別紙様式３

学業及び研究等の進捗状況等報告書
Report of Research Progress and Future Research Plan

※ 本報告書は「4.指導教員からのコメント」を除き、北海道大学私費留学生特待プログラム」の HP に掲載します。（https://www.oia.hokudai.ac.jp/cier/own-scholarship/）
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氏名 Name	ALI MOHAMMAD	学　年 Year	D3
所属学院等名 Graduate School	Graduate School of Chemical Sciences and Engineering		
所 属 専 攻 名 Major	Chemical Sciences and Engineering		
所属研究室等名 Laboratory	Molecule and Life Nonlinear Sciences Laboratory		
指導教員職・氏名 Supervisor	Professor Tamiki Komatsuzaki		

１．研究テーマ名 Research theme

Enumeration Approach to Atom-to-Atom Mapping Accelerated by Ising Computing

２．研究等の進捗状況等 Research progress, etc.

研究の概要、独創性、状況等を含めて具体的に記入のこと。
※研究成果の発表・公表実績がある場合については学会名、掲載紙等の情報を含め詳細を記載すること
In detail, including the outline, originality and so on.
*Please state the name of academic conferences, journals or transactions if you have presented your research or your research was published.

Atom-to-atom mapping (AAM) is a procedure that establishes a one-to-one correspondence between the atoms in reactants and products of a chemical reaction. AAM provides a comprehensive way to track the transformation of chemical entities, including the identification of bonds broken and formed. Such information is crucial for various applications, including retrosynthesis planning, reaction classification, reaction substructure and similarity searching, as well as elucidating mechanisms of enzymatic reactions or identifying metabolic pathways. Furthermore, AAM facilitates the automatic extraction of reaction rules from literature precedents, which is a key component of chemical AI development. Consequently, AAM plays a crucial role in advancing diverse fields including drug discovery, materials science, and the broader realm of chemical synthesis.
Despite the availability of numerous AAM algorithms and tools accurate automatic AAM for complex or unknown reactions remains challenging. In existing approaches, AAM is typically formulated as an NP-hard combinatorial optimization problem, such as finding maximum common subgraph. Consequently, the computation time required to identify exactly optimal mapping(s) increases exponentially with the number of atoms involved in a chemical reaction. Due to the NP-hard nature, existing AAM frameworks often rely on heuristics such as incorporating known chemical rules and machine learning techniques. However, the criteria for selecting the reaction rule sets are ad hoc, and identifying the correct reaction rules for various reaction cases is challenging. Furthermore, the accuracy of the rule-based automatic AAM for unknown reactions may be low due to the lack of known reaction rules. Machine learning-based approaches also strongly depend on the reaction patterns contained in training data. Therefore, accurate automatic AAM demands a universal algorithm that relies on minimal chemical knowledge, without the need for known reaction rules or training data, particularly for unknown reactions.

Moreover, chemical reactions may have multiple potential AAM solution candidates. In such ambiguous cases, the chemically correct mapping that reflects the actual reaction mechanism is typically determined through experiments, such as isotope labeling. Therefore, an exhaustive enumeration of potential candidate mappings is an effective approach to identifying all probable reaction patterns in unknown reactions, after which experiments can be designed to determine the correct one among them. It is important to note that such multiple candidate mappings frequently include chemically ``equivalent'' transformations, which arise due to molecular symmetries in the reaction. Thus, to effectively identify all distinct reaction patterns represented by ``nonequivalent'' mappings, a symmetry reduction method is required.

We propose an AAM algorithm that enumerates all plausible mappings without reaction rules. We formulate the AAM problem as maximum common edge subgraph (MCES) problem, which corresponds to finding atom label mapping(s) that minimize the number of bond cleavages and formations. The MCES problem is then reduced to the maximum clique problem, i.e., finding the largest subgraph(s) where all nodes are fully connected to each other. To solve the maximum clique problem, our proposed framework employs an enumeration algorithm utilizing Ising computing. Ising computing is a novel paradigm that has gained significant attention as an efficient approach for solving hard combinatorial optimization problems across various fields, including chemistry. Finally, candidate mappings are constructed from the MCESs computed using Ising computing and are then clustered into distinct reaction patterns through a symmetry reduction method.

We applied our proposed AAM algorithm to a benchmark dataset. We found that the enumeration algorithm using Ising-computing outperforms a conventional exact algorithm for the maximum clique problem in terms of computation time (See Fig 1). Additionally, our algorithm successfully found the correct mappings in all reactions of the benchmark dataset.

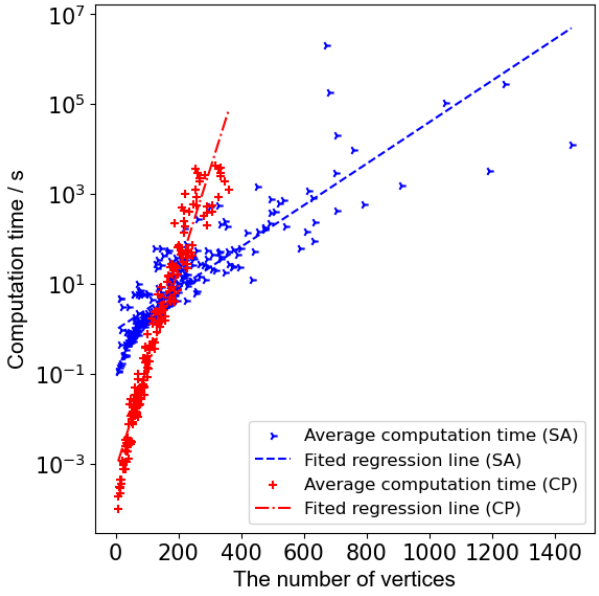

Fig1: Computation time for SA-based maximum cliques enumeration algorithm compared with the CP exact algorithm

Academic conferences:

[1] Mohammad Ali, Yuta Mizuno, Tamiki Komatsuzaki, Accelerating Atom Mapping with an Ising Machine, The 2023 RIES–CEFMS (Research Institute for Electronic Science–Center for Emergent Functional Matter Science) Joint International Symposium (12.7-12.8 ,2023), Rusutsu Resort Hotel and Convention Center, Rusutsu, Hokkaido 12, 7 (Poster).

[2] Mohammad Ali, Yuta Mizuno, Seiji Akiyama, Yuuya Nagata, Tamiki Komatsuzaki, Accelerating Atom Mapping with Ising Machines, The 24th RIES–Hokudai International Symposium (12.6-12.7,2023), Akira Suzuki Hall (Frontier Research in Applied Sciences Building), 12, 6 (Poster).

[3] Mohammad Ali, Yuta Mizuno, Tamiki Komatsuzaki, Automatic parameter tuning of Ising machines: An application to reaction pathway analysis, 5th ICReDD International Symposium (1.10-1.10 ,2023), Online 1, 10 (Poster).

３．今後の研究計画等 Future research plan

現在までの進捗状況等を踏まえ、今後の研究発表等を含めて具体的に記入のこと。
In detail, based on current progress, including a future research presentation plan.

This year, my goal is to publish my research in a scientific journal. I am currently working on my thesis and will soon begin preparing for both my pre-defense and final defense presentations.

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氏名 Name	Pondpan Suwanthada	学　年 Year	D4
所属学院等名 Graduate School	Graduate School of Infectious Diseases		
所 属 専 攻 名 Major	-		
所属研究室等名 Laboratory	Division of Bioresources		
指導教員職・氏名 Supervisor	Yasuhiko Suzuki		

１．研究テーマ名 Research theme

The study on quinolones-resistant bacteria and novel drugs for them

２．研究等の進捗状況等 Research progress, etc.

研究の概要、独創性、状況等を含めて具体的に記入のこと。
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In detail, including the outline, originality and so on.
*Please state the name of academic conferences, journals or transactions if you have presented your research or your research was published.

Originality

Antimicrobial resistance (AMR) is a complex and challeging problem globally due to increasing usage of antimicrobials and its repid spreading. The estimations suggested that **AMR could cause over 10 million deaths annually by 2050, making it the leading cause of death**.[1] Especially AMR in foodborne pathogens, they has become a major concern for food production system. Although AMR is not the current pandemic's primary focus, it could have adverse impact on serious illness and death on a broader level. Therefore, antimicrobial-resistant pathogens are **global health burdens to the achievement of the Sustainable Development Goals (SDGs)**, emphasizing the urgent need for action by the global community.

The field of AMR research is evolving rapidly, and several key areas require focus. One primary focus is the surveillance of antimicrobial-resistant pathogens, which is essential to comprehend the current situation and identifying emerging trends. Another vital research area involves **understanding the mechanisms behind the development and spread of AMR**. This involves studying the genetic and molecular mechanisms that enable microorganisms to become resistant to drugs. By comprehending, these mechanisms, researchers can develop new strategies to prevent and treat AMR. In addition, there is a significant demand for the development of novel antimicrobial drugs and therapies. The understanding of the mechanisms underlying AMR enables researchers to design drugs and therapies that are effective against resistant pathogens. Therefore, I propose a study to elucidate the resistant mechanism of *Salmonella* spp., a significant cause of foodborne illness, against quinolones, the drugs of choice for the treatment of salmonellosis.

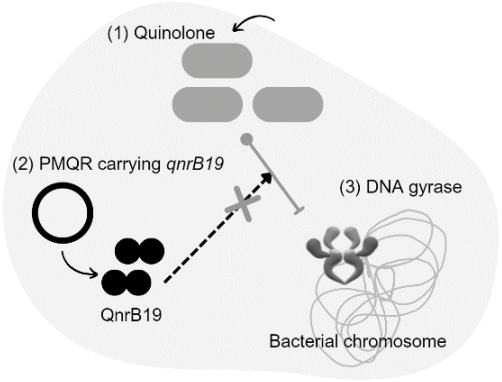

Figure 1 (1) Quinolone entered and accumulated in bacterial cell (2) PMQR carrying *qnrB19* encoded to QnrB19 (3) DNA gyrase can be inhibited by quinolone by stabilized the DNA-DNA gyrase complex, however, the presence of QnrB19 can protect DNA gyrase from the inhibition.

Background leading to this research

Salmonella Typhimurium is a significant cause of foodborne illness worldwide, and quinolones are recommended treatment for severe cases.[2] However, the emergence of quinolone-resistant strains of *Salmonella* has become a major public health concern.[2] It can develop resistance to quinolone antibiotics through various mechanisms, such as acquiring point mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase, which is the target of quinolones. Additionally, this resistance can arise through the reduction of quinolone accumulation and the presence of plasmid-mediated quinolone resistance (PMQR) genes.[3] The presence of PMQR genes is now globally reported among Enterobacteriaceae, and they can spread through horizontal gene transfer.[4] The PMQR genes can encode proteins, particularly Qnr proteins which can protect DNA gyrase from the inhibition of quinolones and potentially confer to low-level quinolone resistance as shown in Figure 1.[5] Furthermore, their presence can facilitate the acquisition of more potent resistance mechanisms, such as the point mutation in

QRDR, leading to the emergence of high-level of quinolones-resistant strains.[4] However, **the mechanism of Qnr proteins' resistance is still not well understood, and there are currently no available drugs to inhibit this protein**. This knowledge gap has motivated among researchers to investigate how the protein can protect DNA gyrase and complement other resistance mechanisms, leading to high-level quinolone resistance. The results of this study could have important implications for the development of novel drugs and the understanding of the structure-drug relationship. As the number of new drugs being developed is declining due to economic constraints, **the findings could potentially reduce the time and cost required for the development of new drugs**. This outcome will ultimately lead to the discovery of new treatment options for drug-resistant infections.

Objective

The purpose of this study is **to elucidate the resistant mechanism of *S.* Typhimurium with the presence of QnrB19 against quinolones** through the direct observation of the complex. The inhibition activity of quinolones is closely related to its structural characteristics. In addition, their unique chemical structures enable them to bind to specific regions of DNA gyrase, inhibiting its function and preventing the enzyme from performing its normal role ahead of transcription. As a result, bacterial cell will be death. Previous research has suggested that QnrB19 may protect the normal activity of DNA gyrase by altering its conformation, thereby reducing quinolones' binding affinity to the enzyme. However, the precise interaction between QnrB19 and quinolones is not fully understood. To address this, I propose to utilize a range of visualization tools and *in vitro* studies to better understand the interaction and determine the mechanism of resistance conferred by QnrB19 against quinolones.

(2) Research plan

This research has be conducted according to the research plan as shown in Figure 2.

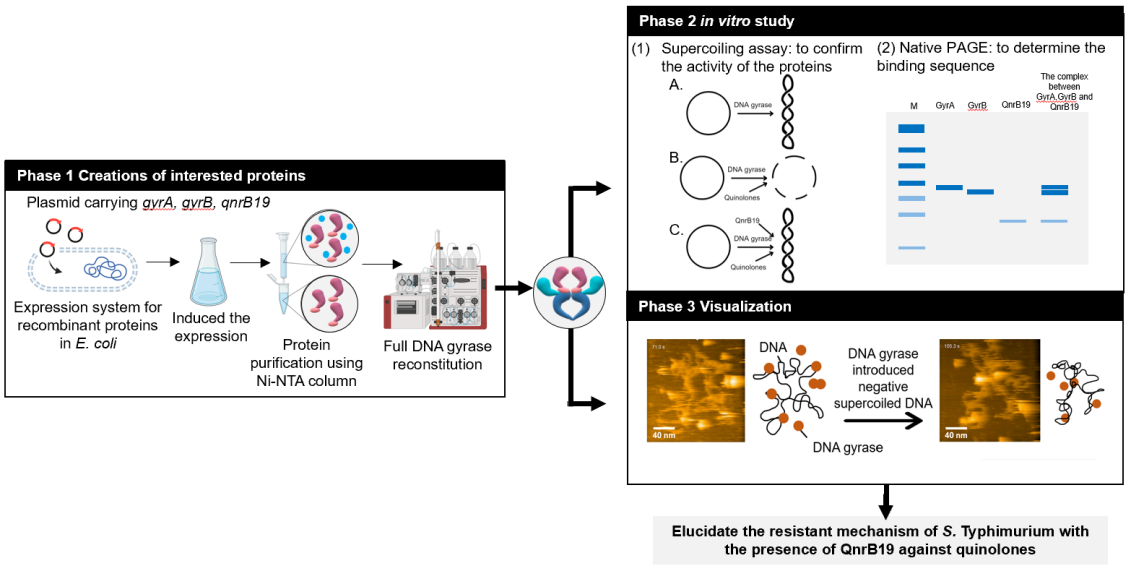

Figure 2 The research plan

Phase 1 Creations of interested proteins

I produced the interested proteins including GyrA, GyrB and QnrB19, utilizing an expression system for recombinant proteins in *Escherichia coli* with reconstructed plasmids. The bacteria was induced the expression by isopropyl beta-D-thiogalactopyranoside and purified using sonication and Ni-NTA Agarose, respectively.[6–10] As *S.* Typhimurium DNA gyrase consists of 2 subunits, $GyrA_2$ and $GyrB_2$, full DNA gyrase reconstitution will be performed by a Superdex S200 16/60 size-exclusion chromatography column.[11]

Phase 2 *In vitro* studies

DNA gyrase an essential enzyme that regulates the topology of bacterial chromosomes by introducing negative supercoiling as shown in Figure 2. Therefore, to confirm the activity of the proteins produced in this study, a supercoiling assay will be performed. Brieftly, DNA gyrase ($GyrA_2B_2$) will be incubated with relaxed pBR322 at 37°C for 60 minutes, followed by gel electrophoresis stained with GelRed to visualize the reaction. QnrB19 and quinolones will be added to the reaction mixture at different concentrations to determine its protective activity and inhibitory activity, respectively. The result indicated that the binding activity between GyrA, GyrB, and QnrB19 affect the activity of DNA gyrase in concentration-dose dependent.[7]

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Publications

1. Suwanthada P, Kongsoi S, Jayaweera S, Akapelwa ML, Thapa J, Nakajima C, Suzuki Y. Interplay Between Amino Acid Substitution in GyrA and QnrB19: Elevating Fluoroquinolone Resistance in *Salmonella* Typhimurium, ACS infect. *In press.*
2. Miura-Ajima N*, Suwanthada P*, Kongsoi S, Kim H, Pachanon R, Koide K, Mori S, Thapa J, Nakajima C, Suzuki Y. Effect of WQ-3334 on *Campylobacter jejuni* carrying a DNA gyrase with dominant amino acid substitutions conferring quinolone resistance. J Infect Chemother. 2024 Apr 4:S1341-321X(24)00110-7. doi: 10.1016/j.jiac.2024.04.002.
3. Suwanthada P, Kongsoi S, Miura N, Belotindos LP, Piantham C, Toyting J, Akapelwa ML, Pachanon R, Koide K, Kim H, Thapa J, Nakajima C, Suzuki Y. Impact of substitutions at 1 and 8 position of fluoroquinolones on the activity against mutant DNA gyrases of *Salmonella* Typhimurium, Microb Drug Resist 2023 Oct 4. doi: 10.1089/mdr.2023.0014.
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*Equally contribution

Award

1. Hokkaido University EXEX Doctoral Fellowship 2023 – 2024
2. Bio-SPM Collaborative Research, Kanazawa University 2023
3. Program for Supporting "Challenging and Interdisciplinary Research Field" 2023
4. Zoonosis Control Expert, Hokkaido University 2023
5. Program for Supporting "Challenging and Interdisciplinary Research Field" 2022
6. Hokkaido University DX Doctoral Fellowship 2021 – 2023
7. World-leading Innovative and Smart Education (WISE) program, "One Health Frontier Graduate School of Excellence" 2020 – 2021

International and Domestic Academic Presentations

1. Suwanthada P, Kongsoi, Akapelwa ML, Pachanon R, Koide K, Kim H, Thapa J, Nakajima C, Suzuki Y. Untangling the tangle: Mutant DNA gyrase and Qnr contribute to the high-level of quinolone resistance of *Salmonella* Typhimurium, Global Young Scientists Summit 2024, Singapore, 9th January 2024, Poster Presentation.
2. Suwanthada P, Kongsoi S, Thapa J, Nakajima C, Suzuki Y, "Effects of Delafloxacin on *Mycobacterium leprae* DNA Gyrase", The 11th Sapporo Summer Symposium (SaSSOH 2023), Japan, 15th September 2023, Poster Presentation.
3. Suwanthada P, Kongsoi S, Thapa J, Nakajima C, Suzuki Y, "Interaction between QnrB19 and DNA-DNA gyrase complex of *Salmonella* Typhimurium", American Society For Microbiology ASM Microbe 2023, the United State, 16th June 2023, Poster Presentation
4. Thapa J, Chizimu J, Kitamura S, Akapelwa M, Suwanthada P, Miura N, Toyting J, Nishimura T, Hasegawa N, Nishiuchi Y, Nakajima C, Suzuki Y, "Role of fluoroquinolone resistance-associated mutations in *Mycobacterium avium gyrA* to resistance", The 96th Annual Meeting of Japanese Society for Bacteriology, Japan,16th March 2023, Poster Presentation.
5. Suwanthada P, Kongsoi S, Thapa J, Nakajima C, Suzuki Y, "Impact of mutations in GyrA and QnrB19 on resistance to fluoroquinolone in *Salmonella* Typhimurium", The 96th Annual Meeting of Japanese Society for Bacteriology, Japan,16th March 2023, Poster Presentation.
6. Suwanthada P, Kongsoi S, Thapa J, Nakajima C, Suzuki Y, "Mutual impact of amino acid substitution in GyrA and existence of QnrB19 on resistance to fluoroquinolone in *Salmonella* Typhimurium", The 10th Sapporo Summer Symposium (SaSSOH 2022), Japan, 14th September 2022, Poster Presentation.
7. Suwanthada P, Kongsoi S, Miura N, Belotindos LP, Piantham C, Toyting J, Pachanon R, Koide K, Kim H, Thapa J, Nakajima C, Suzuki Y, "The impact of substitutions at positions 1 and 8 of fluoroquinolones on the activity against mutant DNA gyrases of *Salmonella* Typhimurium", The 9th Sapporo Summer Symposium (SaSSOH 2021), Japan, 15th September 2021, Poster Presentation.
8. Suwanthada P, Kongsoi S, Miura N, Belotindos LP, Piantham C, Toyting J, Akapelwa ML, Pachanon R, Koide K, Kim H, Thapa J, Nakajima C, Suzuki Y, "The impact of substitutions at positions 1 and 8 of fluoroquinolones on the activity against mutant DNA gyrases of *Salmonella* Typhimurium", Japanese Society for Bacteriology Hokkaido Branch Annual Conference, Japan, 28th August 2021, Oral Presentation.

Invited Speaker

1. 21st Asian Association of Veterinary Schools (AAVS) Meeting, Hokkaido, Japan, title "Toward a researcher who can contribute to One Health", 13rd September 2023
2. Science Camp, Thamavitya Mulniti School, Sirindhorn science home, Thailand, 23rd January, 2024