

FY2017 Annual Report

Molecular Cryo-Electron Microscopy Unit
Assistant Professor Matthias Wolf



(From left to right) Dr. Satoshi Shibata, Makoto Schreiber, Lasse Sprankel (intern student), Dr. Melissa Matthews, Dr. Tae Gyun Kim, EuiKung Yu, Sofii Kosar (rotation student)

Abstract

This fiscal year was characterized by several break-through results due to fruitful collaborations with Kurumizaka Lab (Tokyo University), Kawaoka Lab (Tokyo University), Noda Lab (Kyoto University), Aizawa Lab (Prefectural University of Hiroshima) and Dr. Bostina (University of Otago). Many of the resulting structures were achieved then but appeared in print only in the following year. Dr. Matthews, a new post Doc, who will contribute her expertise in DNA-protein complexes, joined the lab at the of the period. Dr. Maigne went on to become the Director of the newly established Stewart Blusson Quantum Matter Institute at the University of British Columbia.

1. Staff

- Dr. Yoshimasa Takizawa, Staff Scientist
- Dr. Yukihiko Sugita, Postdoctoral Researcher
- Dr. Satoshi Shibata, Staff Scientist
- Dr. Tae Gyun Kim, 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 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

2.3 Structure of Nucleosome complexes

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

2.4 Structure of Icosahedral Viruses

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

3. Activities and Findings

3.1 Structural Basis of Heterochromatin Formation by Human HP1

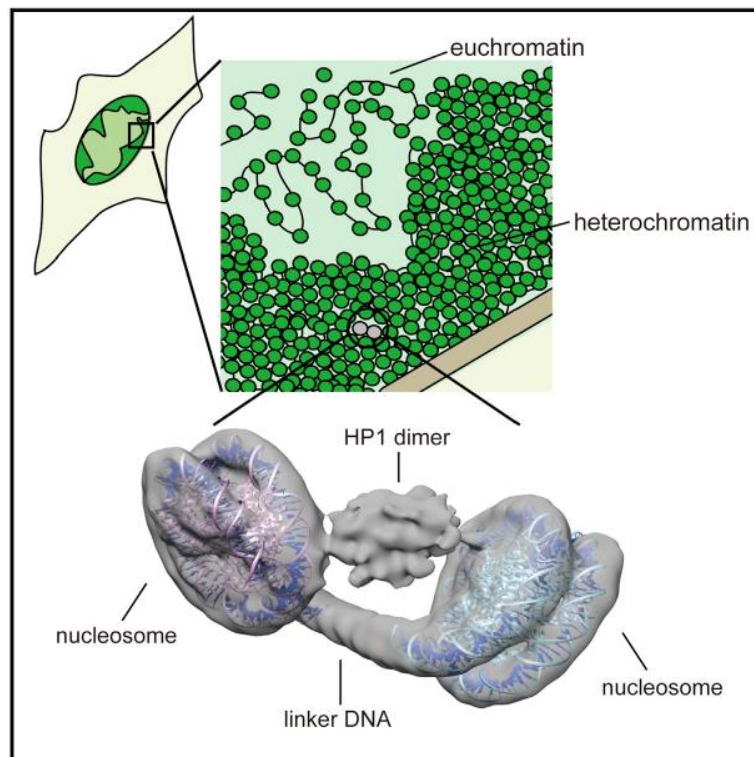


Figure 1: Dr. Takizawa in Wolf Unit determined the structure of the dinucleosome-HP1 complex by single particle cryo-EM at 1 nm resolution. We found that HP1 forms a symmetric dimer and bridges two H3K9me3 nucleosomes in the complex – a novel finding. The linker DNA between nucleosomes does not directly interact with HP1. This work was a collaboration with the group of Prof. Kurumizaka at Tokyo University and the results were published in *Molecular Cell*.

3.2 Nucleosome containing the ALB1 enhancer DNA sequence

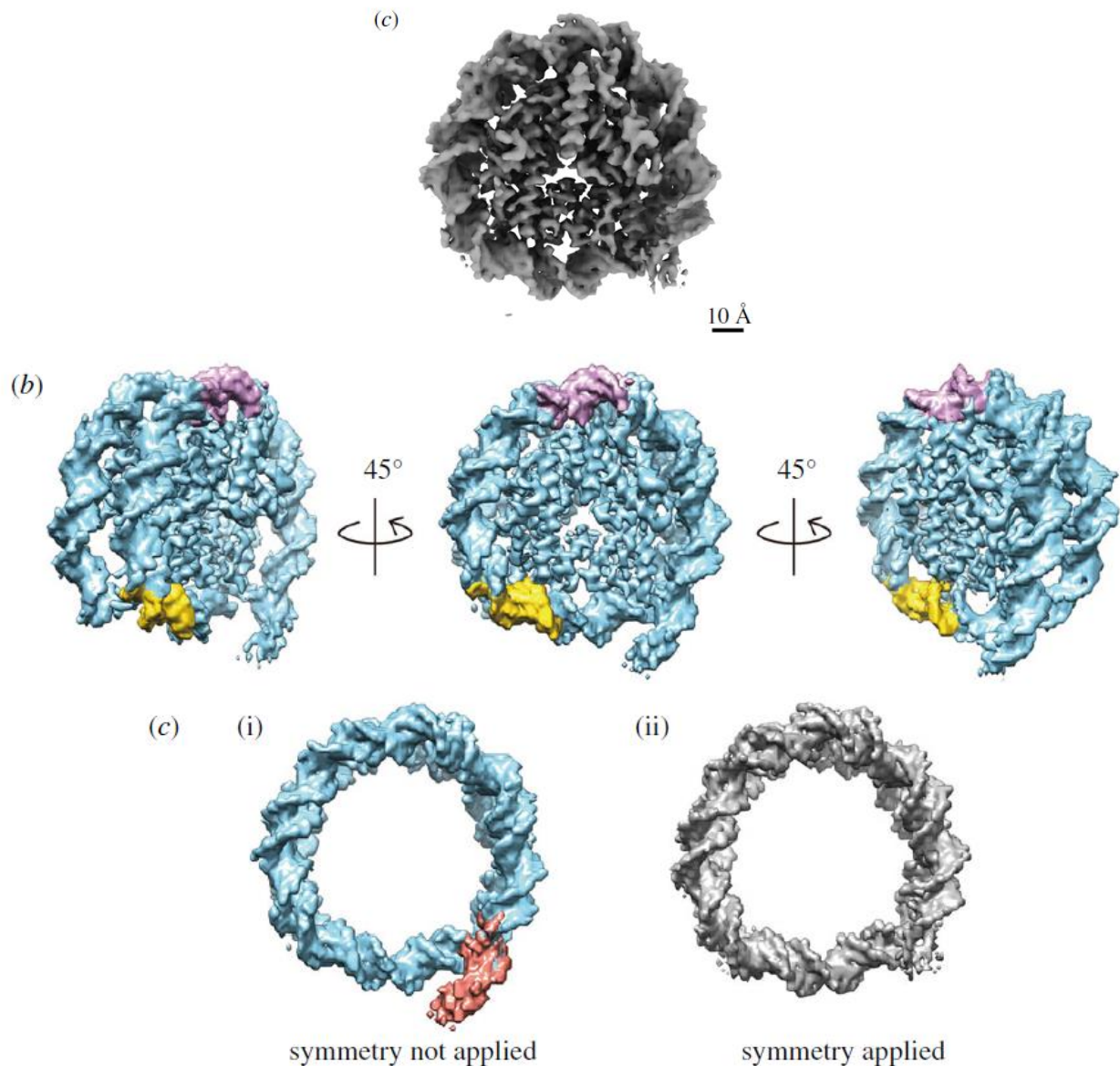


Figure 1: This collaboration with the Kurumizaka Lab, who supplied the modified nucleosomes resulted in the first structure of a nucleosome with a natural DNA sequence (all other crystallized structures contain a special high-affinity sequence). The ALB1 sequence facilitates binding of the pioneer transcription factor FoxA1. Our asymmetric reconstruction revealed the interaction sites of FoxA1 on the nucleosome and represented the highest resolution single particle cryo-EM structure

of this complex without the use of a phase plate. The results were published in the Royal Society Journal *Open Biology*.

3.3 Low-Dose Cryo-TEM-EELS with a Direct Electron Detector

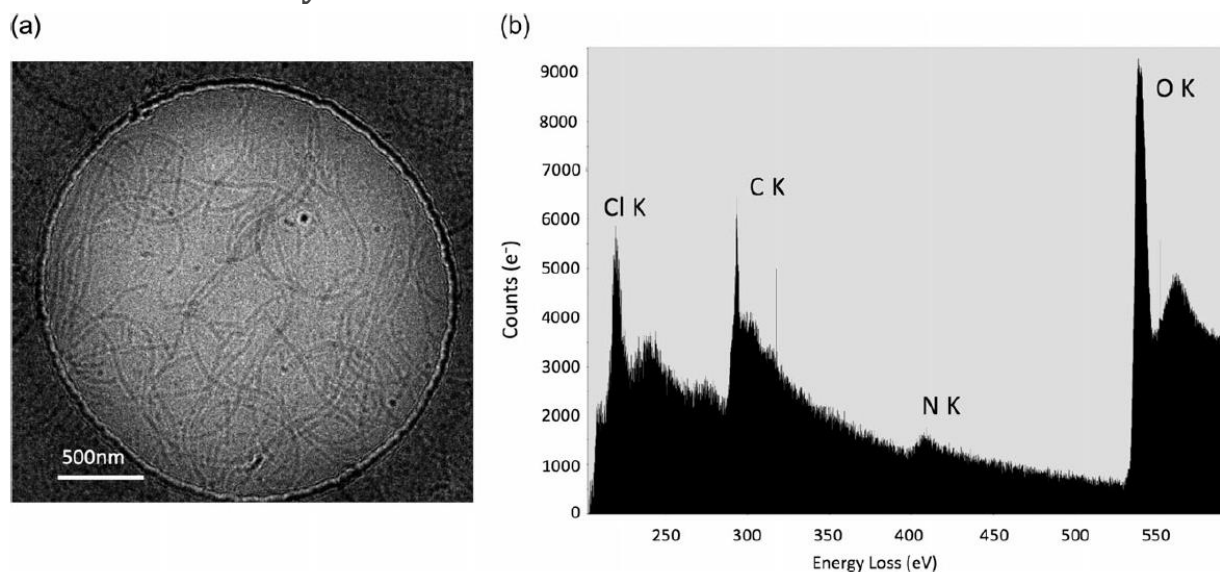


Figure 1: We acquired a secondary post-energy-filter-CCD camera to perform calibration of electron energy loss spectroscopy (EELS) before dropping the dose rate and switching to a direct electron counting detector (the Gatan K2). This enabled us to simultaneously image and measure the elemental spectrum of biological samples in ice at a total dose close to what is usually used in low-dose cryo-EM – a first in the field, which expands the application of EELS to biological cryo-EM. This capability is currently only available at OIST. The workflow was established by my staff scientist Dr. Alan Maigne and the study was published in the journal *Microscopy*.

4. Publications

4.1 Journals

1. Machida, S., Takizawa, Y., Ishimaru, M., Sugita, Y., Sekine, S., Nakayama, J. ichi, **Wolf, M.*** and Kurumizaka, H. (2018). Structural Basis of Heterochromatin Formation by Human HP1. *Molecular Cell* 69, 385–397.e8.
2. Maigné, A., and **Wolf, M.*** (2018). Low-dose electron energy-loss spectroscopy using electron counting direct detectors. *Microscopy (Oxford, UK)* 67, i86–i97.
3. Murata, K., and **Wolf, M.*** (2018). Cryo-electron microscopy for structural analysis of dynamic biological macromolecules. *Biochim. Biophys. Acta* 1862, 324–334.
4. Takizawa, Y., Tanaka, H., Machida, S., Koyama, M., Maehara, K., Ohkawa, Y., Wade, P.A., **Wolf, M.*** and Kurumizaka, H. (2018). Cryo-EM structure of the nucleosome containing the ALB1 enhancer DNA sequence. *Open Biology* 8, 170255.

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

1. 2018.03.21 International Symposium on Grids and Clouds (ISGC2018), Keynote speaker, Academia Sinica, Taipei, Taiwan, *Grid computing and cryo-EM*
2. 2018.03.20 IBC Academia Sinica, Taipei, Taiwan, *The power and beauty of single particle cryo-EM*
3. 2018.02.01 IBC Academia Sinica, Taipei, Taiwan, *Structural Basis of Heterochromatin Formation by Human HP1*
4. 2017.11.29 NIPS EM Workshop 2017 on High-Resolution Single Particle Analysis of Proteins by Cryo-electron Microscopy, Okazaki, Japan, *Structure of the Methanococcus Archaellum*
5. 2017.09.13 International Conference of the Korean Society for Molecular and Cellular Biology (KSMCB) 2017, COEX, Seoul, Korea, *Complete Structures of the Campylobacter Hook and Filament*
6. 2017.08.25 24th Congress and General Assembly of the international Union of Crystallography, Hyderabad, India, *Cryo-EM in the age of X-ray crystallography*
7. Maigne A, Schreiber MT, Wolf M (2017) Development of low dose cryo-EELS toward organic molecules structure analysis. In EDGE 2017: Enhanced Data Generated by Electrons, Okuma, Okinawa, Japan:
8. Meshcheryakov VA, Shibata S, Villar-Briones A, Jarrell KF, Aizawa S, Wolf M (2017) Structure of the Methanococcus Archaellum. In Okazaki, Japan: NIPS EM Workshop 2017
9. Nakayama K (2017) A Multi-Rail Structure in the Cell Envelope for the Bacteroidete Gliding Machinery. In Nagoya, Aichi, Japan: International Symposium on "Harmonized supramolecular motility machinery and its diversity
10. Sugita Y, Matsunami H, Kawaoka Y, Noda T, Wolf M (2017) Cryo-EM structure of Ebola viral nucleoprotein-RNA complex. In Okazaki, Japan: NIPS EM Workshop 2017
11. Takizawa Y, Machida S, Ishimaru M, Sekine S, Nakayama J-i, Kurumizaka H, Wolf M (2017) Cryo-EM structure of heterochromatin unit by human HP1. In Sendai International Center: The 17th Annual Meeting of the Protein Science Society of Japan
12. Takizawa Y, Machida S, Ishimaru M, Sekine S, Nakayama J-i, Kurumizaka H, Wolf M (2017) Molecular Architecture of heterochromatin formation by human heterochromatin protein 1. In New Orleans, LA, USA: 61st Biophysical Society Annual Meeting
13. Takizawa Y, Machida S, Ishimaru M, Sekine S, Nakayama J-i, Kurumizaka H, Wolf M (2017) Molecular Architecture of heterochromatin formation by human heterochromatin protein 1. In Vanderbilt University, Nashville, TN, USA:
14. Takizawa Y, Machida S, Ishimaru M, Sekine S, Nakayama J-i, Kurumizaka H, Wolf M (2017) Molecular Architecture of heterochromatin formation by human heterochromatin protein 1. In NIEHS, Durham, NC, USA:
15. Takizawa Y, Machida S, Ishimaru M, Sekine S, Sugita Y, Nakayama J-i, Kurumizaka H, Wolf M (2017) Cryo-EM structure of heterochromatin unit by HP1. In Okazaki, Japan: NIPS EM Workshop 2017
16. Wolf M (2017) Complete Structures of the Campylobacter Hook and Filament. In COEX, Seoul, Korea: International Conference of the Korean Society for Molecular and Cellular Biology (KSMCB) 2017
17. Wolf M (2017) Cryo-EM in the age of X-ray crystallography. In Hyderabad, India: 24th Congress and General Assembly of the international Union of Crystallography

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Image Contrast in Single Particle Cryo-EM

- Date: April 10, 2017
- Venue: OIST Campus Center Bldg.
- Speaker: Prof. Nikolaus Grigorieff (The Howard Hughes Medical Institute Brandeis University)

6.2 Structural analysis of murine norovirus RNA-dependent RNA polymerase complexed with VPg

- Date: June 14, 2017
- Venue: OIST Campus Center Bldg.
- Speaker: Dr. Ji-Hye Lee (Department of Biotechnology & Bioinformatics, Korea University)

6.3 Enzymatic mechanism of RecA-recombinase in homologous recombination: Role of RecA in homology recognition and the functions of RecA-DNA filaments.

- Date: January 29, 2018
- Venue: OIST Campus Lab1
- Speaker: Prof. Takehiko Shibata (Tokyo Metropolitan University)

7. Other

Nothing to report.