CRF 8G (Revised Dec 08)

Deliverable No: 3

RGC Reference Project No.: HKUST6/CRF/10
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

Protein-phosphoinositides interactions in neuronal signaling

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

Research Team	Name/Post	Unit/Department/Institution
Project Coordinator	Mingjie Zhang/Chair Prof	Life Sci/HKUST
Co-investigator(s)	Jun Xia/ Assoc Prof Yanxiang Zhao/Assoc Prof Nancy Ip/Chair Prof Xuhui Huang/Assist Prof Zilong Wen/Prof	Life Sci/HKUST ABCT/PolyU Life Sci /HKUST CHEM/HKUST Life Sci /HKUST
Others		

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	01/05/2011	n/a	
Project Completion Date	30/04/2014	31/10/2014	
Duration (*in month*)	36	42	April 10,2014 (Ref: UGC/GEN/492/11)
Deadline for Submission of Completion Report	31/10/2015		

5. Project Objectives

5.1 Objectives as per original application

I. Structure, function, and regulation of the auto-inhibited conformation of Par-3

In this section, we will characterize the structural basis and functional significance of the closed, auto-inhibited conformation of the master cell polarity protein Par-3.

II. Characterization of the isoform-specific interactions between Par-6A/B/C and Par-3

We plan to characterize the structure and function of isoform-specific interactions between Par-3 and Par-6 discovered in our lab. We will also elucidate the structural basis of the novel PDZ/PDZ interaction mode between Par-3 PDZ1 and Par-6.

III. Functional characterization of the myosin X triple PH domain repeats/PIP interaction

The experiments in this section will systematically investigate the function of the interaction between myosin X PH domains and $PI(3,4,5)P_3$-containing membranes.

IV. Structure, function, and regulation of the scaffolding functions of ankyrins

In this section, we plan to characterize the structural and biochemical basis of the intra-molecular interaction between the N-terminal ankyrin repeats and the C-terminal regulatory

domains of ankyrin family scaffold proteins. We will also investigate the interaction between ankyrins and β-spectrin.

5.2 Revised objectives

N/A

6. Research Outcome

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

I. Structure, function, and regulation of the auto-inhibited conformation of Par-3

II. Characterization of the isoform-specific interactions between Par-6A/B/C and Par-3

On the above two aims, we have systematically studied the biochemical basis and the function of the auto-inhibited conformation of mammalian Par-3 (mPar-3). We show that mPar3 directly regulates microtubule stability and organization. The N-terminal portion of mPar3 exhibits strong microtubule binding, bundling and stabilization activity, which can be suppressed by its C-terminal portion via an intra-molecular interaction. Interestingly, the inter-molecular oligomerization of mPar3 is able to relieve the intra-molecular interaction and promote microtubule bundling and stabilization. Furthermore, disruption of this microtubule regulatory activity of mPar3 impairs its function in axon specification. Together, these results demonstrate a previously unknown role for mPar3 in directly regulating microtubule organization that is crucial for neuronal polarization. This part of research has benefited from a very fruitful collaboration between the PI's group and Dr. Songhai Shi's group in Memorial Sloan-Kettering Cancer Center, New York. The work has been published in *Dev Cell* in 2013.

Atypical protein kinase C (aPKC) forms a highly conserved complex with Par-3 and Par-6 (the so-called Par complex). The Par complex is critical for all known cell polarization processes. The activity of the Par complex is tightly regulated by aPKC via phosphorylation of Par-3, although the molecular basis of aPKC-mediated phosphorylation of Par-3 (and any of aPKC's substrates) is poorly understood. In the past reporting period, we determined the structure of PKCι in complex with a peptide from Par-3 at 2.4 Å. PKCι in the complex adopts catalytically competent, closed conformation without the need of phosphorylation of Thr402 in the activation loop. The Par-3 peptide binds to an elongated groove formed by the N- and C-lobes of the kinase

domain. The PKCι/Par-3 complex structure, together with extensive biochemical studies, reveals a set of substrate recognition sites common to all PKC isozymes as well as a hydrophobic pocket unique to aPKC. A consensus aPKC's substrate recognition sequence pattern can be readily identified based on the complex structure. Finally, we demonstrate that the pseudo-substrate sequence of PKCι resembles its substrate sequence, directly binds to and inhibits the activity of the kinase. This work has been published in *Structure* in 2013.

III. Functional characterization of the myosin X triple PH domain repeats/PIP interaction

Myosin X (MyoX) is an unconventional myosin that is known to induce the formation and elongation of filopodia in many cell types. MyoX-induced filopodial induction requires the three PH domains in its tail region, although with unknown underlying molecular mechanisms. Interestingly, MyoX's first PH domain is split into two halves by its second PH domain. We demonstrate that the $PH1_N$-PH2-$PH1_C$ tandem allows MyoX to bind to phosphatidylinositol (3,4,5)-triphosphate [$PI(3,4,5)P_3$] with high specificity and cooperativity. We further show that PH2 is responsible for the specificity of the $PI(3,4,5)P_3$ interaction, while PH1 functions to enhance the lipid membrane binding avidity of the tandem. The structure of the MyoX $PH1_N$-PH2-$PH1_C$ tandem reveals that the split PH1, PH2, and the highly conserved inter-domain linker sequences together form a rigid supramodule with two lipid binding pockets positioned side-by-side for binding to phosphoinositide membrane bilayers with cooperativity. Finally, we demonstrate that disruption of PH2-mediated binding to $PI(3,4,5)P_3$ abolishes MyoX's function in inducing filopodial formation and elongation. This part of the work has been published in *Mol Biol Cell* in 2012.

We have also addressed one of the major question in the entire unconventional myosin field, namely the dimerization mode of myosins, using MYOX as the model system. Processive movements of unconventional myosins on actin filaments generally require motor dimerization. A commonly accepted myosin dimerization mechanism is via formation of a parallel coiled-coil dimer by a stretch of amino acid residues immediate carboxyl-terminal to the motor's lever-arm domain. We discover that the predicted coiled-coil region of myosin X forms a highly stable, antiparallel coiled coil dimer (anti-CC). Either disruption of the anti-CC via single point mutations or by replacement of the anti-CC with a parallel coiled-coil with a similar length compromised the filopodial induction activity of myosin X. We further show that the anti-CC and the single α-helical domain of myosin X are connected by a semi-rigid helical linker. The anti-CC-mediated dimerization may enhance myosin X to walk on both single and bundled actin filaments. The unexpected dimerization mechanism discovered in Myo10 may also be relevant to other coiled coil-containing myosins. Finally, the anti-CC-mediated dimerization mode of Myo10 provides hints for designing nano-scale molecular devices with desired functions. This part of the work has been published in *PNAS* in 2012.

In addition to MyoX, we have also investigated the lipid and protein cargo recognitions of a number of other unconventional myosins including myosin VI, myosin V, and myosin IC, and published a series of papers in journals such as *JBC, PNAS, and Nat Struct Mol Biol*. The PI was also invited to contribute a review in *Acc Chem Res* to systematically summarize the cargo recognition mechnisms by unconventional myosins. Due to the space limit, I will not go into any details. All the papers are listed in the table in Part C.

IV. Structure, function, and regulation of the scaffolding functions of ankyrins

Ankyrin-R/B/G (encoded by *ANK1/2/3* respectively) are a family of very large scaffold proteins capable of anchoring numerous receptors and ion-channels to specific, spectrin-containing membrane micro-domains. Hereditary mutations of ankyrins are known to be associated with diseases including spherocytosis, cardiac arrhythmia, and bipolar disorder in humans, although the underlying molecular bases are poorly understood. The middle

spectrin-binding domain of ankyrins contains highly conserved ZU5-ZU5-UPA-DD domains arranged into the ZZUD tandem. Curiously, most of the disease-causing mutations in the tandem have no apparent impact on the spectrin binding of ankyrins. The high resolution structure of the ankyrin-B ZZUD tandem determined here reveals that the ZU5-ZU5-UPA domains form a tightly packed structural supramodule, whereas DD is freely accessible. Although the formation of the ZZU supramodule does not influence the spectrin binding of ankyrins, mutations altering the inter-domain interfaces of ZZU impair the functions of ankyrin-B&G. Our structural analysis further indicates that the ZZU supramodule of ankyrins has two additional surfaces that may bind to targets other than spectrin. Finally, the structure of the ankyrin ZZUD provides mechanistic explanations to many disease-causing mutations identified in ankyrin-B&R. This work has been published in *PNAS* in 2012.

We have made breakthroughs in understanding how ankyrins bind to the membrane associated receptors and ion channels. We determined the crystal structure of 24 ankyrin repeats of AnkB in complex with an autoinhibition fragment from the ankyrin tail. This ankyrin repeats structure enables us to elucidate the general mechanism governing the ankyrin repeats-mediated bindings to membrane targets such as sodium channels and neuronal cell adhesion molecules. The findings are also valuable in understanding many human diseases (e.g. including hemolytic anemia, cardiac arrhythmia, bipolar disorder and schizophrenia) caused by mutations of ankyrins. The work has been published in *eLife* at the end of 2014.

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

A number of research projects covered in this CRF proposals have potential to be developed into new research directions. For example, the research on unconventional myosin Ic opens up a new direction to study this largest subfamily of unconventional myosins that play critical functions in very broad physiologies include hearing, vision, digestive systems, mechano-biology, diabetes, etc. Our study on ankyrins is extremely exciting, as this may offer insights into why mutations of ankyrin-B/G can lead to various forms of psychiatric disorders, our identification of aPKC substrate recognition mechanism and aPKC phosphorylation-mediated MAGUK/target interactions may establishe a new paradigm on how MAGUK/target interactions may be regulated in various tissues. All these projects are being developed into new research directions, and some of them are yielding wonderful results.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

This CRF team is relatively small (5 members in total), and has had a long history of collaboration in the past. The team officially met once a year. However, the PI and Co-PIs and members from all three labs met very regularly. For example, Prof. Zhang's lab and Prof. Huang's lab have had regular joint lab meetings over the entire grant period. Prof. Zhao and her lab visited HKUST members at least 10 times in the entire grant period. Prof. Wen and Prof. Zhang and some of their lab members meet on weekly basis. Such extensive interactions have played critical roles for the success of this CRF team. In the past reporting period, our collaborations have led to several important publications including one in *PNAS*, one in *Blood*, and one in *Nature Communication* (see Section 7). A number of on-going collaboration projects are also progressing well, and form a wonderful base for our future collaborations.

7. The Layman's Summary

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

Phosphoinositides (PIPs) are important signaling lipids that are distributed in various cellular membranes. PIPs, via binding to proteins, actively regulate numerous cellular processes. In this project, we have investigated the structures and functions of a series of protein-lipid interactions that are implicated in both normal functions as well as the etiology of brain and heart muscle cells. We elucidated the biochemical and structural bases of the interactions between PIPs and these proteins and to uncover the physiological significance of these newly identified protein-lipid interactions. The research team has published a large list of research articles in the prestigious journals in the areas of biochemistry, structural biology, neuroscience, and developmental biology. These research outcomes have made important contributions in understanding a number of human diseases including neurodegenerative diseases and cancers.

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Part C: Research Output

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

(Please attach a copy of the 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.)

9. The Latest Status of Publications 10.				Author(s) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*
Year of publication	Year of Acceptance (For paper accepted but not yet published)	Under Review	Under Preparation (optional)					
2011*				Lu, Q., Yu, J., Yan, J., Wei, Z., and Zhang, M.*	"Structural basis of the myosin X $PH1_N$-PH2-$PH1_C$ tandem as a specific and acute cellular $PI(3,4,5)P_3$ sensor" *Mol Biol Cell* **22**, 4268-4278.	Yes	No	Yes
2011*				Pan, L., Chen, J., Yu, J., Yu, H., and Zhang, M.*	"The Structure of the PDZ3-SH3-GuK Tandem of ZO-1 Suggests a Supramodular Organization of the MAGUK Family Scaffold Protein Core" *J Biol Chem.* **286**, 40069-40074.	Yes	No	Yes
2011*				Zhu, J., Shang, Y., Xia, C., Wang, W., Wen, W.*, and Zhang, M.*	"Guanylate Kinase Domains of the MAGUK Family Scaffold Proteins as Specific Phospho-Protein Binding Modules" *EMBO J* **30**, 4986 - 4997.	Yes	No	Yes
2011*				Bernauer, J., Huang, X., Sim, A.Y.L., Levitt, M.*	"Fully differentiable coarse-grained and all-atom knowledge-based potentials for RNA structure evaluation", *RNA* **17**, 1066-1075	Yes	No	Yes
2011*				Silva, D., Bowman, G.R., Sosa-Peinado, A., Huang, X*	"A Role for Both Conformational Selection and Induced Fit in Ligand Binding by the LAO Protein", *PLoS. Comp. Bio.* **7**, e1002054	Yes	No	Yes
2012*				Li, X., Funderburk, S. F., Che, K. H., Pan, L., Zhang, M., Yue, Z., and	"Imperfect and metastable dimer interface of Beclin 1 coiled coil domain is critical for association with Atg14L and	Yes	No	Yes

				Zhao, Y.*	UVRAG" *Nat Comms* **3**:662 doi:10.1038/ncomms1648 .			
2012*				Wang, C., Yu, C., Ye, F., Wei, Z., and Zhang, M.*	"Structure of the ZU5-ZU5-UPA-DD tandem of ankyrin-B reveals interaction surfaces necessary for ankyrin function" *PNAS* **109**, 4822-4827.	Yes	No	Yes
2012*				Da, L., Wang, D., Huang, X.*	"Dynamics of Pyrophosphate Ion Release and Its Coupled Trigger Loop Motion from Closed to Open State in RNA Polymerase II", *J. Am. Chem. Soc.*, **134**, 2399	Yes	No	Yes
2012*				Li X, He L, Zhang M, Yue Z, Zhao Y.	The BECN1 coiled coil domain: An "imperfect" homodimer interface that facilitates ATG14 and UVRAG binding. *Autophagy*. **8**, 1258-1260.	Yes	No	Yes
2012*				Zhang, L., Silva, D.A., Yan, Y.J., Huang, X.	"Force Field Development for Cofactors in the Photosystem II ", *J. Comput. Chem.*, **33**, 1969–1980	Yes	No	Yes
2012*				Wang, C., Shang, Y., Yu, J., and Zhang, M.*	"Substrate Recognition Mechanism of Atypical Protein Kinase Cs Revealed by the Structure of PKCι in complex with a Substrate Peptide from Par-3" *Structure* **20**, 791-801.	Yes	No	Yes
2012*				Jin, H., Li, L., Xu, J., Zhen, F., Zhu, L., Liu, P., Zhang, M., Zhang, W., and Wen, Z.*	"Runx1 regulates embryonic myeloid fate choice in zebrafish through a negative feedback loop that confines Pu.1 expression" *Blood* **119**, 5239-5249.	Yes	No	Yes
2012*				Yu, C, Lou, J., Wu, J., Pan, L., Feng, W.*, and Zhang, M.*	"Membrane-induced lever arm expansion allows myosin VI to walk with large and variable step sizes" *J Biol Chem* **287**, 35021-35035.	Yes	No	Yes
2012*				Wu, L., Pan, L.,	"Large protein assemblies	Yes	No	Yes

				Zhang, C., and Zhang, M.*	formed by multivalent interactions between cadherin23 and harmonin suggest a stable anchorage structure at the tip link of stereocilia" *J Biol Chem* **287**, 33460-33471.			
2012*				Jia, M., Li, J., Zhu, J., Wen, W., Zhang, M.*, and Wang, W.*	"Crystal Structures of the Scaffolding Protein LGN Reveal the General Mechanism by Which GoLoco Binding Motifs Inhibit the Release of GDP from Gαi" *J Biol Chem***287**, 36766-36776.	Yes	No	Yes
2012*				Huo, L., Yue, Y., Yu, J., Yu, Y., Ye, F., Xu, T.*, Zhang, M.*, and Feng, W.*	"The CC1-FHA tandem as a central hub for controlling the dimerization and activation of kinesin-3 KIF1A" *Structure* **20**, 1550-1561.	Yes	No	Yes
2012*				Lu, Q., Ye, F., Wei, Z., Wen, Z., and Zhang, M.*	"Antiparallel Coiled Coil-Mediated Dimerization of Myosin X" *PNAS* **109**, 17388-17393.	Yes	No	Yes
2012*				Da, L., Wang, D., Huang, X.	"Dynamics of Pyrophosphate Ion Release and Its Coupled Trigger Loop Motion from Closed to Open State in RNA Polymerase II", **J. Am. Chem. Soc.**, 134 (4), 2399.	Yes	No	Yes
2012*				Zhang, L., Silva, D.A., Yan, Y.J., Huang, X.	"Force Field Development for Cofactors in the Photosystem II ", **J. Comput. Chem**., 33 (25), 1969–1980.	Yes	No	Yes
2013*				Pan, Z., Shang, Y., Jia, M., Zhang, L., Xia, C., Zhang, M., Wang, W*., and Wen, W*.	"Structural and Biochemical Characterization of the Interaction between LGN and Frmpd1" *J Mol Biol.* **425**, 1039-1049.	Yes	No	Yes
2013*				Chen, S.*, Chen, J., Shi, H., Wei, M., Castaneda-Castellanos, D. R., Bultje, R.S., Pei, X., Kriegstein,	"Regulation of Microtubule Stability and Organization by Mammalian Par3 in Specifying Neuronal Polarity" *Dev Cell* **24**, 26-40.	Yes	No	Yes

				A.R., Zhang, M., and Shi, S.-H.*				
2013*				Zhang, Y., Wang, W., Chen, J., Zhang, K., Gao, F., Gao, B., Zhang, S., Dong, M., Besenbacher, F., Gong, W., Zhang, M., Sun, F.*, and Feng, W.*	"Structural Insights into the Intrinsic Self-Assembly of Par-3-N-Terminal Domain" *Structure* **21**, 997-1006.	Yes	No	Yes
2013*				Pan, Z., Zhu, J., Shang, Y., Wei. Z., Jia, M., Xia, C., Wen, W., Wang, W.*, and Zhang, M.*	"An auto-inhibited conformation of LGN reveals a distinct interaction mode between GoLoco motifs and TPR motifs" *Structure* **21**, 1007-017.	Yes	No	Yes
2013*				Wei, Z.*, Liu, X., Yu, C., and Zhang, M.*	"Structural Basis of Cargo Recognitions for Class V Myosins" *PNAS* **110**, 11314-11319.	Yes	No	Yes
2013*				Ye, F, and Zhang, M.*	"Structures and Target Recognition Modes of PDZ Domains: recurring themes and emerging pictures" *Biochem J*, **455**, 1-14.	Yes	No	Yes
2013*				Da, L., Pardo-Avila, F., Wang, D., Huang, X.	"A Two-State Model for the Dynamics of the Pyrophosphate Ion Release in Bacterial RNA Polymerase" **PLoS. Comp. Bio**., 9(4): e1003020.	Yes	No	Yes
2013*				Pardo-Avila, F., Da, L., Wang, Y., Huang, X.	"Theoretical Investigations on Elucidating Fundamental Mechanisms of Catalysis and Dynamics Involved in Transcription by RNA Polymerase" **J. Theor. Comput. Chem**., 12(8), 1341005.	Yes	No	Yes
2013*				Qiao, Q., Bowman, G.R., Huang, X.,	"Dynamics of an intrinsically disordered protein reveal metastable conformations that potentially seed aggregation", **J. Am. Chem. Soc**., 135 (43),	Yes	No	Yes

					16092–1610			
2014				Zhu, J., Shang, Y., Wan, Q., Xia, Y., Chen, J., Du, Q., and Zhang, M.*	"Phosphorylation-dependent interaction between tumor suppressors Dlg and Lgl" Cell Res **24**, 451-463.	No	Yes	Yes
2014				Lu, Q., Li, J., and Zhang, M.*	"Cargo Recognition and Cargo-mediated Regulation of Unconventional Myosins" *Acc Chem Res* **47**, 3061-3070.	No	Yes	Yes
2014				Yan, B., Han, P., Pan, L., Lu, W., Xiong, J., Zhang, M., Zhang, W., Li, L., and Wen, Z.*	"IL-1β and reactive oxygen species differentially regulate neutrophil directional migration and Basal random motility in a zebrafish injury-induced inflammation model". *J Immunol.* **192**, 5998-6008.	No	Yes	Yes
2014				Wang, C., Wei, Z., Chen, K., Ye, F., Yu, C., Bennett, V., and Zhang, M.*	"Structural Basis of Diverse Membrane Target Recognitions by Ankyrins" *eLife* **3**:e04353.	No	Yes	Yes
2015				Lu, Q., Li, J., Ye, F., and **Zhang, M.**	"Structure of myosin-1c tail bound to calmodulin provides insights into calcium-mediated conformational coupling" *Nat Struct Mol Biol* **22**, 81-88.	No	Yes	Yes

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9.Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each conference abstract)*

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 RGC *(Yes or No)*
July/2011/New London, NH, USA	"Cargo recognition mechanisms of MyTH4-FERM tandem unconventional myosins"	Gordon Research Conference (Muscle & Molecular Motors)	No	No	Yes
Oct/2012/Beijing, China	"Assembling highly specific signaling complexes by multiple weak but coincident interactions"	Weak Protein-Ligand Interactions: New Horizons in Biophysics and Cell Biology Meeting (Biophysical Society, USA)	No	No	Yes
Oct/2013/Dalian, China	"Viewing Brain Disorders at the Atomic Scale"	Ideaslab: Breakthroughs in Brain Research, World Economic Forum Summer Davos 2013	No	No	Yes
June/2014/Suzhou, China	"Structural basis of Ankyrin-mediated membrane patterning"	5th CSHA Symposium: Structural Biology - From Atoms to Cells.	No	No	Yes
July/2014/Stonehill College, Easton, MA, USA	"Ankyrin and Intrinsically Disordered Membrane Target Interactions: Assembling Large Complexes for Fast Signaling in Excitable Tissues"	Gordon Research Conference on "Intrinsically Disordered Proteins"	No	No	Yes

**Conference abstracts are generally not regarded as official publications in life science, I therefore only listed a few of such conference contributions that the PI acted as an invited speaker..*

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
Jia Chen	Ph.D.	Sept. 1, 2005	Dec 2011
Chihao Wang	Ph.D.	Sept. 1, 2007	Dec 2012
Fei Ye	Ph.D.	Sept. 1, 2008	Aug 2013
Qing Lu	Ph.D.	Sept. 1, 2009	Aug. 2014
Yutong Zhao	MPhil	Sept. 1, 2011	Aug. 2012
Xiaoxiao He	MPhil	Feb. 1, 2011	Aug. 2013
Bo Yan	Ph.D.	Sept. 1, 2008	Sept. 2012

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