DATE：Day__18__Month__4__Year 2019

SUMMARY of
2018 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Structural and functional research on the survival-essential factors from bacterial pathogens for the development of novel antibiotics which induce suicide effect (phase II)
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	Affiliation	Seoul National University
	Present Title	Professor
Research Collaborator (Host PI)		**Atsushi Nakagawa**

Summary

We tried to collect all data over ~3.5 Å after calculating the appropriate distance, the measurement time, the wavelength, and choice of a start degree for efficient data collection but had many difficulties to get good results. For low-diffraction cases or bad quality of data, we tried flash-annealing or dehydration method to improve resolution or quality. Actually, a kind of quick solutions to improve quality of data show a little improvement. Therefore, we recalculated the data using various tools, reinterpreted them, and extracted meaningful information from low-resolution data.

For a set being capable of molecular replacement, we tried to solve a structure using known a structural template by Phenix or CCP4i program suites. For sets which have no structural homologs based upon sequences, we tried single- (or multi-) wavelength anomalous dispersion method to solve protein structures. For efficient performance, we prepared and mounted SeMet-derivatized crystals at first and used heavy atoms-derivatized crystals which were treated with platinum or mercury compounds etc. In addition, we tried to solve phasing problems using non-covalently bound atoms including sodium bromide. For efficient management of beam time, we screened optimum conditions of concentration and soaking time of a specific heavy atom in prior to works in the assigned beam time. For a weak phasing set, we tried to calculate data using multiple isomorphous replacement method.

Data were processed using XDS. Structural determination was tried using various programs including CCP4, CNS, and Phenix with either manual or automated method.

Experimental Results

The Whi protein from *M. tuberculosis*

- Not diffracted. Crystals were improved but showed low diffraction.

SAV2069-DNA complex

- Diffracted poorly to ~10 A.

- Tried to improve crystal samples by addition of various additives and detergents.
- Low improvement were observed.
- Quality improvement is still in progress.

SP1740-1741 complex

- Native data sets were collected at 3 - 4 A (five sets were collected and processed.)
- SeMet data sets were collected at 3 - 4 A.
- Obtaining the other crystallization conditions is in progress.
- 1 set of SeMet data were collected to ~3.6: phasing problem was not solved.
- Improvement of crystals are in schedule.

Rv0239-0240 complex

- Native crystals of the protein complex were diffracted to ~ 3.0 A.
- Three data sets were collected.
- Improvement of the crystal samples is in progress.
- The other 10 conditions were tested.
- Native crystals were diffracted to ~ 4.0 Å.
- Crystals from the other conditions were diffracted to 4.0 ~ 8.0 Å.
- Optimization is in progress.

Rv1494-Rv1495 complex with DNA

- Three DNA constructs were soaked with the protein complex.
- No electron density maps of DNA were observed.
- Truncated constructs of DNA were not better than previous samples.

Rv3183

- Sodium bromide soaking was performed (100 and 300 mM, 10/30/60 mins)
- Three data sets were collected.
- Phasing was not successful.
- EMTS were soaked in various conditions: phasing was not successful.

***Deadline: May 17, 2019**
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