This report should be submitted within 2 weeks after you return to Japan.

✓

（Abroad・Domestic）Internship report form（Student）　09/16/2018 (Year/Month/Day)

Name	Park Jong-Hoon
Laboratory	Bioresources
Year (Grade)	3
Internship institution	National Institute of Infectious Disease, Tokyo, Japan
Internship period	Internship period: 08/05/2018 - 09/16/2018 (Departure Date from Sapporo: 08/05/2018, Arrival Date in Sapporo: 09/16/2018)
Purpose	**Internship** • To develop professional and personal network with experts of infectious disease and understand the working nature of the National Institute of Infectious Disease (NIID) in Tokyo. • To explore job opportunities in NIID or other similar institutes in Japan. **Collaborative research activity** • Reveal the molecular basis of strong WQ-3810 inhibitory activity against *Mycobacterium leprae* DNA gyrase that has quinolone-resistant amino acid substitutions in its GyrB by using protein crystallization technique.

- The reason why you chose this institute

The NIID is a research institute under the Ministry of Health, Labor and Welfare for conducing i) fundamental and applied research on infectious disease and ii) national test for lot release and development of antibiotics and vaccines (https://www.niid.go.jp/niid/en/). Therefore, it is great chance to see the process how the infectious disease issues are monitored and how new antibiotics are developed or tested. Fortunately, I could obtain information about NIID through my supervisors and our research collaborator Dr. Hyun Kim.

I had an opportunity to exchange ideas about research topics as well as research attitude and working culture of NIID with Dr. Kim. he belongs to the department of bacteriology II. Additionally, the department of bacteriology II in NIID has expertise on protein crystallization, a research theme that I would like to learn and apply to my PhD research.

- Result of the activity （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

The main objective of these internship and collaborative research activities was to develop human network with experts of infectious diseases in Japan and learn about the protein crystallization skill. These two goals were successfully achieved during this period. Furthermore, I had a chance to learn about in-silico study and apply to

my present study.

Development of human network

The department bacteriology II was a special place for me to develop a precious human network. All of members of the lab were not only specialist of bacteriology but also had broad thinking as they accepted opinions from others who belonged to the different fields. Dr. Mori, the head of 4th team of the department of bacteriology II, is a specialist for in-silico study. He was a great discussion partner when I was planning to apply the in-silico study to my theory of present study. I would like to thank him, because of my discussion with him, I could make a new study plan and obtain results that I wanted to get from in-silico study. It was a good chance to enhance of my study capacity and it will be a useful tool for my future career. Also, Dr. Limbara, belonged to same division, she is a specialist for bacterial infectious disease and pharmacology. She has many kinds of methodological insight for each wet lab experiments. Even though she didn't give a detail experimental opinion, she gave me other important ones. She taught me a manner what I must keep in mind when I am in Japanese research institute and trained me for the insight what I have to poses when I check my own methodology. Those skill and information were significantly helpful for my internship and collaborative research activity in NIID as a researcher and as a member of the team. Dr. Kim was a great coach. He provided essential information for foreign researcher in Japan.. Those information made me serious for my future career in Japan. Some of information are like what kind of visa will be allowed for me or how I can get a house without any support from school or what would be our position in the Japanese society. Those three lab members were key persons of the human network that I made in NIID. I strongly expect that this developed network will be highly functional when I try to get a future career in here Japan.

Experimental result – Protein crystallization with DNA gyrase subunit B of *M. leprae*.

I focused on the *M. leprae* because it is a causative agent of leprosy, a chronic infectious disease which is also seriously concerned by WHO. However, it is difficult to study this bacteria because it cannot be cultured in artificial media. Drug resistant bacterial infectious disease issues including leprosy are critically concerned all over the world. Even though, drug resistant leprosy is already reported, the counter measure is still unclear. Unfortunately, Ofloxacin (OFX), the member of fluoroquinolone, a reliable bactericidal agent for treatment of leprosy is also needed for treatment of OFX-resistant leprosy. To clear understanding for the target enzyme of OFX and interaction with each other, protein crystallization is necessary. The 4th team of the department of bacteriology, NIID has an abundant experience on protein crystallization. So I learned about the protein crystallization skill from them and performed the OFX target protein crystallization. *M. leprae* DNA gyrase is the target protein of OFX and consist of 2 subunit A and 2 subunit B, GyrA and GyrB as a heterotetrameric molecular structure. Especially, GyrB is interesting subunit because that is deeply related with the quinolone resistance although the specific quinolone resistant amino acid substitutions are still not reported in leprosy patients. It might be GyrB amino acid sequence around of quinolone binding site is essential for the DNA gyrase enzymatic activity. I think that somehow

WQ-3810, a novel structural quinolone has some special relationship with GyrB. For clear understanding about it, we targeted GyrB as a candidate for the protein crystallization. In the period of internship and collaborative research activity, I found the specific buffer condition for the GyrB crystallization among the 1,344 different buffer conditions. Still, in-depth investigation is needed to take an X-ray and get an complete crystal structure of GyrB. For that, I had some discussion with Dr. Kim. According to our discussion, soon the 4th team of the department of bacteriology II, NIID will start next step of GyrB crystallization. (Figure 1)

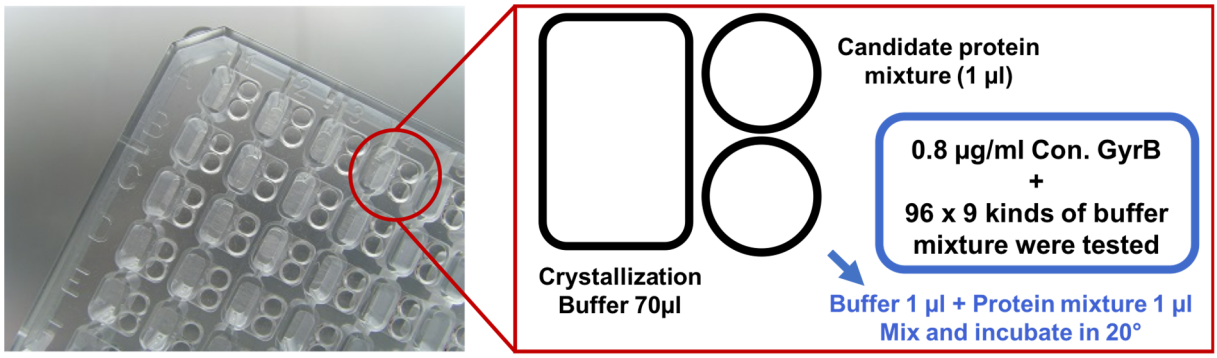

Fig 1. Summary of protein crystallization buffer condition screening method principle. The screening of buffer condition for protein crystallization is performed with 96 well crystallization plate. Each well consist of one large place for buffer and two small places for proteins. In place of protein, 1 µl of purified protein and 1 µl of buffer will be mixed. Crystallized protein will be constructed in side of the mixture and can be detected with microscope.

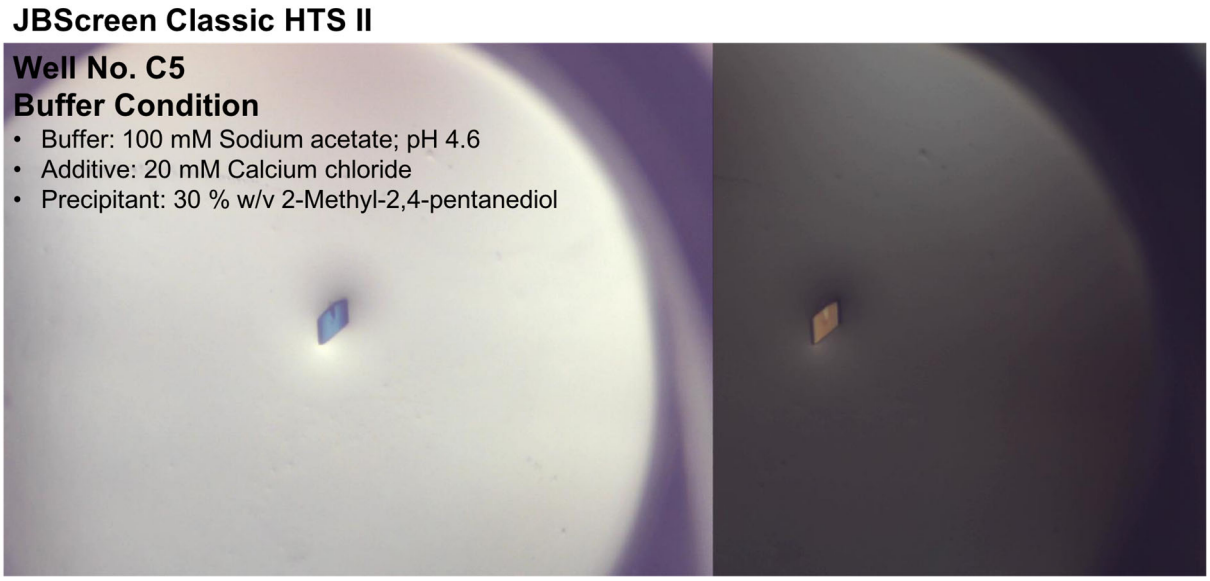

Fig 2. Final buffer condition candidate for *M. leprae* DNA gyrase subunit B crystallization. Crystallizaed GyrB of *M. lerpae* DNA gyrase was confirmed in the specific buffer condition; 100 mM sodium acetate (pH 4.6), 20 nM calcium chloride and 30% w/v 2-methyl-2,4-pentanediol.

In-silico study result – Docking simulation with DNA gyrase and WQ-3810

Furthermore, I tried to apply the in-silico study to my theory. In my previous study, the newly developed compound named WQ-3810 showed strong inhibitory activity against amino acid substituted DNA gyrase on GyrA, while ofloxacin and moxifloxacin showed weaker inhibitory activity than WQ-3810. I speculated the result might be because of additional interaction between WQ-3810 and GyrB. To understand what kind of interaction might have occurred, In-silico study can be one of the reliable methods to get an answer.

Reliability of In-silico result is deeply related with the functionality of each software. Molecular Operating Environment – MOE software is the most reliable software for in-silico study. Fortunately, NIID poses a license for MOE software and I could get a chance to run the software. Docking simulation has benefit to check the possibility t how specific ligand dock to the receptor. In this case, I performed the docking simulation using DNA gyrase complex as a receptor and WQ-3810 as a ligand. Interestingly, WQ-3810 showed additional interaction with GyrB, however moxifloxacin and ofloxacin did not show such interactions. This information could be data to support my previous speculation that WQ-3810 has additional interaction with GyrB which has a benefit for GyrA quinolone resistant amino acid substitutions. (Figure 3)

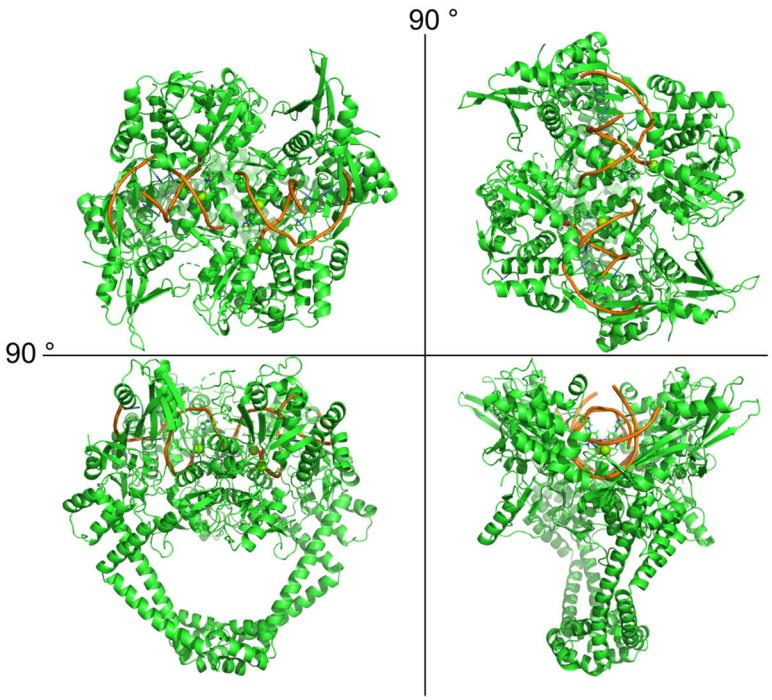

Fig 3. Prepared receptor (PDB: 5BTA Mycobacterium tuberculosis DNA gyrase molecular structure). Receptor was prepared from Protein Data Bank (PDB). The code number of PDB is 5BTA which is 3D molecular structure of *Mycobacterium tuberculosis* DNA gyrase. 5BTA is contained not only DNA gyrase structure but also DNA, ligand (Moxifloxacin; MOX) and solution state.

(A) WQ-3810 docking result with 5BTA_WT

Final candidate labeled with red box

	mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2	PLIF_pronum	PLIF_raw	FP:PLIF	PLIF_ligidx
1	11676981	1	1	-7.8790	3.1378	-810.4669	-11.0829	-15.7565	343.6060	-7.8790	1	[[6,4,6,6,25,26	1 2 3 4 5	[6,[4,6,6,25,26
2	11676981	1	1	-7.4960	2.6211	-798.3684	-120.5241	-15.3994	255.6934	-7.4960	1	[[8,8,26,10,10,	9 10 11	[26,8,8]
3	11676981	1	1	-7.1960	2.3234	-811.3497	-14.6662	-22.4808	-613.1914	-7.1960	1	[[4,5,5,11,4,6,	12 13 14 1	[11,11,[6,6,26]
4	11676981	1	1	-6.9457	2.2606	-800.2889	-43.8936	-17.3522	-514.1057	-6.9457	1	[[4,5,5,12,12,4	14 15 16 2	[[6,6,26],[6,6]
5	11676981	1	1	-6.3406	1.3665	-814.4906	-43.4848	-26.9970	-548.2932	-6.3406	1	[[4,5,5,11,4,6,	29 30 31 3	[[6,26],26,[6,6

(B) WQ-3810 docking result with 5BTA_Asp464Asn

	mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2	PLIF_pronum	PLIF_raw	FP:PLIF	PLIF_ligidx
1	11676981	1	1	-6.8084	1.6415	-800.0395	-8.0604	-16.0048	454.1836	-6.8084	1	[[11,4,6,4,4,4,	1 2 3 4 5	[6,6,[4,6],4,4,
2	11676981	1	1	-6.3396	4.8722	-786.0969	-120.7324	-15.6906	2.7752	-6.3396	1	[[11,11,11,26],	10 11 12 1	[26,26,11,11,11
3	11676981	1	1	-5.1241	1.7813	-802.1046	-101.3319	-19.5823	-396.3417	-5.1241	1	[[4,5,8,8,4,6,6	15 16 17 1	[[6,6,26],[6,26
4	11676981	1	1	-5.0887	2.3319	-780.9887	-69.0286	-15.4743	208.0145	-5.0887	1	[[4,6,25,26,30]	10 11	[[4,6,25,26,30]
5	11676981	1	1	-4.9273	1.2891	-809.6125	-19.1413	-16.2163	-628.3853	-4.9273	1	[[4,5,8,8,4,6,6	15 16 17 1	[[6,26],[6,26],

(C) WQ-3810 docking result with 5BTA_Asp464Ala

	mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2	PLIF_pronum	PLIF_raw	FP:PLIF	PLIF_ligidx
1	11676981	1	1	-7.1744	2.3824	-818.4634	1.7457	-20.6370	-675.4848	-7.1744	1	[[4,5,5,8,8,11,	1 2 3 4 5	[[4,25,30],4,4,
2	11676981	1	1	-7.0056	1.0890	-780.1741	-31.3579	-15.3020	246.1881	-7.0056	1	[[8,8,11,6,6,4,	20 21 22 2	[[6,6],6,11,11,
3	11676981	1	1	-5.1899	1.5671	-798.0082	-82.7867	-14.9599	223.5580	-5.1899	1	[[8,11,11,21,6,	31 32 33 3	[6,6,6,6,21,[4,
4	11676981	1	1	-5.0916	1.4528	-807.4323	-24.4522	-15.9515	-658.6530	-5.0916	1	[[4,5,11,11,31,	42 43 44 4	[[6,26],[6,26],
5	11676981	1	1	-5.0380	1.5567	-794.8711	-58.2701	-15.6665	66.7520	-5.0380	1	[[8,8,12,12,6,4	55 20 21 2	[6,6,6,[4,6,6,2

Fig 4. Docking simulation results. The simulation was performed using Molecular Operating Environment (MOE) software, the most reliable docking simulator. Those results are provided as a S-score indicating how much amount of free energy is needed when those ligands are docked to receptor. (A) WQ-3810 showed most lower S-score against wild type (WT) DNA gyrase while (B) the compound showed most higher S-score against Asp464Asn. (C) Asp464Asn was applied as a control.

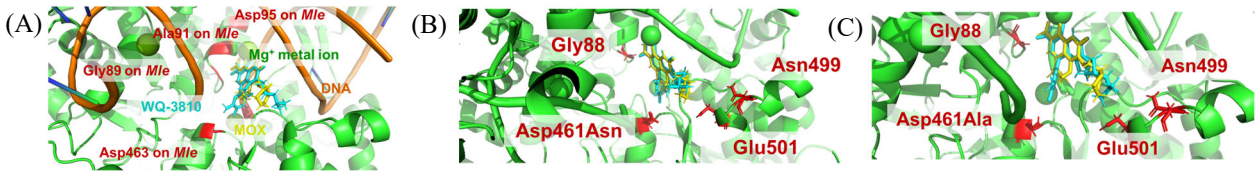

Fig 5. Detail coordinates of each ligand in the binding site of receptor. Coordinate information of ligand was given from each docking simulation result. (A) Final candidate of docking simulation from ligand; WQ-3810, receptor; WT of 5BTA. (B) ligand; WQ-3810, receptor; Asp464Asn. (C) ligand; WQ-3810, receptor; Asn464Ala.

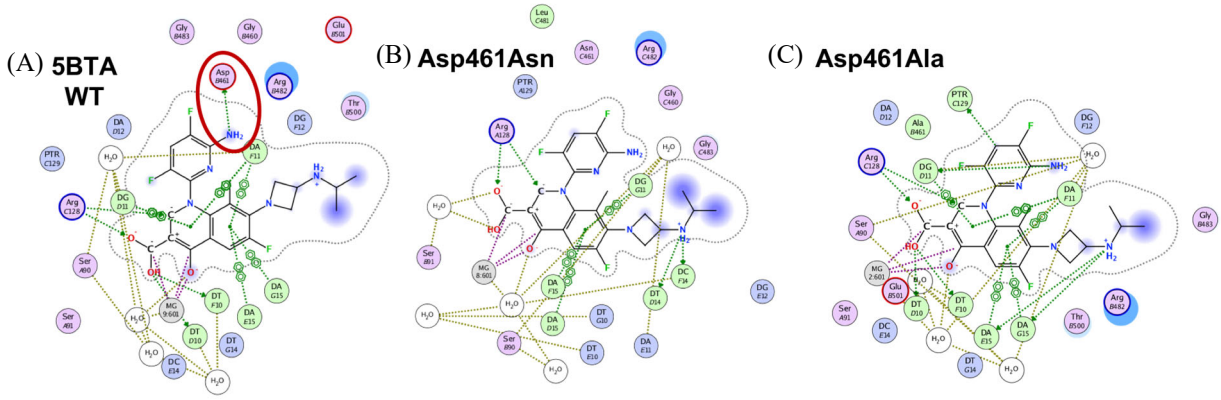

Fig 6. Ligand interaction simulation results of WQ-3810. Those interactions between each docked ligand and receptor was simulated using MOE software respectively. (A) Only WT and WQ-3810 showed specific interaction between novel R1 residue of WQ-3810 molecular structure and Asp464 on GyrB of 5BTA. (B) and (C) has no similar interaction between R1 residue and 464th amino acid on GyrB.

- What do you think the positive impact of the activity will have on your further career path?

It is a great chance for a PhD student to have working experience in a government research institute. The working of government research institute is different from universities as they have a large scale of database and are also responsible for making management decisions. During this internship, I found opportunities to work and make a network with researchers who are working on research field that I am interested on by free discussion with the team members and research seminars. Also, I had conversation on academics or personal topics which might help to develop networks. Those conversation and mutual research helped me to think about my student life and future career. It also allowed me to think why I want to be a scientist and work at research institutes. I would like to be in contact with my developed networks to share knowledge and information in my future work. Additionally, I will continue to perform collaborative research work on protein crystallization and this work will be directly used to advance my PhD research work.

- Advice for your junior fellows

I would like to advice to junior fellows with following contents. At first, select the potential or interested research areas and institute that will help to determine the future career path as a researcher after graduation. The most important thing to remember is to sketch the long-term career goal before starting any crucial tasks in life. Secondly, start the internship preparation at least three months early from the tentative date of visit to respective the country or institute. Before travelling please check the required documents needed for respective institute or country and arrange them properly to avoid trouble. At last, try your best, be hardworking, enjoy the trip and learn productive styles and methods that can be easily transferred to your laboratory.

Approval of supervisor	Institution・Official title・Name Division of Bioresources Research Center for Zoonosis Control Hokkaido University Professor Yasuhiko Suzuki

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氏名	
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学年	
活動先名	機関名、国名
期間 ①　（出発日―帰札日） ②　(インターンシップ実施開始日―終了日)	①　201X 年 XX 月 XX 日-XX 月 XX 日 ②　201X 年 XX 月 XX 日-XX 月 XX 日

・活動目的及びインターンシップ先を選択した理由

・活動内容・成果（2,000 字程度、活動内容が判る様な写真や図表を加えて下さい）

・今後のキャリアパスを考える上でどのようにプラスになったか。

・後輩へのアドバイス

指導教員確認欄	指導教員所属・職・氏名

※１　電子媒体を国際連携推進室・リーディング大学院担当に提出して下さい。
※２　インターンシップ先の担当者が活動内容を証明した文書（署名入り）を提出して下さい。
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