RGC Ref.: M-HKUST601/13
(please insert ref. above)

The Research Grants Council of Hong Kong
SRFDP & RGC ERG Joint Research Scheme
Completion Report

(Please attach a copy of the completion report submitted to the Ministry of Education by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Elucidating molecular mechanisms of the Maltose transporter (MalFGK2) using Markov State Models

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Xuhui Huang	Prof. Wenning Wang
Post	Associate Professor	Professor
Unit / Department / Institution	Department of Chemistry, HKUST	Department of Chemistry, Fudan University
Contact Information	xuhuihuang@ust.hk Tel: 2358-7363	wangwn@fudan.edu.cn
Co-investigator(s) *(with title and institution)*	N.A.	N.A.
PhD student(s) (with period of involvement)	Name: Shuo Gu Institution: HKUST Period: Jan 2014 to July 2015 Name: Wei Wang Institution: HKUST Period: July 2015 to Dec 2016	

Note: The Hong Kong project team must involve at least one research postgraduate student pursuing a Doctor of Philosophy degree at the UGC-funded university (PhD student) at any time throughout the project period.

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*

Project Start date	Jan 1, 2014		
Project Completion date	Dec 31, 2016		
Duration *(in month)*	36		
Deadline for Submission of Completion Report	Dec 31, 2017		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To reveal the thermodynamics and kinetics of the transitions between the inward-facing, pre-translocation and outward-facing conformational states.
2. To elucidate the roles of the binding of ATP and maltose binding protein (MBP) on the MalFGK2 conformational changes.
3. To develop Metadynamics guided Markov State Models (MG-MSM) to enhance the initial sampling and improve the efficiency of the MSM construction.

5.2 Revised Objectives

N.A.

Date of approval from the RGC: _____

Reasons for the change: _____

_____

6. Research Outcome

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

ATP-binding cassette (ABC) transporters are responsible for transporting a large variety of substrates across cell membranes against the concentration gradient. The malfunction of ABC transporters is a major factor in several human genetic diseases such as cystic fibrosis and multiple chemotherapeutic drug resistance. Therefore, understanding mechanisms of ABC transporters is important for understanding these human diseases and can also greatly facilitate the drug development against multi-drug resistance. To allow the transport of the substrates, $MalFGK_2$ has to undergo large conformational changes. Several conformational states of MalFGK2 complex (inward facing, pre-translocation, and outward-facing) have been identified by X-ray crystallography and other experimental techniques. However, these crystal structures are only static snapshots of the molecule in action, and how $MalFGK_2$ transits between these states remains a mystery. Elucidation of such mechanisms at atomic resolution is important but this dynamic information is largely inaccessible to present experimental techniques. By performing extensive molecular dynamics (MD) simulations and metadynamics simulations, we found that the apo-$MalFGK_2$ system has strong preference to rest in the inward-facing state, and the system has to overcome a free energy barrier of over 30kcal/mol to reach the outward-facing state. These results suggest that thermal fluctuations are not sufficient to activate $MalFGK_2$. We further show that the binding of MBP could facilitate the transition from inward-facing to the outward-facing state by stabilizing the intermediate pre-translocation state. Moreover, we found the binding of MBP not only induces the lateral motion, but also trigger several segments at the periplasmic side of MalF core undergo collective downward motion. This dynamic correlation between the vertical motion and the lateral motion has been further supported by a MalF500 mutant (*Phys. Chem. Chem. Phys.*, 19, 9366-9373, (2017)). We further suggest that

while MBP binding facilitates the transition by stabilizing the intermediate pre-translocation state, the ATP binding is necessary for complete conversion to the outward-facing state, which is consistent with our observations in another ABC transporter (*Biochemistry,* 55:6897-6907*,* (2016)). Our findings greatly facilitate the understanding of molecular mechanisms of functional conformational changes of ABC transporters. On the algorithmic side, we have proposed an efficient computational scheme using metadynamics simulations to guide MD simulations for subsequent MSM construction (*WIREs Comput. Mol. Sci.,* e1343, (2017)), and also developed several new computational algorithms to improve the efficiency of the MSM construction (e.g. *Phys. Chem. Chem. Phys.,* 18, 30228-30235, (2016) & *PLOS. Comp. Bio.*, 10, e1003767, (2014)). Although we didn't construct MSMs for $MalFGK_2$ due to high free energy barriers separating different conformational states, we have successfully applied our new algorithms to construct MSMs to investigate various other functional conformational changes in biology such as RNA polymerase translocation (*PNAS*, 111, 7665, (2014)) and backtracking (*Nature Communications*, 7, 11244, (2016)), peptide dimerization (*Phys. Chem. Chem. Phys.*, 18, 29892, (2016)), and protein-ligand recognition (*Angew. Chem. Int. Ed.*, 55, 13990, (2016)). For $MalFGK_2$, we adopted an alternative approach to direct investigate MD simulations initiated from conformations generated by metadynamics simulations to elucidate molecular mechanisms of structural transitions in $MalFGK_2$ (*Phys. Chem. Chem. Phys.*, 19, 9366-9373, (2017)).

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

In the long term, our research can be easily extended to other ABC transporters, which will greatly improve our understanding of the working mechanisms of ABC transporters and shed light on the related drug discovery and clinic therapy. In addition, our novel algorithms hold great potential to be widely applied to investigate important conformational changes in other biological macromolecules.

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)

Transport across biological membranes is fundamental to any form of life, and ABC transporters constitute the largest transporter protein family. Some ABC transporters are involved in multidrug resistance, while mutations in some others are associated with a range of human diseases including cystic fibrosis. The maltose transporter $MalFGK_2$ has been extensively studied by experiment, while molecular mechanisms of conformational changes of $MalFGK_2$ still remains largely elusive. The applications of experimental techniques to probe protein dynamics are limited by spatial and temporal resolutions. In this project, we have performed computer simulations to elucidate the conformational dynamics and translocation mechanism of $MalFGK_2$. MalFGK2 needs to undergo conformational changes from an inward-facing state to outward-facing state when performing its function. We show that the inward-facing state is the only stable conformation in the apo-$MalFGK_2$ system, and there exists a significant free energy barrier preventing spontaneous activation of this transporter via thermal fluctuations. We found that MBP and ATP assist the activation of MalFGK2. In particular, MBP binding facilitates the transition by stabilizing the intermediate pre-translocation state, whereas ATP binding is necessary for complete conversion to the outward-facing state. Our results provide new insights in understanding the operation of ABC transporters.

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)*
2017				Weng, J., **Gu, S.,** Gao, X., **Huang, X.*, Wang, W.***	"Maltose Binding Protein Effectively Stabilizes the Partially Closed Conformation of the ATP-binding Cassette Transporter MalFGK2", *Phys. Chem. Chem. Phys.*, 19, 9366-9373, (2017)	2017	Yes	Yes	Yes
2017				**Wang, W.**, Cao, S., Zhu, L., **Huang, X.***	"Constructing Markov State Models to Elucidate the Functional Conformational Changes of Complex Biomolecules", *WIREs Comput. Mol. Sci.,* e1343, (2017)	2017	Yes	Yes	No
2017				Zhu, L., Jiang, H., Sheong, F.K., Cui, X., Wang, Y., Gao, X.*, **Huang, X.***	"Understanding the Core of RNA Interference: the Dynamic Aspects of Argonaute-mediated Processes", *Prog. Biophys. Mol. Bio.*, 128, 39-46, (2017)	2017	Yes	Yes	Yes
2016				Pan C., Weng J.*, **Wang W.***	"Conformational Dynamics and Protein-Substrate Interaction of ABC Transporter BtuCD at the Occluded State Revealed by Molecular Dynamics Simulations" *Biochemistry,* 55:6897-6907, (2016)	2017	Yes	Yes Note: The corresponding mainland grant number (20130071140004) of this joint research scheme was acknowledged.	No
2016				Zhu, L., Sheong, F. K., Zeng, X., **Huang, X.***	"Elucidating conformational dynamics of multi-body systems by constructing Markov State Models", *Phys. Chem. Chem. Phys.,* 18, 30228-30235, (2016)	2017	Yes	Yes	Yes

2016				Qiao, Q.*, Qi, R., Wei, G.*, **Huang, X.***	"Dynamics of the Conformational Transitions during the Dimerization of an Intrinsically Disordered Peptide: a Case Study on the Human Islet Amyloid Polypeptide Fragment ", *Phys. Chem. Chem. Phys.*,18, 29892-29904, (2016)	2017	Yes	Yes	Yes
2016				Feng, Y., Zhang, L., Wu, S., Gao, X., Liu, J.*, **Huang, X.*, Wang, W.***	"Conformational Dynamics of apo-GlnBP Revealed by Experimental and Computational Analysis", *Angew. Chem. Int. Ed.*, 55 (45), 13990–13994, (2016)	2017	Yes	Yes	Yes
2016				Zhang, L., Pardo, F., Unarta, I.C., Cheung, P., Wang, G., Wang, D., and **Huang, X.***	"Elucidation of the Dynamics of Transcription Elongation by RNA Polymerase II using Kinetic Network Models", *Accounts of Chemical Research*, 49 (4), 687–694, (2016)	2017	Yes	Yes	Yes
2016				Da, L., Pardo, F., Xu, L., Silva, D., Zhang, L., Gao, X., Wang, D.*, **Huang, X.***	"Bridge Helix Bending Promotes RNA Polymerase II Backtracking Through a Critical and Conserved Threonine Residue", *Nature Communications*, 7, 11244, (2016)	2017	Yes	Yes	Yes
2015				Zhang, L., Silva, D.A., Pardo-Avila, F., Wang, D., **Huang, X.***	"Structural Model of RNA Polymerase II Elongation Complex with Complete Transcription Bubble Reveals NTP Entry Routes", *PLOS. Comp. Bio.*, 11(7), e100435, (2015)	2015	No	Yes	Yes
2014				**Gu**, **S.**, Silva, D.A., Meng, L., Yue, A., **Huang, X.***	"Quantitatively Characterizing the Ligand Binding Mechanisms of Choline Binding Protein using Markov State Model Analysis", *PLOS. Comp. Bio.*, 10(8):e1003767, (2014)	2015	No	Yes	Yes
2014				Silva, D.A., Weiss, D.R., Pardo-Avila, F., Da, L.T., Levitt, M., Wang, D.*, and **Huang, X.***	"Millisecond Dynamics of RNA Polymerase II Translocation at Atomic Resolution", *Proc. Nat. Acad. Sci. U.S.A.*, 111, 7665-7670, (2014)	2015	No	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)*

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
Shuo Gu	Ph.D	Fall 2011	July 31, 2015
Wei Wang	Ph.D	Fall 2014	Aug 31, 2018 (Expected)

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

- Prof. Huang was awarded the OpenEye Outstanding Junior Faculty Award, American Chemical Society, 2014.