Project number: AoE/M-09/12

RESEARCH GRANTS COUNCIL
AREAS OF EXCELLENCE (AoE) SCHEME

Completion Report on Funded Project

Project start date: 01 Oct 2013
Project completion date: 30 Sep 2022

1. Project Title: Mechanistic basis of synaptic development, signalling and neuro-disorders

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the whole project period
Project Coordinator (PC)	WEN Zilong[1]/ Professor	LIFS / HKUST	12 hours/wk
Co-Principal Investigator(s)	HUANG Xuhui[2]/ Associate Professor	CHEM / HKUST	8 hours/wk
	LIU Kai/ Associate Professor	LIFS / HKUST	8 hours/wk
	QU Jianan/ Professor	ECE / HKUST	6 hours/wk
	WU Zhenguo/ Professor	LIFS / HKUST	6 hours/wk
	YAN Yan[3]/ Associate Professor	LIFS / HKUST	8 hours/wk

	ZHANG Mingjie[1]/ Adjunct Professor	LIFS / HKUST	12 hours/wk
	XIA Jiang[4]/ Professor	CHEM / CUHK	8 hours/wk
	ZHAO Yanxiang/ Professor	Dept of Applied Biology & Chemical Technology / PolyU	8 hours/wk
Collaborators	SONG Hongjun[5]	Department of Neuroscience/ University of Pennsylvania	N/A
	MING Guo-li[5]	Department of Neuroscience/ University of Pennsylvania	N/A
	SHEN Kang	Biology / Stanford University	N/A
	SHI Songhai	Dev Biol / Memorial Sloan Kettering Institute, New York	N/A
	LI Shawn	Biochemistry / University of Western Ontario	N/A

Please highlight the approved changes in the project team composition and quote the date when the UGC/RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

[1] The request for changing Prof. Mingjie Zhang's role (from PC to Co-PI) and Prof. Zilong Wen's role (from Co-PI to PC) with effect from 15 December 2020 upon the former's change of affiliation was approved by RGC on 23 December 2020.

[2] Prof Huang has withdrawn from the team with effect from 1 September 2021. This request was approved by RGC on 16 November 2021.

[3] Prof. Yan was promoted from Assistant Professor to Associate Professor effective from 1 July 2020.

[4] Prof. Xia was promoted to Full Professor effective from 1 August, 2021

[5] Prof. Song and Prof. Ming have been appointed as Professors in the Department of Neuroscience at the University of Pennsylvania since March 2017.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. Systematic characterization of the structure, function, and interaction networks of a set of key synaptic proteins involved in synaptic development	100%	Nil

Objectives*	Percentage achieved	Remarks**
and signaling		
2. The mechanisms underlying synaptic developmental and signaling defects caused by mutations of these gene products and the impacts of such mutations on mental illnesses	100%	Nil
3. Developing peptides, peptide mimetics, and low molecular weight chemical compounds that can specifically target some of these synaptic proteins for biomedical research as well as potential therapies against mental disorders	100%	Nil

* *Please highlight the approved changes in objectives and quote the date when the UGC/RGC granted approval of such changes.*

** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

6.1 What are the most exciting research accomplishments of the project?
(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

The project team has been extremely successful in executing the proposed objectives. The success of this team is evidenced by their establishment of an internationally leading research center focused on the molecular basis of neuronal synapse formation and signaling. In particular, the team's research has revealed that phase separation is a new molecular paradigm underlying synapse formation and synaptic plasticity. The team has also made significant strides in studying the development of microglia and their function in specific brain regions. The scientific achievement of this team is evidenced by their publishing more than 200 papers in journals including *Cell, Neuron, Mol Cell, Dev Cell, Nat Neurosci, J Cell Biol, Cell Res, PNAS,* and *eLife* (see **Annex 1**). Moreover, 95 graduate students have been trained, many of whom have now established their own independent labs around the world (see **Section 6.6**). The following summarizes the major scientific activities and achievements of this AoE team.

I: Systematic characterization of the structure, function, and interaction networks of a set of key synaptic proteins involved in synaptic development and signaling

Eukaryotic cells orchestrate numerous biochemical reactions spatiotemporally by segregating each into structurally and functionally distinct compartments. In addition to classical membrane-enclosed cellular compartments, increasing evidence points to a diverse class of cellular compartments that either lack membranes or are not enclosed by membranes, formed by a physical process known as liquid-liquid phase separation and frequently referred to as membraneless compartments or biological condensates. Neurons take cellular compartmentalization to extremes due to their elaborate morphologies. In addition to membrane-enclosed organelles and membraneless condensates common to other cell types, neurons contain a unique type of membrane-semi-enclosed compartment known as synapses, which are molecular apparatuses that dictate signal processing and transmission in all nervous systems. Neither pre- nor postsynaptic compartments are enclosed by membrane bilayers. Beneath the postsynaptic plasma membranes of each synapse reside a condensed protein-rich sub-compartment known as the postsynaptic density (PSD), a structure responsible for receiving, amplifying, and storing signals initiated by presynaptic cells. PSDs are composed of densely packed proteins forming mega-assemblies. A prominent feature of PSDs is that one side of the dense assembly is attached to the postsynaptic plasma membranes while the other is exposed to the dendritic spine cytoplasm. Thus, PSDs are condensed subcellular compartments that are not enclosed by lipid membranes. In two papers published in *Cell* in 2016 and 2018 by this team (Zeng, et al., *Cell* 2016&2018), we provided evidence that PSDs are likely formed via liquid-liquid phase transition.

We further demonstrated that the presynaptic active zone may also be formed via phase separation (Wu, et al., 2019 *Mol Cell;* Wu, et al., 2021 *Mol Cell*). Another major research direction of this AoE-supported research was the investigation of the way in which phase separation may contribute to the organization of the presynaptic bouton in synapses. The timing and kinetics of neurotransmitter release depend on the relative positioning of clustered Ca^{2+} channels in active zones (AZs) to docked synaptic vesicles (SVs) on presynaptic plasma membranes. However, how AZs form remains unknown. In this study, we showed that RIM and RIM-BP, via specific multivalent interactions, form dynamic and condensed assemblies through liquid-liquid phase separation. Voltage-gated Ca^{2+} channels (VGCCs), via C-terminal tail-mediated direct binding to both RIM and RIM-BP, can be enriched to RIM/RIM-BP condensates. We further showed that RIM and RIM-BP together with VGCCs form dense clusters on supported lipid membrane bilayers via phase separation.

Therefore, RIMs and RIM-BPs are plausible organizers of AZs, and the formation of RIM/RIM-BP condensates may cluster VGCCs into nano- or micro-domains as well as position the clustered Ca^{2+} channels with Ca^{2+} sensors on docked vesicles for efficient and precise synaptic transmissions. This work was published in *Mol Cell* in 2019 (Wu et al., (2019) *Mol Cell* **73**, 971-984). Next, we investigated how different populations of SVs in presynaptic boutons are organized. Tethering of SVs to the AZ determines synaptic strength, although the molecular basis governing SV tethering is rather elusive. We discovered that small unilamellar vesicles (SUVs) and SVs, purified from rat brains, are coated on the surface of liquid condensates formed by AZ proteins RIM, RIM-BP, and ELKS. Remarkably, SUV-coated RIM/RIM-BP condensates are encapsulated by synapsin/SUV condensates, forming two distinct SUV pools reminiscent of the reserve and tethered SV pools that exist in presynaptic boutons. SUV-coated RIM/RIM-BP condensates can further cluster Ca^{2+} channels anchored on membranes. Thus, we have reconstituted a presynaptic bouton-like structure that mimics SV-tethered AZs with one side attached to the presynaptic membrane and the other connected to the synapsin-clustered SV condensates. The distinct interaction modes between membraneless protein condensates and membrane-based organelles revealed here may possess general implications in other cellular processes, including vesicular trafficking, organelle biogenesis, and autophagy. This work was published in *Mol Cell* in 2021.

While our findings on phase separation-mediated PSD molecular assembly formation may introduce a paradigm shift in understanding synapse formation, a key challenge was to provide experimental support that shows phase separation is indeed linked to synaptic physiology. The team's research in the second phase of this project focused on the connection between phase separation and synaptic functions. Two papers were published in this direction (one in *Neuron,* showing phase separation-mediated AMPAR receptor synaptic trafficking and signaling (Zeng et al., 2019), and another in *Mol Cell,* showing that the formation of modular enzyme complex condensates via phase separation can dynamically concentrate limited quantities of enzymes to distinct cellular compartments for specific and optimal signaling (Zhu et al. 2020). Recently, we have also investigated how Arc, a scaffold protein and an immediate early gene product, can specifically facilitate synaptic removal of AMPARs. We found that Arc directly antagonizes with PSD-95 in binding to TARPs, which are the auxiliary subunits of AMPARs. Arc, in a highly concentration-sensitive manner, acutely disperses TARPs from the PSD condensate formed via phase separation. TARPs with the Ser residue in the "P-S-Y"-motif of its tail phosphorylated are completely refractory from being dispersed by Arc, suggesting that Arc cannot displace AMPARs from PSDs in active synapses. Conversely, strengthening the interaction between Arc and TARPs enhances Arc's capacity in weakening synapses. Thus, Arc can specifically and effectively modulate synaptic AMPAR clustering via modulating PSD phase separation. Our study further suggests that activity-dependent, bi-directional modulation of PSD condensate formation/dispersion represents a general regulatory mechanism for synaptic plasticity. This study was published in *Cell Res* in 2022 (Chen et al., 2022).

IRSp53 (aka BAIAP2) is abundantly expressed in excitatory synapses and is essential for synapse development and synaptic plasticity. It is a scaffold protein that couples membranes with the cytoskeleton in actin-filled protrusions such as filopodia and lamellipodia, though with poorly understood mechanisms. We showed that specific multivalent interactions between IRSp53 and its binding partners PSD-95 or Shank3 drive phase separation of the complexes in solution. IRSp53 can be enriched to the reconstituted excitatory PSD (ePSD) condensates via bridging to the core and deeper layers of ePSD. Overexpression of mutations defective in the IRSp53/PSD-95 interaction perturbs synaptic enrichment of IRSp53 in mouse cortical neurons. The reconstituted PSD condensates promote bundled actin filament formation both in solution and on membranes, via IRSp53-mediated actin binding and bundling. Overexpression of mutants that perturb IRSp53/actin interaction leads to defects in synaptic maturation of cortical neurons. Together, our studies provide potential mechanistic insights into the physiological roles of IRSp53 in synapse formation and function. This study was published in *J Cell Biol* recently (Feng et al., 2022).

One of the most important questions in synaptic biology is how neuronal activity is linked to synaptic structural changes and eventually synaptic strength alterations. However, it is extremely challenging to elucidate molecular events that govern synaptic plasticity, due to the inherent complexity and awkward size of synapses for direct experimental access. Our reconstituted PSD platform is a wonderful system for exploring the regulation of PSDs by protein kinases such as CaMKII and protein phosphatases, including PP1 and PP2A. We discovered that Shank3 is a specific binding partner for autoinhibited CaMKIIα. We demonstrated that Shank3 and GluN2B, via combined actions of Ca^{2+} and phosphatases, reciprocally bind to CaMKIIα. Under basal conditions, CaMKIIα is recruited to the Shank3 sub-compartment of PSDs via phase separation. A rise in Ca^{2+} concentration induces GluN2B-mediated recruitment of active CaMKIIα and formation of GluN2B/PSD-95/CaMKIIα condensates, which are autonomously dispersed upon Ca^{2+} removal. Protein phosphatases control Ca^{2+}-dependent shuttling of CaMKIIα between the two PSD sub-compartments and PSD condensate formation. Activation of CaMKIIα further enlarges the PSD assembly and induces structural long-term potentiation (LTP). Thus, Ca^{2+}-induced and phosphatase-checked shuttling of CaMKIIα between distinct PSD nanodomains can regulate phase separation-mediated PSD assembly and synaptic plasticity. Perhaps most excitingly, we have been able to reconstitute synaptic plasticity and structural LTP in the study. In this reconstituted system, the addition of Ca^{2+} to basal PSD mixtures leads to enhanced PSD assembly and growth. Removal of Ca^{2+} from stimulated PSD mixtures disperses PSD condensates and autonomously converts the system back to the basal state. Such activity-dependent structural plasticity is supported by the balanced actions of CaMKIIα and phosphatases. Thus, our study offers an extremely simple molecular model underpinning LTP and long-term depression (LTD) of synapses. Being able to investigate synaptic plasticity using a biochemically defined system offers unprecedented opportunities and advantages for elucidating molecular mechanisms governing synapse formation and function. Additionally, our study also provides an extremely exciting example that demonstrates how phase separation is adopted by neurons to perform the most fundamental functions in inter-cellular communication. This study was published in *Cell Res* in 2021 (Cai et al., 2021).

In collaboration with Dr. Yasunori Hayashi at Kyoto University, we have also found that activated CaMKII undergoes liquid-liquid phase separation with the NMDA-type glutamate receptor subunit, GluN2B. Due to CaMKII autophosphorylation, the condensate stably persists even after Ca^{2+} removal. Selective binding of activated CaMKII with GluN2B co-segregates AMPA receptors and the synaptic adhesion molecule, neuroligin, into a phase-in-phase assembly, which models the nanodomain structures formed in PSDs. In this way, Ca^{2+}-induced liquid-liquid phase separation of CaMKII has the potential to act as an activity-dependent mechanism to crosslink postsynaptic proteins, which could serve as a platform for synaptic reorganization associated with synaptic plasticity. This study was published in *Nat Neurosci* in 2021 (Hosokawa et al., 2021).

Two major isoforms of CaMKII, CaMKIIα and CaMKIIβ, play distinct roles in supporting synaptic transmission and LTP with unknown underlying mechanisms. In collaboration with Dr. Roger Nicoll in University of California, San Francisco, we demonstrated that the length of the unstructured linker between the kinase domain and the oligomerizing hub domain of CaMKII determines the ability of the enzyme to rescue the basal synaptic transmission and LTP defects caused by removal of both CaMKIIα and CaMKIIβ (DKO) in mouse brain. Remarkably, although CaMKIIβ has a similar affinity for binding to GluN2B as CaMKIIα, only CaMKIIα with the short linker forms robust dense clusters with GluN2B via phase separation. Lengthening the linker of CaMKIIα with unstructured "Gly-Gly-Ser" repeats impairs its phase separation with GluN2B and the mutant enzyme cannot rescue the basal synaptic transmission and LTP defects of DKO mice. Our results demonstrate that the phase separation capacity of CaMKII with GluN2B is critical for its cellular functions in the brain. This study was published in *Cell Rep* in 2023 (Cai et al., 2023).

Formation of membraneless organelles or biological condensates via phase separation and related processes hugely expands the cellular organelle repertoire. Biological condensates are dense and viscoelastic soft matter instead of canonical dilute solutions. Though numerous different

biological condensates have been discovered, mechanistic understanding of biological condensates remains poor. During this project period, we developed an adaptive single-molecule imaging method that allows simultaneous tracking of individual molecules and their motion trajectories in both condensed and dilute phases of various biological condensates. The method enables quantitative measurements of concentrations, phase boundary, motion behavior, and speed of molecules in both condensed and dilute phases as well as the scale and speed of molecular exchanges between the two phases. Notably, molecules in the condensed phase do not undergo uniform Brownian motion, but instead constantly switch between a (class of) confined state(s) and a random diffusion-like motion state. Transient confinement is consistent with strong interactions associated with large molecular networks (i.e., percolation) in the condensed phase. In this way, the behavior of molecules in biological condensates is distinctly different from those in dilute solutions, making the study a paradigm shift. This work has just been published in *eLife* (Shen et al., 2023). The methods and findings described in this work can aid in deciphering the molecular mechanisms underlying the assembly, dynamics, and consequently functional implications of biological condensates.

The series of publications produced by the team during this AoE grant period has provided a convincing body of evidence showing that the highly dense signaling complexes on both the pre- and post-synaptic sides of neuronal synapses are likely formed via liquid-liquid phase separation. These findings establish a new conceptual framework for understanding synapse formation and function, as well as brain disorders caused by synaptic signaling complex malfunction. We are pleased to report that our research findings are appreciated by colleagues in the field. We have been invited to write reviews on synapse formation via liquid-liquid phase separation by various journals including *Nat Neurosci, Dev Cell, Mol Cell, J Neurosci, J Biol Chem, J. Mol. Biol.* and *Biochemistry* (see **Annex 1**). Moreover, the PC has been invited to present our findings at numerous conferences and institutes/universities. Our systematic studies on phase separation have also sparked collaborations with many colleagues from different parts of the world.

II: The mechanisms underlying synaptic developmental and signaling defects caused by mutations of these gene products and the impacts of such mutations on mental illnesses

Our reconstituted PSD condensates are simple and chemically well defined. The system could be used to investigate the roles of other PSD proteins in regulating PSD assembly formation and dynamics. The information derived from such a reconstitution system, when combined with results of experiments performed in living neurons, may offer valuable insights for understanding the roles of these proteins in synaptic formation and functions. Additionally, given that a very large number of brain disorders may be caused by mutations of genes encoding synaptic proteins, the reconstitution system could also be extremely useful for investigating how mutations of the PSD components may alter PSD structure and functions. We have published several papers in this area in the past year. For example, we discovered that Shank3, using its N-terminal domain-ankyrin repeats (NTD-ANK) tandem, specifically interacts with CaMKIIα. Interaction depends on the conformational and activity status of the enzyme. Using a reconstituted PSD *in vitro*, we demonstrated that inactive CaMKIIα is enriched in the Shank3 sub-compartment of PSD. Importantly, mutations of Shank3 (e.g. R12C) found in patients with autism spectrum disorder are defective in binding to CaMKII, and mutant Shank3 fails to recruit CaMKII into reconstituted PSD condensates. This part of the research was published in *Cell Res* in 2021. In collaboration with Dr. Guoping Feng's laboratory in MIT, we generated R12C-Shank3 knock-in mice. We found that mice bearing R12C-Shank3 are defective in LTP (unpublished results), indicating that the Shank3/CaMKII interaction identified in our study is important for PSD assembly regulation.

We have also demonstrated via biochemical and structural studies that Shank3 NTD-ANK tandem specifically binds to the GTP-bound form of HRas and Rap1. In addition to the canonical site that is mediated by the Ras-association domain and common to both GTPases, Shank3 NTD-ANK contains an additional unconventional Rap1 binding site that is formed by NTD and ANK together.

Shank3 NTD-ANK binding to GTP-loaded Rap1 prevents its inactivation by SynGAP, a major GTPase-activating enzyme in synapses. Using Förster resonance energy transfer-fluorescent lifetime imaging microscopy (in collaboration with Dr. Yasunori Hayashi at Kyoto University), we further showed that the interactions between Shank3 and HRas/Rap1 at excitatory synapses in neurons are triggered by synaptic activation. Thus, Shank3 may sustain the signaling of Ras family proteins in the PSD via directly binding to and stabilizing the GTP-bound form of the enzymes (Cai et al., *Structure* (2020)). CASK, a member of the MAGUK family scaffold proteins, forms evolutionarily conserved tripartite complexes with Mint1 and Veli, critical for neuronal synaptic transmission and cell polarity. The CASK CaM kinase (CaMK) domain, in addition to interacting with Mint1, can also bind to many different target proteins, although the mechanism governing CASK-CaMK/target interaction selectivity remains unclear. We demonstrated that an extended sequence in the N-terminal unstructured region of Mint1 binds to CASK-CaMK with a dissociation constant of ~7.5 nM. The high-resolution crystal structure of CASK-CaMK in complex with this Mint1 fragment reveals that the C-lobe of CASK-CaMK binds to a short sequence common to known CaMK targets and the N-lobe of CaMK engages an α-helix that is unique to Mint1. Biochemical experiments together with structural analysis reveal that the CASK and Mint1 interaction is not regulated by Ca^{2+}/CaM.

Cross-disciplinary and in-depth collaborations among our team members are a hallmark of this AoE team. Such collaborations have produced a series of ground-breaking publications with potentially deep impacts. One of the most exciting examples is a series of collaborations between Qu's lab with engineering expertise and Wen, Liu, and Wu's labs with developmental biology, neuroscience, and cell biology expertise. Directly relevant to this AoE project is that this interdisciplinary team has been investigating, using zebrafish models, how microglia originate and how these cells are colonized to and function in specific brain regions (four *Dev cell* papers published between 2015 to 2019, a paper in *J Exp Med* in 2017, two papers in *eLife* with one in 2018 and the other in 2020, one paper in *Science Advances* in 2020, two papers in *PNAS* in 2022, and two papers in *Cell Rep* in 2023). The Scientific Advisory Board members of this AoE team commented: "*the second subject concerns microglia, an exciting new line of investigation that would not be possible without the interdisciplinary approach fostered by this AoE grant*".

Microglia are the major immune cells in the central nervous system (CNS). Born in peripheral hematopoietic tissues, microglial precursors colonize the CNS during early embryogenesis and maintain themselves thereafter. The role of microglia in diverse aspects of nervous system functions and pathophysiology are becoming increasingly important in neuroscience. This team, led by Dr. Wen, has been pioneering the development of microglia using zebrafish as the model system. Dr. Wen's group recently identified two distinct microglial populations – phagocytotic microglia and regulatory microglia – in adult zebrafish, that differ in morphology, distribution, development, and function. Phagocytotic microglia are the predominant population, which is broadly distributed, amoeboid in shape, highly mobile and phagocytotic, whereas regulatory microglia are white, matter-enriched, and possess ramified protrusions, but have limited mobility and phagocytosis capability. The functional differences between the two distinct microglial populations are further supported by distinct transcriptomes and responses to bacterial infection, where phagocytotic microglia function in tissue clearance and regulatory microglia release immune regulators. This study was published in *Science Advances* in 2020. In addition, through collaboration with Dr. Qu, the team developed a high-precision infrared laser-evoked gene operator heat-shock system, which harnesses laser-induced $CreER^{T2}$ combined with loxP-DsRedx-loxP-GFP reporter to achieve precise single-cell labeling and tracing. They further showed that this system can precisely label single cells in the brain, muscle, and hematopoietic system of zebrafish embryos *in vivo*. This work was published in *eLife* in 2020. The work directly led to the team being invited to write a review on this specific topic for *Experimental Hematology*. Immune cells in mucosal barriers of vertebrates are highly heterogenous in their origin and functions. This is exemplified by recent findings indicating the existence of ectoderm-derived immune cells - metaphocytes in zebrafish epidermis. However, whether non-hematopoiesis-derived immune cells generally exist in barrier tissues remains unknown.

Dr. Wen's team reported the identification and characterization of an endoderm-derived immune cell population in the gills and intestine of zebrafish. Transcriptome analysis revealed that these endoderm-derived immune cells are tissue-resident macrophage-like cells with high similarity to the ectoderm-derived metaphocytes in the epidermis. These cells are non-phagocytic but specialize in taking up external soluble antigens through transepithelial protrusions. Depletion of the endoderm-derived immune cells in the gills hinders the local immune response to external soluble stimulants. This study demonstrates the existence of non-hematopoiesis-derived immune cells in the mucosal barriers of zebrafish, which challenges the prevalent view that resident immune cells in vertebrate mucosal barriers arise exclusively from hematopoiesis. This work was published this year in *Cell Rep*.

Previous studies have shown that naturally occurring neuronal apoptosis plays a critical role in regulating microglial colonization of the brain in zebrafish. However, the molecular signaling cascades underlying neuronal apoptosis-mediated microglial colonization and the regulation of these cascades remain undefined. Prof. Wen's team discovered that basic leucine zipper (b-Zip) transcription factors, Mafba and Mafbb, two zebrafish orthologues of mammalian MAFB, are key regulators in neuronal apoptosis-mediated microglial colonization of the brain in zebrafish. We document that the loss of Mafba and Mafbb function perturbs microglial colonization of the brain. We further demonstrate that Mafba and Mafbb work together in a cell-autonomous manner to orchestrate microglial colonization, at least in part, by regulating the expression of G protein-coupled receptor 34a (Gpr34a), which directs peripheral macrophage recruitment into the brain through sensing the lysophosphatidylserine (lysoPS) released by the apoptotic neurons. Our study reveals that Mafba and Mafbb regulate neuronal apoptosis-mediated microglial colonization of the brain in zebrafish via lysoPS-Gpr34a pathway. This study was published in *PNAS* in 2022. In another study, Prof. Wen's team revealed that, in zebrafish, microglia arise from two sources, rostral blood island (RBI) and aorta-gonad-mesonephros (AGM). The RBI-derived microglia are born early but have a short lifespan and diminish in adulthood, while the AGM-derived microglia emerge later and are capable of long-term maintenance in adulthood. They show that the attenuation of RBI microglia is due to their lower competitiveness for neuron-derived interleukin 34 (Il34) caused by age-dependent decline of colony-stimulating factor-1 receptor a (*csf1ra*). Alterations of Il34/Csf1ra levels and removal of AGM microglia expand the proportion and lifespan of RBI microglia. Remarkably, the *csf1ra/CSF1R* expression in zebrafish AGM-derived microglia and murine adult microglia also undergoes age-dependent decline, leading to the elimination of aged microglia. This study reveals cell competition as a general mechanism controlling the turnover and lifespan of microglia. This study was published in *Science Advances* in 2023.

The team has also been developing new technologies to explore frontiers in synaptic biology and other tissues. For example, Prof. Qu and Prof. Wen have been collaborating to develop single-cell resolution infrared laser-mediated heating system coupled with a fluorescence microscope for precise temperature control in living animals. This technique provides a unique noninvasive approach to single-cell labeling in vivo, which is of great significance in cell fate mapping and lineage tracing and enabled the team to published many papers as described above. The Qu lab has also been developing an adaptive optics two-photon endomicroscopy system integrated with a gradient refractive index lens, which enables recovery of diffraction-limited resolution in deep-brain imaging. Using this newly developed technology, Dr. Qu's group investigated neuronal plasticity in the hippocampus, a critical deep brain structure, and revealed the relationship between the somatic and dendritic activity of pyramidal neurons. This work was published in *Science Advances* in 2020. Dr. Qu's group has also developed an adaptive optics two-photon excitation fluorescence microscopy (AO-TPEFM) system for high-resolution and deep-brain imaging of synapse formation in living mouse brains. They have collaborated with Dr. Kai Liu, a member of this AoE team, to demonstrate that their AO-TPEFM system permits structural and functional imaging of mouse retina with submicron resolution. Simultaneous functional calcium imaging of neuronal soma and dendrites was demonstrated. Moreover, the time-lapse morphological alteration and dynamics of microglia were characterized in a

mouse model of retinal disorder. In addition, precise laser axotomy was achieved, and degeneration of retinal nerve fibres was studied. This high-resolution AO-TPEFM system is a promising tool for non-invasive retinal imaging and can facilitate the understanding of a variety of eye diseases as well as neurodegenerative disorders in the CNS. This study was published in *Science Advances* in 2020. In another study, Dr. Qu in collaboration with Dr. Liu developed a minimally invasive intervertebral window by retaining the ligamentum flavum to protect the underlying spinal cord. By introducing an optical clearing method, we achieved repeated two-photon fluorescence and stimulated Raman scattering imaging at subcellular resolution and observed no inflammatory response for up to six months. Using this optically cleared intervertebral window, we studied neuron-glia dynamics following laser axotomy and observed strengthened contact of microglia with the nodes of Ranvier during axonal degeneration. By enabling long-term, repetitive, stable, high-resolution, and inflammation-free imaging of mouse spinal cord, our method provides a reliable platform for the interpretation of spinal cord physiology and pathology. This study was published in *Nature Communications* in 2022.

High-resolution optical imaging deep in tissues is challenging because of optical aberrations and scattering of light caused by the complex structure of living matter. Prof. Qu's team took on this challenge and developed an adaptive optics three-photon microscope based on analog lock-in phase detection for focus sensing and shaping (ALPHA-FSS). ALPHA-FSS accurately measures and effectively compensates for both aberrations and scattering induced by specimens and recovers subcellular resolution at depth. A conjugate adaptive optics configuration with remote focusing enables in vivo imaging of fine neuronal structures in the mouse cortex through the intact skull up to a depth of 750 μm below the pia, enabling near-non-invasive high-resolution microscopy in cortex. Functional calcium imaging with high sensitivity and high-precision laser-mediated microsurgery through the intact skull were also demonstrated. Moreover, they achieved in vivo high-resolution imaging of the deep cortex and subcortical hippocampus up to 1.1 mm below the pia within the intact brain. This work was published in *Nature Biotechnology* in 2022.

III: Developing peptides, peptide mimetics, and low molecular weight chemical compounds that can specifically target some of these synaptic proteins for biomedical research as well as potential therapies against mental disorders.

The advancements made in *I and II* laid a solid foundation for the research in this objective. Here, we will briefly summarize our achievements in this direction.

We have been able to develop very specific, potent, and genetically encodable tools to modulate PSD-95/SAPAP interaction to investigate the role of the complex in synaptic activity regulation. In the first half of this project, we showed that the specific SAPAP/Shank interaction is critical for Shank synaptic targeting and Shank-mediated synaptogenesis. In this study, we showed that the PSD-95/SAPAP interaction, SAPAP synaptic targeting, and SAPAP-mediated synaptogenesis require phosphorylation of the N-terminal repeat sequences of SAPAPs. The atomic structure of the PSD-95 GK in complex with a phosphor-SAPAP repeat peptide reveals the molecular mechanism underlying the phosphorylation-dependent PSD-95/SAPAP interaction and also provides an explanation for a PSD-95 mutation found in patients with intellectual disabilities. Guided by the structural data, we developed potent non-phosphorylated GK inhibitory peptides capable of blocking the PSD-95/SAPAP interaction and interfering with PSD-95/SAPAP-mediated synaptic maturation and strength. These peptides are genetically encodable for investigating the functions of the PSD-95/SAPAP interaction *in vivo*. We went on to demonstrate that the most potent GK inhibitory peptide can effectively block the PSD-95/SAPAP interaction and impair synaptogenesis when expressed in cultured neurons. This paper was published in *Cell Rep* in 2017. This research was jointly conducted between Dr. Huang, Dr. Xia, and Dr. Zhang within the team and our external collaborators from Canada Drs. Shawn Li and Guoping Feng. Additionally, the Huang group, in collaboration with the Zhang group, has made substantial progress in applying computational biology

approaches to optimize the sequence of small peptide inhibitors of the GK domain of DLG MAGUKs. One major achievement is that we quantitatively demonstrated how entropy plays the dominant role in stapled peptide binding to MAGUK GK. Guided by the theory and the crystal structures of GK/phosphor-peptide complexes published earlier by the Zhang lab, the team (Huang, Zhang, and a peptide chemist Dr. Xuechen Li from HKU) successfully designed a stapled peptide inhibitor of PSD-95 GK domain that leads to an order of magnitude increase in binding affinity (from tens of μMs to 1.36 μM) with high cell permeability. For this stapled peptide, we showed that entropy greatly enhances binding affinity by lowering the entropy penalty via the constrained-helix structure of the stapled peptide in solution, also known as the free state. Interestingly, via subsequent designs of more rigid stapled peptides, we further observed that the entropy gain of stapled peptides is actually accompanied by loss of enthalpy. These results highlight the interplay between entropy and enthalpy in optimizing binding affinity and established the guiding principles for optimizing the binding of stapled peptides to protein targets. This work was published in *RSC Chem Biol* in 2021.

Other examples of designed peptides include our development of highly specific, potent Kibra binding peptides to evaluate the role of Kibra in synaptic signaling. This part of the research was described in last year's report and published in *Cell Rep* (Ji et al., 2019). In parallel, we performed a systematic investigation on the target recognitions of a panel of tandem WW domain proteins including KIBRA, MAGI, and YAP. These WW domain tandem-containing proteins play critical roles in synaptic signaling, cell growth, and polarity via binding to and positioning target proteins in specific subcellular regions. We discovered that WW domain tandems of KIBRA and MAGI, but not YAP, bind to specific target proteins with extremely high affinity and exquisite sequence specificity. Via systematic structural biology and biochemistry approaches, we decoded the target binding rules of WW domain tandems from cell growth regulatory proteins and uncovered a list of previously unknown WW tandem binding proteins including β-Dystroglycan, JCAD, and PTPN21. The WW tandem-mediated target recognition mechanisms elucidated here can guide functional studies of WW domain proteins in cell growth and polarity as well as in other cellular processes including neuronal synaptic signaling and MAGI. The extremely high affinity WW domain tandem binding peptides developed in this study will be very useful tools for investigating function of WW domain proteins in living cells. This work was published in *eLife* in 2020.

We explored the druggability of the PSD-95 PDZ1-2 domain and used fragment screening to investigate whether this protein is prone to binding small molecules. We screened 2,500 fragments by fluorescence polarization and validated the hits by surface plasmon resonance, including an inhibition counter-test, and identified four promising fragments. Three ligand-efficient fragments were shown by nuclear magnetic resonance spectroscopy to bind in the small hydrophobic P^0 pockets of PDZ1-2, and one underwent structure-activity relationship studies. Overall, we demonstrated that fragment screening can be successfully applied to PDZ1-2 of PSD-95 and disclosed novel fragments that can serve as starting points for optimization towards small-molecule PDZ domain inhibitors. This work was published in *ChemMedChem* in 2020.

6.2 What was the added value of the AoE funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

The scientific questions that have been addressed in this project cover many areas including biochemistry, chemical biology, structural biology, cell biology, neuroscience, genetics, physiology, soft matter physics, and optical engineering. The scale and complexity of these questions necessitated a cross-disciplinary team with diverse expertise. This AoE project has provided excellent opportunities for such a team to work together. The 9-year duration of the project allowed the team to work on risky projects that took a fair amount of time to complete. The wonderful outcomes of our collaborations provide the best proof of the value of the AoE funding.

6.3 If the project has not met its original objectives, why?
N/A

6.4 (a) Please provide details of each peer-reviewed journal publication arising directly from this project by completing the table at **Annex** *(one for each publication)*.
Please refer to **Annex I**

(b) Please provide details of recognised international conference(s) in which paper(s) related to this project was/were delivered by completing the following table:
(Please attach a copy of each conference abstract)

In the field of life sciences, conference papers are not considered formal academic outputs. Thus, we do not list conference papers here.

(c) UGC/RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/papers, please indicate and provide explanations.
N/A

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

The extensive communications among PIs, students, and postdocs from these team members' laboratories have strengthen inter-institutional collaborations, again evidenced by the many joint publications. Additionally, this AoE project has enabled the team members to collaborate with scientists globally. These collaborations have certainly elevated the academic reputation of the scientific community in Hong Kong.

6.6 Research students trained (registration/awards)

From HKUST:

Name	Degree registered for	Date of registration	Date of graduation
Yu Tao	PhD/ LIFS	Sept 2010	Aug 2015
Fatima Pardo	PhD/ CHEM	Sept 2011	Aug 2015
Sun Qiqi	PhD/ ECE	Sept 2011	Dec 2016
Li Songshan	PhD/LIFS	Sept 2011	Aug 2015
Wang Tienan	PhD/ LIFS	Sept 2011	Aug 2017
Ding Yue	MPhil/ LIFS	Sept 2012	Aug 2015
He Sicong	PhD/ECE	Sept 2012	Aug 2017
Chan Yan	PhD/ LIFS	Sept 2012	Dec 2015
Zeng Menglong	PhD/ LIFS	Sept 2012	Aug 2016

Name	Degree registered for	Date of registration	Date of graduation
Jiang Hanlun	MPhil/ CHEM	Sept 2013	Aug 2015
Zheng Susu	MPhil/ LIFS	Sept 2013	Aug 2015
Sun Yang	MPhil/ LIFS	Sept 2013	Aug 2015
Li Xuesong	PhD/ ECE	Sept 2013	Aug 2018
Yu Chuan	PhD/ LIFS	Sept 2013	Aug 2017
Ji Zeyang	PhD/ LIFS	Sept 2013	Aug 2017
Yang Xin	PhD/ LIFS	Sept 2013	Aug 2018
Chen Weitao	PhD/ LIFS	Sept 2013	Aug 2018
Yu Youqian	PhD/ LIFS	Sept 2013	Aug 2017
Zhu Han	PhD/ LIFS	Sept 2013	Aug 2017
Kong Lingming	MPhil/ CHEM	Sept 2014	Aug 2016
Wang Guo	MPhil/ CHEM	Sept 2014	Aug 2017
Weng Mingxi	MPhil/ LIFS	Sept 2014	Aug 2016
Chen Xianwei	MPhil/ LIFS	Sept 2014	Aug 2016
Yu Youqian	MPhil/ LIFS	Sept 2014	Aug 2016
Ji Tiantian	MPhil/ LIFS	Sept 2014	Aug 2016
Wang Wei	PhD/ CHEM	Sept 2014	Aug 2018
Di Yimei	PhD/ LIFS	Sept 2014	Aug 2018
Guo Weilin	PhD/ LIFS	Sept 2014	Aug 2020
Situ Chenghao	PhD/ LIFS	Sept 2014	Aug 2018
Wang Gang	PhD/ LIFS	Sept 2014	Aug 2019
Wei Xiuqing	PhD/ LIFS	Sept 2014	Jan 2020
Wu Shuting	PhD/ LIFS	Sept 2014	Aug 2018
Long Yangyang	MPhil/ LIFS	Sept 2015	Aug 2019
Yang Yang	MPhil/ LIFS	Sept 2015	Aug 2017
Zhang Lina	MPhil/ LIFS	Sept 2015	Aug 2017
Lin Xi	PhD/ LIFS	Sept 2015	Aug 2019
Shachuan Feng	PhD/ LIFS	Sept 2015	Aug 2022
Zhu Ruichi	MPhil/ LIFS	Feb 2016	Aug 2018
Chan Leung Ting	MPhil/ LIFS	Sept 2016	Aug 2018
Huang Chong Tin	PhD/ CHEM	Sept 2016	Aug 2020
Wang Xiaowei	PhD/ CHEM	Sept 2016	Aug 2020
Qin Zongya	PhD/ ECE	Sept 2016	Aug 2021

Name	Degree registered for	Date of registration	Date of graduation
Wei Xiuqing	PhD/ LIFS	Sept 2016	Aug 2020
An Yanru	PhD/LIFS	Sept 2016	Aug 2020
Wang Xu	PhD/ LIFS	Sept 2016	Aug 2020
Yang Zhou	PhD/ LIFS	Sept 2016	Aug 2020
Shizhen Zhao	PhD/ LIFS	Sept 2016	Aug 2022
Liang Luo	PhD/ LIFS	Sept 2016	Aug 2022
Tian Xiaozhen	MPhil/ LIFS	Sept 2017	Aug 2019
Pan Wen	MPhil/ LIFS	Sept 2017	Aug 2019
Ilona Christy Unarta	PhD/ CHEM	Sept 2017	Aug 2020
Ma Chenxue	PhD/ LIFS	Sept 2017	Aug 2023
Shen Zeyu	PhD/ LIFS	Sept 2017	Aug 2022
Wu Haowei	PhD/ LIFS	Sept 2017	Aug 2023
Heng Youshan	PhD/ LIFS	Sept 2017	Aug 2021
Wang Ruirong	PhD/ LIFS	Sept 2017	Aug 2021
Zhou Shaopu	PhD/ LIFS	Sept 2017	Aug 2020
Changlong Zhao	PhD/ LIFS	Sept 2017	Aug 2022
Linh TM Nguyen	PhD/ LIFS	Sept 2017	Aug 2023
Qiuxia Zhou	PhD/ LIFS	Sept 2017	Aug 2023
Pan Hongru	MPhil/ LIFS	Sept 2018	Aug 2020
Wang Yu	PhD/ LIFS	Sept 2018	Aug 2023
Jia Bowen	PhD/ LIFS	Sept 2018	Aug 2023
Nguyen Thi My Linh	PhD/ LIFS	Sept 2019	Aug 2022
Wu Juanqiang	PhD/ LIFS	Sept 2019	Aug 2022

From Poly U:

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Wu Shuai	PhD	Oct 2012	Oct 2016
Qiu Xianxiu	PhD	Dec 2013	Dec 2017
Li Na	PhD	Feb 2015	Jan 2019
Zhang Xiaozhe	PhD	Sept 2015	Aug 2019
Yang Xian	PhD	Sept 2018	Aug 2020
Zhang Shuqi	PhD	Sept 2018	Aug 2020

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Chen Jingyi	PhD	Sept 2018	Aug 2020
Gao Shan	PhD/Bio Sciences	Sept 2019	Aug 2022
We Weijian	PhD/Bio Sciences	Sept 2019	Aug 2022
Feng Yu	PhD/Bio Sciences	Sept 2020	June 2023
Yu Yingting	PhD/Bio Sciences	Sept 2020	June 2023
LEUNG Ming Fai	PhD/Bio Sciences	Sept 2021	June 2024
ZHENG Siqiong	PhD/Bio Sciences	Sept 2021	June 2025

From CUHK:

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Yu Yongsheng	PhD	Sept 2012	Aug 2016
Wang Rui	PhD	Sept 2013	Aug 2017
Liu Miao	PhD	Sept 2013	Aug 2017
Li Linting	PhD	Sept 2015	Aug 2019
Liu Jiahui	PhD	Sept 2015	Aug 2019
Yu Yongsheng	PhD	Sept 2015	Aug 2019
Zhang Yu	PhD	Sept 2015	Aug 2019
Liang Yujie	PhD	Sept 2016	Aug 2020
Liu Zhenjun	PhD	Sept 2016	Aug 2020
Huang Feng	PhD	Sept 2016	Aug 2020
Kang Wei	PhD	Sept 2016	Aug 2020
So Wing Ho	PhD	Sept 2016	Aug 2020
Nie Yunyu	PhD	May 2017	Aug 2021
Wei Qixin	PhD	Sept 2017	Aug 2021
Chen Xi	PhD	Sept 2017	Aug 2021
Liu Min	PhD	Sept 2019	Aug 2023
Chen Hongfei	PhD	Sept 2019	Aug 2023

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

1. An adaptive optics two-photon excitation fluorescence microscopy (AO-TPEFM) system.
2. An infrared laser-evoked gene operator (IR-LEGO) microscope heating system for live animal cell lineage tracing.

6.8 Other education activities and/or training programmes developed:
None

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the UGC/RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.
N/A

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

The activities described in this report have been actively coordinated by the PC. The PIs of the team have been extremely collegial and responsive to the activities. PIs and their lab members have been actively participating in all the seminar presentations and meetings hosted by this AoE team (under the name of "Systems Biology Center"). Many in-depth research collaborations have been developed among groups in the past nine years. The PC has also been mentoring junior faculty members of this team and investing in their career development. We are very happy to see that a number of them have been progressing very well in their career, as evidenced by their large volumes of publications, wonderful record in attracting research funding, tenure and promotions, etc.

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7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

Yes, we have been awarded numerous projects (local grants such as CRF and GRF projects, national grants such as a MOST Key Research Project, and international grants such as HFSP and Simons Foundation for Autism Research) that are directly attributable to the results obtained from this AoE project over the project period. The MOST project is the first major research grant directly awarded to PIs based in Hong Kong and with funding allocated to Hong Kong from the central government.

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Yes, the team members have been invited to speak in numerous conferences (e.g., Society of Neuroscience annual meeting, American Society of Cell Biology annual meeting, Biophysical Society annual meeting, Gordon Research Conferences, etc.). It is impractical to list all such invited talks due to the space limit of this report.

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?
The PC serves as reviewing editor for eLife, Editor for J Mol Biol, Editorial Board Member of *Structure*, *J Mol Biol*, *J Biol Mol NMR*, *Bioscience Reports, Protein & Cell*, etc.

7.4 Any documentary proof of the application of technologies arising directly from this project?
No

7.5 Other awards and recognitions as a result of this project (please specify):
Prof. Mingjie Zhang was awarded the following awards and accolades: Founding Member, The Academy of Sciences of Hong Kong (2015); Hong Kong Leader of the Year 2016; The Croucher Foundation Senior Research Fellow Award (2017); Senior Research Fellow, Research Grants Council of Hong Kong (Inaugural Round 2020, award declined); The 13th CC Tan Life-time Achievement Award in Life Science (2020).

Prof. Jiang Xia became Fellow of Royal Society of Chemistry in 2023

8. Impacts

8.1 What are the current and expected impacts of the project in terms of its contribution to the local and regional economic and societal well-being (e.g. patent, technology transfer, collaboration with external organisations, etc.)?

This research is basic science-focused, and thus generation of new knowledge in the form of openly accessible publications is the main output of the project. As set out in our proposal, this AoE team strives to become an internationally leading group in studying the mechanistic basis of synaptic development and signaling. Thanks to the support of the AoE scheme and dedicated efforts from the entire team, we have made excellent progress toward this goal. We believe that this AoE team is now a global leader in studying the structural biology of excitatory post-synaptic protein complex organization and regulation. This is evidenced by repeated invitations from prestigious journals including *Nat Neurosci, Dev Cell, Mol Cell,* and *J Neurosci* to contribute review articles on synapse formation via liquid-liquid phase separation. Moreover, the PC and PIs of this team have been invited to present our findings at numerous conferences and institutes/universities. Our systematic studies on phase separation in the central nervous system (CNS) have also sparked collaborations with many colleagues from different parts of the world. The project team led by Prof Zilong Wen who is studying microglia development and function is also world-leading in the important research area dealing with interactions between the immune system and the CNS. This AoE team has demonstrated that, via extensive collaborations among scientists sharing common scientific interests and goals, research groups in a relatively isolated region like Hong Kong can still be extremely competitive.

Both the quality and the volume of output from this AoE team have certainly earned us a high reputation in the field. The SAB echoes this sentiment: "[this AoE team] *has made ground-breaking advances and highly visible scientific impact*". The team "*has made important and paradigm-shifting discoveries concerning the structural and organizational principles governing the formation and maintenance of the PSD*". The SAB further commented that "*these high-impact*

studies demonstrate that this team is the world leader in studying microglia development and function, a cell population that are currently under spotlight due to their intimate and critical role in neurodegenerative diseases and mental disorder. …The second subject concerns microglia, an exciting new line of investigation that would not be possible without the interdisciplinary approach fostered by this AoE grant."

8.2 Others (please specify):

Nil

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

Many new ideas have evolved directly from this project. The most impactful idea is perhaps that the formation of synaptic signaling assemblies both at the pre- and post-synaptic compartments of neuronal synapses is mediated by phase separation. This series of studies indicate that phase separation-mediated biomolecular condensation can form various cellular organelles that are not enclosed by lipid membranes. These studies also call for attention in the life science community at large to view living cells as condensed matter systems and study living cells using soft matter physics approaches in addition to the traditional homogenous solutions. Our study also indicate that phase separation-mediated condensate formation may offer new ideas and opportunities for understanding numerous physiological functions and disease conditions of living organisms.

9.2 Whether there are new projects evolved directly from this project?

Numerous projects (local grants such as CRF and GRF projects, national grants such as a MOST Key Research Project, and international grants such as HFSP and Simons Foundation for Autism Research) have evolved directly from this AoE project over the project period. The MOST project is the first major research grant directly awarded to PIs based in Hong Kong and with funding allocated to Hong Kong from the central government. Due to the space limit of the report, we do not list all the projects.

9.3 Whether there are new collaborations developed directly from this project?

In addition to the collaboration within the AoE team as indicated by the large volume of joint publications, we have also been actively collaborating with numerous other colleagues both locally and from overseas. For example, we have extensively worked with our overseas collaborators, Drs. Hongjun Song, Guo-li Ming, Shawn Li, and Guoping Feng. These collaborations have led to a number of publications in journals including *Neuron, Cell Rep,* and *Mol Psychiatry.* Another successful international collaboration is between Zhang's lab and Dr. Richard Huganir's group at Johns Hopkins University School of Medicine over the past 10 years. This collaboration has led to papers in journals including *Cell, Neuron,* and *Cell Res*. We have been actively collaborating with Dr. Roger Nicoll at the University of California, San Francisco in the past several years in trying to link our mechanistic findings of synapse formation with physiological functions. This collaboration has also been extremely fruitful as evidenced by publications in *Neuron*, *Cell Rep* (2), and *Mol Psychiatry*. We have also collaborated with Dr. Yasunori Hayashi at the Kyoto University in the area of phase separation and synaptic functions and this collaboration has yielded publications in

Nat Neurosci and *J Neurosci,* and a joint grant from HFSP.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

Both Prof. Zhang (original PC) and Prof. Wen (new PC) have left Hong Kong, so we did not actively seek additional funds in Hong Kong to continue this line of research.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	173	0*	0	0	n/a	

**Conference abstracts are not recognized as formal publications and thus are not listed in this research output statistics.*

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

Please specify the information, if any, that cannot be provided for public access and give the reasons.

Information that cannot be provided for public access	Reasons
Nil	

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Mental illness is a leading burden among all chronic human diseases worldwide, and yet very few effective treatments are available. For better treatments, better science is needed. However, society is facing a huge crisis as drug companies—major investors of mental illness research in the past—are increasingly withdrawing from mental illness research due to the slow commercial returns on their investments as well as the complicated mechanistic bases of the diseases. Academia is thus tasked with conducting both deeper and broader research activities in the field. This also creates many opportunities that would otherwise be within the exclusive domain of the pharmaceutical industry. Additionally, advancements in technologies such as high-throughput genomic sequencing and in systems biology have generated—and will continue to generate at an even faster pace—a very large number of candidate genes that are associated with various brain disorders. Whereas hundreds of disease-causing genes have been discovered, the underlying biology of diseases caused

by mutations of these genes is extremely poorly understood. To fully understand and develop effective treatments for mental disorders, elucidating the action mechanisms of these disease-causing genes will continue to be one of the most important research directions for many years to come.

In this AoE project, we investigated the physiological roles and action mechanisms of a set of key proteins and their complexes that are known to play fundamental roles in the development and signaling of synapses in excitatory neurons. We were also particularly interested in the underlying mechanisms of mental illnesses (such as autism, schizophrenia, and depression, which are broadly defined as neuro-disorders in this project) caused by the malfunctioning of the proteins that orchestrate neuronal development and synaptic signaling, and in suggesting/developing possible therapeutic approaches for these diseases. This team addressed these topics through a combination of genetic, cellular, biochemistry, structural biology, and chemical biology approaches. Unlike numerous other groups/consortiums in neuronal signaling and neuro-disorder research worldwide, our research program consisted of structural biology and biochemistry-based mechanistic studies at its core. These mechanistic-based studies were supported by, and in turn drove, “upstream” functional studies that used animal model and cellular approaches. They also offered the basis for developing peptides, peptide mimetics, and low molecular weight compounds with therapeutic potential using chemical biology approaches.

As evidenced by numerous publications in prestigious journals, our team has made important contributions to the understanding of normal brain functions and to the fight against mental illnesses resulting from synaptic functional defects through nie years of collaborative research. The project has also enhanced Hong Kong’s scientific research capabilities, infrastructure, and work force, and established a top-notch research center for neural development, signaling, and neuro-disorders in the territory.