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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

Part A: The Project and Investigator(s)

1. Project Title

Structural Studies of Flagellar Motor Switch from *H. pylori*: A Combination of X-ray Crystallography and Cryo-electron Microscopy Approaches

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Au Wing Ngor Shannon	Professor Zhang Qinfen
Post	Associate Professor	Associate Professor
Unit / Department / Institution	School of Life Sciences The Chinese University of Hong Kong	School of Life Sciences Sun Yat-sen University
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Co-investigator(s) *(with title and institution)*	-	-

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	1 Jan 2014		
Project Completion date	31 Dec 2017		
Duration *(in month)*	48-month		
Deadline for Submission of Completion Report	31 Dec 2018		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. *To* determine the crystal structures of flagellar motor switch complexes of *H. pylori* by x-ray crystallography
2. *To* resolve the structure of flagellar motor switch complex of *H. pylori* by single-particle cryo-electron microscopy
3. To resolve and compare the *in vivo* structures of motor switch complex from wild type strain and fli-null mutant strains by cryo-electron tomography.
4. To define the location of FliG, FliM, FliN and FliY in the motor switch of *H. pylori* by immuno-electron microscopy

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

In this joint research project, we combine x-ray crystallography and cryo-electron microscopy to understand the molecular structure of the flagellar motor in *H. pylori.* Specifically, the structural and functional significance of the co-existence of FliY and FliN in *H. pylori* was characterized. We demonstrated that the C-terminal SpoA domain of FliY forms heterodimeric complexes with the FliN and FliM SpoA domains, which are β-sheet domains of type III secretion system proteins. Surprisingly, unlike in other flagellar switch system, neither FliY nor FliN self-associated. The atomic details underlying the heterodimerization of FliY-FliN was further revealed by crystallographic studies. The 2.5 Å crystal structure of the FliY-FliN heterodimer showed a saddle-shaped structure homologous to the FliN-FliN dimer of *Thermophilic maritima*, consistent with a FliY-FliN heterodimer forming the functional unit. Additional analysis of the FliY-FliN binding interface indicated that oppositely charged residues ($Lys215_{FliY}$ and $Glu51_{FliN}$) specific to each protein drive heterodimer formation. Interestingly, both FliY-FliM and FliY-FliN complexes associated with the N-terminal region of flagellar regulatory protein FliH, suggesting that the two complexes functioned as protein docking platform for FliH in flagellar export. These results are further supported by the *in vivo* molecular genetic studies that deletion of FliY C-terminal SpoA domain led to non-flagellation. On the other hand, FliY N-terminal domain is dispensable for flagellation, however its deletion resulted in tumbling bias. We conclude that *H. pylori* distinctively incorporate three SpoA domains in the motor C-ring for proper flagellar assembly and functioning. Our findings open a new way to understand the diversity of the Type III secretion system and flagellar motor. This part of the study has been published in *J. Biol. Chem*. (2018).

The motor structure of *H. pylori* wild type and FliYc complementation strain (with FliY N-terminal deleted) were further studied by cryo-EM (joint work with our mainland team). Overall, the core structure of the flagella motor of the two strains are conserved and resemble those of other enteric bacteria. The density corresponding to the rod, L-ring, P-ring, MS-ring, C-ring and the stator can be clearly annotated in the reconstructed 3D cryo-map. FliYc complementation strain has a more prominent density for the C-ring, suggesting that the FliY N-terminal domain may be a mobile module. Noteworthy, this complementation strain possesses a weaker density connecting the C-ring and the export apparatus when compared with the wild type. It is possible that the FliY N-terminal domain is positioned in the interior of the C-ring that contributes to some density connecting with the export apparatus. In addition, we also examined the motor structure of *spee* knock-out strain. SpeE was found by our team as a FliM interacting partner and deletion of *spee* induced clockwise rotation bias. Comparing the cryo-EM maps between the wild type and the *spee*-null strain, no significant changes in the C-ring was identified. These results suggest that SpeE-mediated regulation of motor behavior likely requires specific signaling. Higher resolution cryo-EM data will be needed to elucidate the structural arrangement of the two SpoA-containing units in the bottom of the C-ring and the regulatory mechanism by SpeE.

Another highlight of our research output is the study of the assembly of the C-ring to the MS-ring in *H. pylori*. The FliF-FliG crystal structure has revealed extensive hydrophobic contacts similar to those observed in the *T. maritima* homologous structure. Further analysis of the crystal lattice revealed that the heterodimeric complex packs as a linear superhelix via stacking of the armadillo repeat-like motifs (ARM) of FliG. The *in vivo* relevance of the FliG stacking was validated by complementation studies in *Escherichia coli*. Furthermore, structural comparison with apo FliG from the *Aquifex aeolicus* indicated that FliF apparently provides a template for FliG polymerization and spatiotemporally controls subunit interactions within FliG. Our proposed assembly model of FliF-FliG aligns well with the cryo-EM data. This part of the study has been published in *J. Biol. Chem*. (2017).

Potential for further development of the research and the proposed course of action *(maximum half a page)*

Bacterial flagella are rotary nano-machines that contribute to bacterial fitness in a variety of settings, including mammalian and plant colonization. Although the basic function of flagella as a motor organelle is conserved, there are substantial variation between microbes in the components used to build and operate key aspects of the flagella. Our work has revealed the overall architecture of flagellar motor and the molecular basis underlying its assembly in *H. pylori*. Specifically, our results demonstrate the attachment of the motor C-ring to the MS ring and the unique incorporation of three SpoA domains in the C-ring to ensure proper flagellar assembly and functioning. Yet, the atomic structure of the whole nano-rotary device that will explain the mechanistic details in rotational switching and motor performance is not available. Our future direction will focus on the structure determination of the supramolecular complex and the motor performance by biophysical studies.

New collaboration with Prof. Wah CHIU (Stanford University, US), Prof. Yuan Junhua (University of Science and Technology of China, China) and Prof. Wu Yilin (CUHK) are established. There are several collaborative studies underway, these include 1) purification optimization of recombinant FliF-FliG-FliM-FliY-FliN complex and isolation of native basal body from *H. pylori* for high resolution structure determination by cryo-EM (AU, CHIU,

ZHANG), and 2) characterization of the motor performance of *H. pylori* flagellar motor using wild type and FliY complemented strains (AU, YUAN and WU). CHIU's lab in Stanford houses various 300 kV TEM with direct electron detector device. The cryo-EM facility will allow us to collect high resolution data for structure determination.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Different bacteria exhibit great diversity in their flagellar motors. One such variation is exemplified by the motor-switch apparatus of the gastric pathogen *Helicobacter pylori*, which carries an extra switch protein, FliY, along with the more typical FliG, FliM, and FliN proteins. This collaborative project aims to investigate the structure of *H. pylori* switch complex by x-ray crystallography and cryo-electron microscopy. Our team has mapped the interactions among the five switch proteins and further resolved the atomic structure FliY-FliN complex. The direct association of FliM-FliY and FliN-FliY with the flagellar regulatory protein FliH further suggested that both switch proteins are essential for flagellation. Furthermore, cryo-electron tomographic studies showed that while the overall architecture of *H. pylori* motor resembled that of *Salmonella*, weaker density connecting to the export apparatus was observed when FliY N-terminal domain was deleted. This results aligned with the molecular genetic studies that both N-terminal and C-terminal domains of FliY are required for full motor function. Our findings provide a basis to understand the macromolecular assembly of the distinctive C-ring complex of ε-proteobacteria. This species-specific macromolecular assembly prompts a different genetic control of flagellum biogenesis in response to environmental cues and awaits further investigation.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*
1) 2018				**Lam KH, Xue C, Sun K, Zhang H, Lam WW, Zhu Z, Ng JTY,** Sause WE, Lertsethtakarn P, Lau KF, Ottemann KM, **Au SWN***	Three SpoA-domain proteins interact in the creation of the flagellar Type III secretion system of *Helicobacter pylori* / *J Biol. Chem. 293:13961-13973*		Yes	Yes	Yes
2) 2018				**Xue C, Lam KH, Zhang H, Sun K, Lee SH, Chen X, Au SWN***	Crystal structure of the FliF-FliG complex from *Helicobacter pylori* yields insight into the assembly of the motor MS-C ring in the bacterial flagellum / *J Biol. Chem. 293:2066-2078*		Yes	Yes	Yes
3) 2017				**Zhang H, Lam KH, Lam WWL,** Wong SYY, Chan VSF, **Au SWN***	A putative spermidine synthase interacts with flagellar switch protein FliM and regulates motility in *Helicobacter pylori* /*Mol. Microbiol. 106:690-703*		Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year / Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
1) Jan, 2015 /Tucson	Molecular structure of FliY-FliN complex in *Helicobacter pylori*	Bacterial Locomotion & Signal Transduction (BLAST) XIII	2015	Yes	Yes	Yes
2) Dec, 2015 /Xiamen	Molecular interaction of motor switch protein FliM and spermidine synthase in *Helicobacter pylori*	The 3rd South China Structural Biology Symposium (SCSBS)	2015	Yes	Yes	Yes
3) Jun, 2016 / Beijing	Structural architecture of flagellar motor switch complex	2016 Kuo Symposium on 3D Cryo-EM Molecular Imaging		Yes	Yes	Yes
4) Aug, 2016 / Taiwan	Structural insights into the assembly of molecular rotary machine	Bilateral Symposium (CUHK & IBC, Academia Sinica) (Oral presentation)		Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
1) CHU Wai Sze	MPhil	Aug 2013	Mar 2016
2) ZHANG Huawei	PhD	Aug 2013	Aug 2017
3) SUN Kailei	PhD	Aug 2017	July 2020 (expected)

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

1) In 2015, AU spent a 7-month sabbatical training in Prof. Wah CHIU's lab at Baylor College of Medicine, Houston. During this trip, AU has acquired hands-on experience on single particle analysis and tomography. Collaboration with Prof. CHIU has also been set up.
2) A half-day meeting titled "Micro-Symposium on Cryo-EM in Life Sciences" was held on 17 Nov, 2015 at Chinese University of Hong Kong. AU was the chairperson of the organizing committee. The meeting aimed to highlight the advances of cryo-EM in biological and biomedical science. Invited speakers included Prof. Wah CHIU and Prof. Qinfen ZHANG (PI from Mainland). The meeting attracted approximately 60 researchers from different local institutions.

3) A joint GRF project titled "Structure-function studies of ESX-5 associated PE/PPE proteins in *Mycobacterium tuberculosis*"with Prof. Wah CHIU and PI from mainland team Prof. Qinfen ZHANG and as co-Is has been funded (GRF 2018/19).