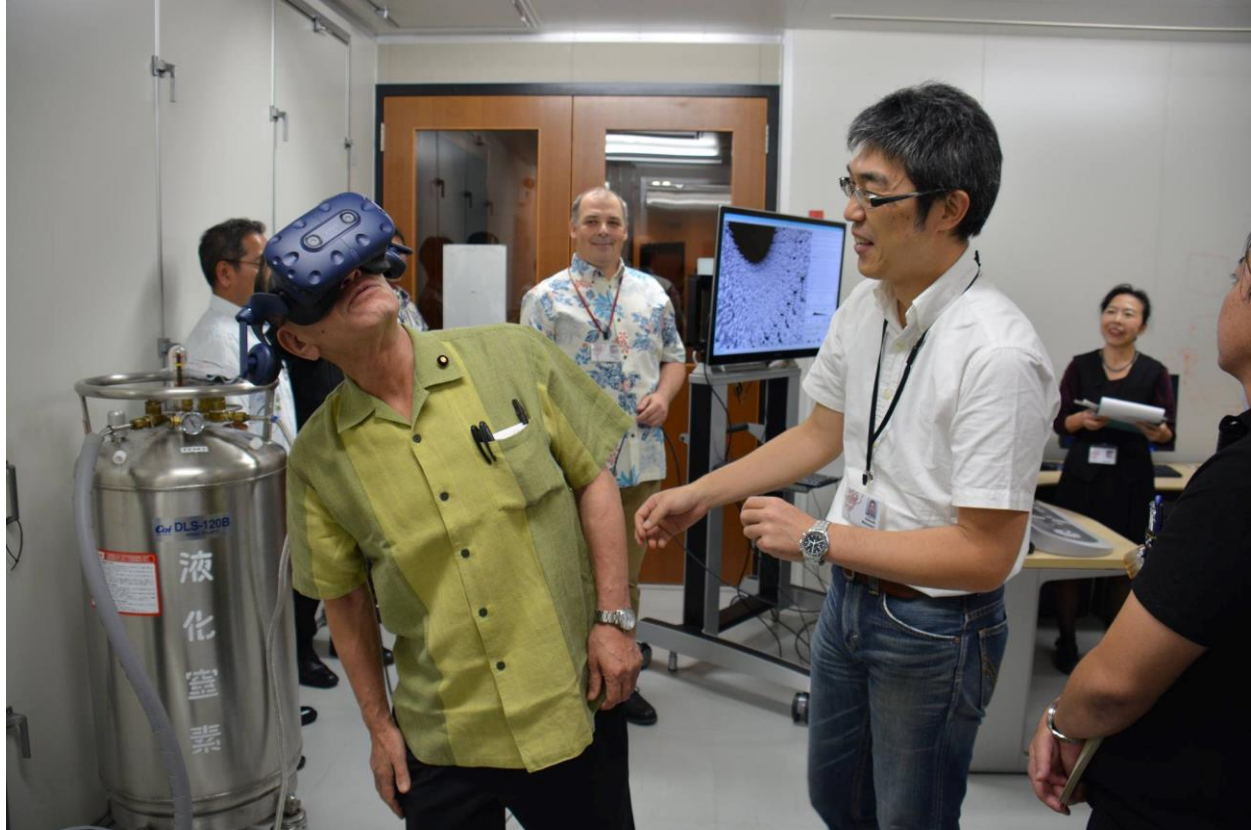


FY2018 Annual Report

Cryo-Electron Microscopy Unit
Mathias Wolf



Abstract

Fiscal Year 2018 was very productive and resulted in multiple high-impact publications. I am very proud of my excellent team. After the departure of Dr. Takizawa, who became Assistant Professor at Tokyo University and of Dr. Sugita, who is now a leading staff scientist the cryo-EM center at Institute for protein Research at Osaka University, our team has been reconstituted by addition of Dr. Matsunami (formerly at RIKEN) and Dr. Hyun (formerly at KBSI). The emphasis lies on structural virology and microbiology and more broadly on nucleic acid-protein complexes.

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
- Eui Kyung Yu, BSc, Technician (externally funded)
- Rika Yoshizawa, Research Administrator

2. Collaborations

2.1 Structure of components of the bacterial and archaeal propulsion system

- Type of collaboration: Joint research
- Researchers:
 - Professor Shin-Ichi Aizawa, Prefectural University of Hiroshima

2.2 Structure of Ebolavirus

- Type of collaboration: Joint research
- Researchers:
 - Professor Yoshihiro Kawaoka, University of Tokyo Institute of Medical Science
 - Professor Takeshi Noda, Kyoto University

2.2 Structure of Nucleosome complexes

- Type of collaboration: Joint research
- Researchers:
 - Professor Hitoshi Kurumizaka, University of Tokyo

2.2 Structure of Seneca Valley Virus with Anthrax Toxin Receptor

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

3. Activities and Findings

3.1 Ebolavirus Nucleoprotein-RNA Complex

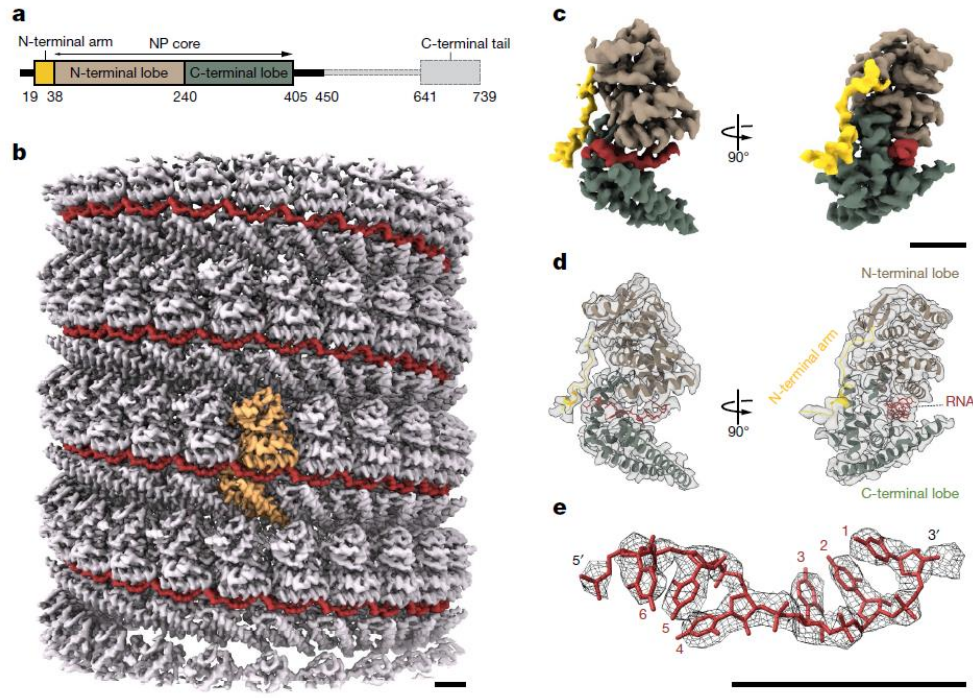


Figure 1: Dr. Sugita in Wolf Unit determined this structure by single particle cryo-EM at 3.6Å resolution. The protein was expressed and purified at OIST. Prof. Kawaoka and Prof. Noda provided the construct. The structure is the first description of this complex at near-atomic resolution and provided novel insights into coordination of RNA and viral assembly. Our joint work was published in the journal *Nature*.

3.2 Anthrax Toxin Receptor 1 in complex with the Oncolytic Seneca Valley Virus

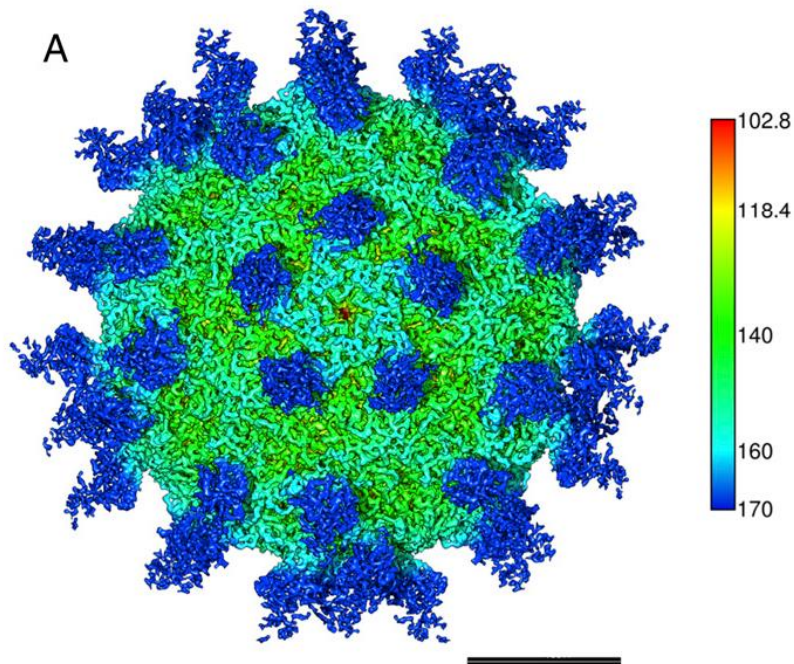


Figure 1: The results of this fruitful collaboration with Prof. Mihnea Bostina at University of Otago, NZ, were published in the journal *PNAS*. The cryo-EM data were collected at OIST. The work is important as it represents the first report of the detailed structure of an oncolytic virus with its receptor (ANTXR1, also referred to as Tumor Endothelial Marker 8), which is a biomarker for 60% of human cancers. SVV is currently in clinical trials for oncovirotherapy.

3.3 Metal-binding Site in *Methanococcus* Archaeellum

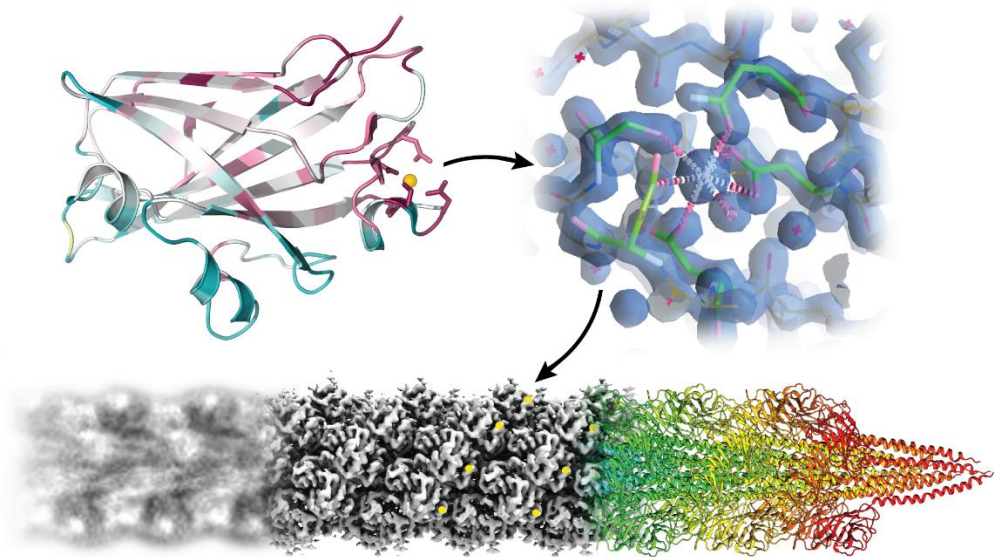


Figure 1: Former Wolf Unit staff scientist Dr. Meshcheryakov, who determined the first reported crystal structure of *Methanococcus* flagellin and also worked on the cryo-EM was first author in this collaboration with Prof. Aizawa (Univ. of Hiroshima), who assisted with helical diffraction analysis of the cryo-EM data. It is the first report of a metal binding site in archaea. We used our unique capability of TEM-EELS on a direct electron detector to validate the identity of the metal ion. Our results were published in the journal *EMBO Reports*.

3.4 Mechanism of CRISPR-Cas-induced Adaptive Immunity by Targeted Phage Evolution

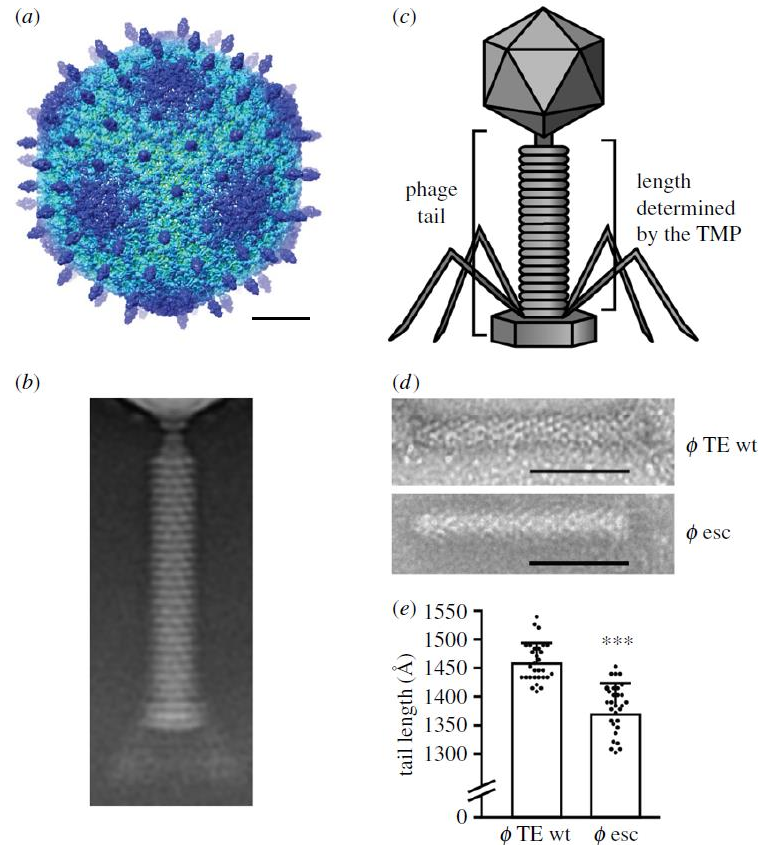


Figure 1: We contributed cryo-EM to this extensive study by Profs. Fineran and Bostina (both University of Otago, NZ). The tail length of this bacteriophage PhiTE is under control of ‘spacers’ introduced by the phage into the bacterial CRISPR array. The study shows that the phages can adopt to the bacterial CRISPR defense system by escaping from multiple spacers.

4. Publications

4.1 Journals

1. Sugita, Y., Matsunami, H., Kawaoka, Y., Noda T., **Wolf, M.*** (2018). Cryo-EM structure of the Ebola virus nucleoprotein-RNA complex at 3.6 Å resolution. *Nature*, doi: 10.1038/s41586-018-0630-0.
2. Jayawardena, N., Burga, L., Easingwood, R., Takizawa, Y., **Wolf, M.*** and Bostina, M. (2018). Structural basis for Anthrax Toxin Receptor 1 recognition by Seneca Valley Virus. *Proc. Natl. Acad. Sci.*, doi:10.1073/pnas.1810664115
3. Meshcheryakov, V., Shibata, S., Schreiber, M.T., Villar-Briones, A., Jarrell, K.F., Aizawa, S., **Wolf, M.** (2019). High-resolution archaeellum structure reveals a conserved metal-binding site. *EMBO Reports*, doi: 10.15252/embr.2018046340
4. Watson, B.N.J, Easingwood, R.A., Tong, B., **Wolf, M.**, Salmond, G.P.C., Staals, R.H.J., Bostina, M., Fineran, P.C. (2018). Different genetic and morphological outcomes for phages targeted by single or multiple CRISPR-Cas spacers. *Phil. Trans. B*, doi: 10.1098/rstb.2018.0090

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

1. 2018.09.05 Invited Plenary Speech, Grand Opening Ceremony ASCEM, Cryo-EM facility at Academia Sinica, Taiwan
2. 2018.03.21 Invited Keynote Lecture, International Symposium on Grids and Clouds (ISGC2018), Keynote speaker
3. 2018.03.22 NION Company, Seattle, USA, *A vision for low dose single particle cryo-STEM and spectroscopy of radiation-sensitive bio samples*
4. 2018.03.21 International Symposium on Grids and Clouds (ISGC2018), Keynote speaker, Academia Sinica, Taipei, Taiwan, *Grid computing and cryo-EM*
5. 2018.03.20 IBC Academia Sinica, Taipei, Taiwan, *The power and beauty of single particle cryo-EM*
6. Meshcheryakov VA, Shibata S, Tokoro Schreiber M, Villar-Briones A, Jarrell KF, Aizawa S-I, Wolf M (2018) Metal ion binding of the Methanococcus archaeellin required for filament integrity. In Biwako, Shiga, Japan: The 23rd Annual Flaglla Meeting
7. Sugita Y, Kawaoka Y, Noda T, Matsunami H, Wolf M (2018) Structure of Ebola virus nucleoprotein-RNA complex. In Itoman, Okinawa, Japan: 7th Negative Strand Virus-Japan Symposium
8. Takizawa Y (2018) Structure of the heterochromatin unit revealed by cryo-EM. In Stanford University, Stanford, CA, USA:
9. Takizawa Y, Machida S, Ishimaru M, Sekine S, Nakayama J-i, Kurumizaka H, Wolf M (2018) Cryo-EM Structure of Constitutive Heterochromatin Unit by Human HP1 In Newport, RI, USA: "Three-Dimensional Electron Microscopy" Gordon Research Conference
10. Takizawa Y, Machida S, Ishimaru M, Sekine S, Nakayama J-i, Kurumizaka H, Wolf M (2018) Cryo-EM Structure of Heterochromatin Unit by Human HP1 In Whistler Conference Centre, Whistler, British Columbia, Canada: Chromatin Architecture and Chromosome Organization (X5)

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Structural analysis of biological macromolecules using 3D cryo-electron microscopy

- Date: May 16, 2018
- Venue: OIST Campus Lab1
- Speaker: Dr. Jaekyung Hyun (Korea Basic Science Institute)

6.2 New insights into bacterial chemoreceptor arrays by electron cryotomography

- Date: Thursday, July 19, 2018
- Venue: OIST Campus Lab1
- Speaker: Prof. Ariane Briegel, Ultrastructural biology University Leiden

7. Other

Nothing to report.