RGC Ref.: A-HKU712/14
(please insert ref. above)

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

(Please attach a copy of the completion report submitted to the ANR by the French researcher)

Part A: The Project and Investigator(s)

1. Project Title (ANR Acronym)

Pattern Formation in Bacterial Colonies: from Theoretical Physics to Synthetic Biology

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

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Professor Jiandong Huang	Dr. Julien Tailleur
Post	Professor	Chargé de Recherche 1ère classe Senior Research Scientist
Unit / Department / Institution	School of Biomedical Sciences, The University of Hong Kong	CNRS, Université Paris-Diderot
Contact Information	jdhuang@hku.hk	julien.tailleur@univ-paris-diderot.fr
Co-investigator(s) *(with title and institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	Jan.1, 2015	Jan.1, 2015	
Project Completion date	Dec.31, 2018	Sept. 30, 2019	Nov. 20, 2018
Duration *(in month)*	48 months	57 months	
Deadline for Submission of Completion Report			

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To fully characterize the mechanism leading to circular patterns in bacterial colonies where the motility of each cell is enslaved to the local density of bacteria

2. To study how interactions between bacteria, which affect the spatial distribution of the bacterial colony, interplay with phenotypic and genotypic segregation

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

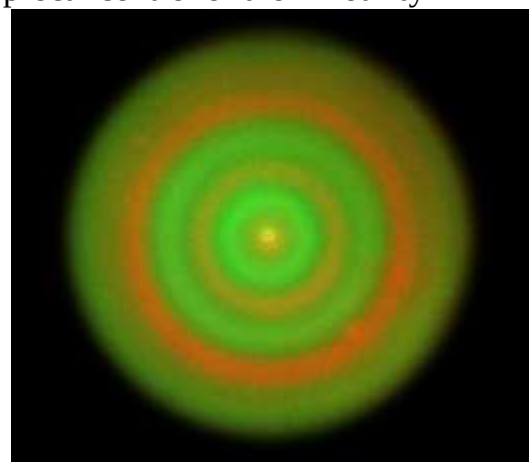
6. Research Outcome

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

There are two major outcomes of the project. One is on pattern formation. Pattern formation, as one of the most remarkable features of life, describes the reliable and recurrent generation of orderly structures or patterns. As early as 1952, Alan Turing, known as the father of modern computer science, proposed the “reaction-diffusion”

model to explain those naturally occurring biological patterns, which opened up a long studying process of this topic. The most crucial feature of pattern formation is its self-organization, by which biological systems can develop spatial patterns autonomously without any pre-existed positional information. At the cellular and population level, it mounts to how different cell types assume specified distribution in space and time through cell differentiation, proliferation, and migration. Studying pattern formation and clarifying their regulatory mechanisms are crucial to the understanding of important biological processes such as embryogenesis and morphogenesis. However, in most biological systems, the overwhelming complexity makes elucidating any underlying pattern forming process a challenge in the absence of any guiding principles.

Instead of natural living systems, we team worked with artificial living systems which are engineered built by synthetic biology. Using *E. coli* as a model organism, they fabricated signaling pathways built synthetic "quorum sensing" circuits to allow between two types of cells, which allow them to populations with a reciprocal control of their motility in response to the local cell density of their counterpart population. When the two cell populations are programmed to enhance the motility of each other and seeded as a cell mixture on a semisolid agar plate, the initially well-mixed cell populations tend to segregate and form an orderly stripe pattern in which two cell types forms alternatively appeared as concentric rings (out of phase, see the picture on the right). By re-designing the synthetic signaling pathways, we also programmed two cell populations to suppress the motility of each other. Interestingly, the mixture also autonomously developed periodic rings, however, this time, the two cell types tend to always form the concentric rings in phase colocalized in the rings. The research team further established a theoretical model to decipher the mechanisms of the observed pattern formation processes. Simulation of the model produced similar ring patterns that both qualitatively and quantitatively agreed with the experiments. The results have been published.



- Agnese I. Curatolo∗, Nan Zhou∗, Yongfeng Zhao∗, Chenli Liu, Adrian Daerr, Julien Tailleur † and **Jian-Dong Huang †** (2020) Cooperative pattern formation in multi-species bacterial colonies. **Nature Physics** (article) (Received 28 September 2018, Accepted 04 June 2020, Published 24 August 2020) (∗ equal contribution; † co-corresponding author) (IF= 21.797, (2018)) DOI: https://doi.org/10.1038/s41567-020-0964-z
  bioRxiv: http://biorxiv.org/cgi/content/short/798827v1
  DOI: https://doi.org/10.1101/798827:

The second outcome is on bacterial movement. The survival strategies of *Escherichia coli* are controlled by their run-and-tumble 'gait'. While much is known about the molecular mechanisms of the bacterial motor, quantifying the motion of these microorganisms in three dimensions has remained challenging. We propose a high-throughput method, using differential dynamic microscopy and a renewal theory, for characterizing the run-and-tumble behavior of a population of *E. coli*. We demonstrate the potential of our method by relating, for the first time, molecular

properties of the motor to the dynamics of engineered *E. coli* mutants. It therefore lays the foundation for future studies on gait-related phenomena in different microorganisms. The results have been summarized and submitted.

- Christina Kurzthaler, Yongfeng Zhao, Nan Zhou, Jana Schwartz-Linek, Clemence Devailly, Jochen Arlt, **Jian-Dong Huang**, Wilson C. K. Poon, Thomas Franosch, Julien Tailleur, and Vincent A. Martinez (2020) Quantitative Characterization of the Run-and-Tumble Dynamics of E. coli. **Physical Review Letters** (submitted)

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

The development of a mature organism from a single cell is a complicated but orderly process. During the process, diverse cell types are differentiated and coordinate to form regular body layouts spatiotemporally. For example, in zebrafish, different types of pigment cells are accurately aligned to form colored stripes. In past studies, food competition, chemotaxis and other mechanisms have been proven to play important roles in self-organization of different biological systems, such as cell-cell adhesion et al. “Our study shows reciprocal control of motility to be a novel self-organization pathway and a general pattern formation mechanism in multicellular systems. This pattern forming mechanism explains how different cell populations could spontaneously colocalize or segregate in space and time without the guidance of any predetermined positional information or man-made interference, which is a prerequisite of the formation of more complex biological patterns and structures This finding may inspire us to check if such mechanism is at play in the pattern formation of motile cells in nature. From an engineering view, it could provide a guiding principle for tissue engineering and regenerative medicine the design and construction of artificial multicellular systems in the future.

7. The Layman’s Summary

(describe <u>in layman’s language</u> the nature, significance and value of the research project, in no more than 200 words)

Living organisms exhibit amazing patterns or structures during development, such as stratified cell layers during early embryogenesis, animal skin patterns composed of different pigment cells, etc. Understanding how these patterns or structures are formed remains a fundamental scientific question. We studied pattern formation in synthetic artificial multicellular systems. Combining experimental biology and theoretical work physics, the study identified the reciprocal control of motility to be a general mechanism for biological pattern formation and self-organization of different cell types, providing new insights into development. The findings are now published in Nature Physics.

<u>Part C: Research Output</u>

8. Peer-reviewed journal publication(s) arising <u>directly</u> 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	Submitte	Attached	Acknowledged

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*)*	Journal/Book *(with the volume, pages and other necessary publishing details specified)*	d to RGC *(indicate the year ending of the relevant progress report)*	to this report *(Yes or No)*	the support of this Joint Research Scheme *(Yes or No)*
2015				A. P. Solon, J. Stenhammar, R. Wittkowski, M. Kardar, Y. Kafri, M. E. Cates, J. Tailleur	Pressure and Phase Equilibria in Interacting Active Brownian Spheres, Physical Review Letters 114, 198301		Yes	yes
2015				A. P. Solon, J. Tailleur,	"Flocking with discrete symmetry: The two-dimensional active Ising model", Phys. Rev. E 92, 042119		Yes	yes
2015				A. P. Solon, J.-B. Caussin, D. Bartolo, H. Chate, J. Tailleur,	"Pattern formation in flocking models: A hydrodynamic description", Phys. Rev. E 92, 062111		Yes	yes
2016				N. Nikola, A. P. Solon, Y. Kafri, M. Kardar, J. Tailleur, R. Voituriez ,	"Active particles on curved surfaces: Equation of state, ratchets, and instabilities", Phys. Rev. Lett. V117(9), PP 098001		Yes	Yes

2016				Nikolai Nikola, Alexandre P. Solon, Yariv Kafri, Mehran Kardar, Julien Tailleur, and Raphaël Voituriez	Active Particles with Soft and Curved Walls: Equation of State, Ratchets, and Instabilities PRL 117, 098001		Yes	Yes
2020				Agnese I. Curatolo, Nan Zhou, Yongfeng Zhao, Chenli Liu, Adrian Daerr, Julien Tailleur and Jian-Dong Huang	Cooperative pattern formation in multi-species bacterial colonies. Nature Physics (article) DOI: https://doi.org/10.1038/s41567-020-0964-z		Yes	Yes
2020				Christina Kurzthaler, Yongfeng Zhao, Nan Zhou, Jana Schwartz-Linek, Clemence Devailly, Jochen Arlt, Jian-Dong Huang, Wilson C. K. Poon, Thomas Franosch, Julien Tailleur, and Vincent A. Martinez	Quantitative Characterization of the Run-and-Tumble Dynamics of E. coli. (submitted to) Physical Review Letters		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)*

March 13–17, 2017; New Orleans, Louisiana	Motility-induced bacterial pattern formation in multi-species bacterial colonies Authors: Agnese Curatolo Yongfeng Zhao Nan Zhou Adrian Daerr Jiandong Huang Julien Tailleur	APS March Meeting 2017		No	Yes
February 18-21, 2020, Hilton Waikoloa Village, The Big Island, Hawaii, USA	Pattern formation by multi-species bacterial colonies with programmed population-to-population interactions Jian-Dong Huang	the 8th Annual Winter q-bio conference		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
Dr. Yongfeng Zhao	Ph.D	Nov, 1st, 2012	Nov, 10th, 2016
Mr. Zhou Nan	Ph.D	Feb.1st, 2014	Jan.31st, 2018

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

(a) Talk on synthetic biology by Dr. JD Huang by Phoenix TV - Trendy Guide Interview (完全时尚手册邀访) (Interview on February 1, 2015, broadcasted on February 5, 2015), Phoenix Satellite Television Company Limited, No. 2-6 Dai King Street, Tai Po Industrial Estate, Tai Po, N.T., Hong Kong, China 香港新界大埔大埔工業村大景街2-6號 鳳凰衛視 (http://v.ifeng.com/fashion/focus/201502/0122781e-c92b-4299-9da0-961cf8dd11ba.shtml)

(b) Invited seminars on synthetic biology:

1. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (March 26, 2015), Xiamen University, Xiamen, China.
2. "Synthetic Patterns: Sequential Establishment of Stripe Patterns in an Expanding Cell Population", (December 17, 2015), Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, 161 Cathedral St, Glasgow G4 0RE, Scotland, UK

3. "Synthetic Patterns: Sequential Establishment of Stripe Patterns in an Expanding Cell Population", (December 18, 2015), SynthSys Centre, the School of Biological Sciences at the University of Edinburgh, Edinburgh, UK
4. "How to make a biological structure? - Sequential establishment of stripe patterns in an expanding cell population", (January 22, 2015), G02, Lo Kwee-Seong Integrated Biomedical Sciences Building, Area 39, School of Biomedical Sciences, Chinese University of Hong Kong
5. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (March 9, 2016), Institute of Translational Medicine, Zhejiang University, Hangzhou, Zhejiang, China.
6. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (March 10, 2016), New York University Shanghai Campus, Shanghai, China.
7. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (May 2, 2016), Institute of Cardiometabolism And Nutrition, Hopital de la Pitie Salpetriere, Salle 012, RDC, 91 boulevard de l'hôpital, Paris, France
8. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (July 18, 2016), The Sanger Seminar Room, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1GA, United Kingdom
9. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (November 14, 2016), Department of Chemistry & Biochemistry, Florida International University, 11200 SW 8th Street, Miami, FL 33199, USA
10. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers in Pets", (November 30, 2016), Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, Sanya, Hainan, China
11. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (January 12, 2017), Department of Chemistry, Fudan University, Shanghai, China
12. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (January 13, 2017), Department of Biochemistry and Molecular Cell Biology, Shanghai Jiao Tong University School of Medicine, 280 South Chongqing Road, Huangpu District, Shanghai, China
13. "Engineering Bacteria to attack cancer", (March 13, 2017), The Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
14. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers in Pets", (May 16, 2017), CIB-CSIC, Ramiro de Maeztu, 9, 28040-Madrid, Spain
15. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers in Pets", (May 22, 2017), Institut de Biotecnologia I de Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain
16. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers in Pets", (July 5, 2017), Cardiff University, Cardiff, UK
17. "Engineering bacteria to form biological structures and to attack cancer", (January 26, 2018), Donnelly Centre (CCBR), University of Toronto, Toronto, Ontario, Canada
18. "Engineering bacteria to form biological structures and to attack cancer", (March 14, 2018), Shanghai Institute for Advanced Immunochemical Studies (SIAIS), ShanghaiTech University, Shanghai China

19. "Engineering Bacteria to Form Biological Structures and to Attack Cancer", (April 19, 2018), Institute for Academic Medicine, Houston Methodist Research Institute, Weill Cornell Medical College of Cornell University, Houston, Texas, USA
20. "Engineering Bacteria to Form Biological Structures and to Attack Cancer", (June 22, 2018), Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street | Manchester, M1 7DN, United Kingdom
21. "Engineering Bacteria to Form Biological Structures and to Attack Cancer", (June 29, 2018), Immunosurveillance Laboratory, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, United Kingdom
22. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (September 19, 2018), 中国农业科学院兰州畜牧与兽药研究所, Lanzhou, China
23. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (October 9, 2018), 南京军区总医院, Nanjing, China
24. "Engineering Bacteria to Reveal Biological Principles and to Target Tumors", (October 13, 2018), 粤港澳大灣區轉化醫學聯盟成立大會。廣州, China
25. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers", (December 18, 2018), 中国北京 北京医院 老年医学研究所
26. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers", (January 25, 2019), Department of Chemistry, University of British Columbia, Vancouver, BC, Canada
27. "Engineering bacteria to form biological structures and to attack cancer", (March 12, 2019), School of Life Sciences, Nanjing University, Nanjing, China
28. "Development of Staphylococcus aureus vaccine designed to counter multiple bacterial virulence factors", (March 21, 2019), Department of Biochemical Engineering, University College London, London, the United Kingdom
29. "Engineering Bacteria to Reveal Biological Principles and to Treat Cancers", (June 11, 2019), School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China
30. "利用合成生物学探索生物图案的形成及开发癌症治疗新方法", (November 26, 2019), 康立明医学检验实验室大会议室(A3栋2层)，广州康立明
31. "编码细菌形成图案及治疗肿瘤", (November 27, 2019), 慕恩生物
32. "Engineering Bacteria to for Biological Structures and to Attack Cancers", (December 5, 2019), College of Life Sciences and Technology, Huazhong University of Science & Technology, Hongshan District, Wuhan, Hubei Province, China
33. "Engineering Bacteria to for Biological Structures and to Attack Cancers", (December 6, 2019), Tong Ji Medical School, Huazhong University of Science & Technology, Wuhan, Hubei Province, China

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project	7	2	0	0	0