RGC Reference **HKU8/CRF/10G**
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

Self-Assembled Synthetic Ion Channels: Design, Characterization and Biomedical Applications

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

Research Team	Name/Post	Unit/Department/Institution
Project Coordinator	Dan Yang/Morningside Professor in Chemical Biology	Chemistry/HKU
Co-Investigator(s) [Co-I(s)]: (with title)	Paul M. Vanhoutte / Chair Professor of Pharmacology	Pharmacology and Pharmacy/HKU
	Hung-Fat Tse / William MW Mong Professor in Cardiology	Medicine/HKU
	Xiao-Qiang Yao / Professor of Physiology	School of Biomedical Sciences/CUHK
	Ge Lin / Professor of Pharmacology	School of Biomedical Sciences/CUHK
	Wing-Hung Ko / Associate Professor of Physiology	School of Biomedical Sciences/CUHK

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	Feb. 1, 2011		
Project Completion Date	Jan. 31, 2014		
Duration (*in month*)	36		
Deadline for Submission of Completion Report	Oct. 31, 2014		

5. Project Objectives

5.1 Objectives as per original application

1. Design, synthesis, and chemical characterization of synthetic ion channels
2. Biological evaluations of synthetic ion channels in cell lines, tissues and animal models of human diseases (cystic fibrosis, asthma, hypertension, and myocardial infarction)
3. Pharmacokinetic studies of synthetic ion channels to improve bioavailability and efficacy
4. Structural studies to understand the molecular basis of the self-assembly of synthetic ion channels

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)

We have demonstrated that small synthetic molecule C11 which form channels to mediate Cl^- transport across lipid bilayer membranes is capable of restoring Cl^- permeability in human CF epithelial cell (*PLoS ONE,* **2012**, *7*, e34694). We have discovered that C11 also has the capability to modulate airway smooth muscle responsiveness in rat tracheal rings. This relaxing effect was dependent on the presence of extracellular Cl^- and HCO_3^- (*PLoS ONE*, **2012**, 9, e45340).

In addition, we have found that more easily accessible compounds with natural amino acid residues were even more effective than C11 in Cl^- transporting. Compound LPY-CM9, which forms channel in giant liposomes, can induce Cl^-/HCO_3^- transport in both lipid membranes and epithelial monolayers. This compound can also restore the chloride secretion of CFBE41o- cell monolayers with mutant CFTR. So this may provide a new platform for the treatment of cystic fibrosis (manuscript in preparation).

We have also explored the ion transporting ability of short liner peptides. We surprisingly found that two tripeptides and three pentapeptides can self-assemble into Na^+-conducting or Cl^--conducting channels in lipid membranes. To the best of our knowledge, these are the shortest peptides with capability to form ion channels. As short peptides emerge at early stage of the prebiotic Earth, this discovery may shed light on the structure of primitive ion channels (manuscript in preparation).

We have found that ZHY-CM23 with the Boc-protected aminoxy lysine side chain has the ability to self-assemble into Cl^- dependent K^+ channel in both liposome and cell-based studies. More importantly, this compound can hyperpolarize living cell membrane potential and relax agonist-induced blood vessel contraction. Therefore, it may have the potential to become a lead compound for the treatment of human diseases associated with K^+ channel dysfunction (*Org. Biomol. Chem.* **2014**, *12*, 8174-8179).

Small synthetic molecule LPY-CM64 capable of simultaneously transporting Na^+ and Cl^- has also been developed. Compounds like this can mimic the function of NCC, which is a natural cation-coupled Cl^- cotransporter and plays important role in regulating of important physiological process. The relative permeability of ion channel formed by LPY-CM64 towards Na^+/Cl^- is around 0.83 (manuscript in preparation).

We have found that the action potential duration at 90% repolarization of ventricular cardiomyocytes was slightly increased at increasing concentration of C11. After intravenous administration of C11, no sustained ventricular tachyarrhythmia could be induced by in vivo programmed electrical stimulation in rats. The administration of C11 resulted in a modest reduction in heart rate but with no effects on the left ventricular contractility. These results demonstrate the antiarrhythmic property of C11 in a rat ventricular tachycardia model (manuscript in preparation).

The metabolic stability studies have demonstrated that the peptides containing α-aminoxy acids showed significantly improved metabolic stability than peptides containing only natural α-amino acids (*Amino Acids*, **2012**, *43*, 499–503). By modifying the isobutyl side chains of C11, four analogues with improved

intestinal absorption were design and synthesized. The results demonstrated that intestinal absorption and transport mechanism of the aminoxy peptides varied significantly with different structures (*Mol. Pharmaceutics*, **2011**, *8*, 1073-1082).

Peptides LPY-CM4 and LPY-CM11 were found to have good aqueous solubility (200 μM). The follow-up absorption study (Caco-2 cell monolayer model) revealed that both peptides had acceptable absorbability. LPY-CM11 was shown to be extraordinary stable when incubated with simulated gastric fluid, simulated intestinal fluid, rat liver S9, rat liver microsome, rat blood and plasma, respectively (manuscript in preparation).

By combining X-ray crystal structure and NOESY data of C11, together with molecular dynamic simulations, a helical self-assembly model with three monomers per turn has been proposed for the self-assembled chloride channel (manuscript in preparation).

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

With the strong ability to self-assemble and form channels in lipid bilayers, those easily synthesized small molecules can modulate the traffic of physiologically important ions (Na^+, K^+, Cl^-, HCO_3^-) in both liposome and cell lines. Besides, our previous results have proved several biological functions of these compounds, such as restoring the chloride secretion in cell lines with mutant CFTR, hyperpolarization the membrane potential and relaxation of rat airway smooth muscle and alleviating arrhythmia in rats. We plan to further examine the anion transporting activities using normal primary cultures of human bronchial epithelial cells or CFTR-knockdown primary cells. Drug testing in CF mouse model can also be conducted in future. We are still working toward developing better K^+-selective small molecules for potential therapeutic applications in human diseases. As cell proliferation and apoptosis are closely related to channel activity, we plan to investigate effects of our compounds on the regulation of cell cycle, and test their antitumor activities.

Given the significant biological roles of ion channels, it is also very important to reveal the principles controlling the ion transport across biological membranes. Although the preliminary models were proposed, there is still a need for more solid evidence. We plan to gain more information on the self-assembly structures in lipid bilayers by using STD NMR and solid state NMR techniques.

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

In this project, we have established close interdisciplinary collaborations among the six research groups in HKU and CUHK through joint supervision of five research postgraduate students and one postdoctoral fellow as shown below:
Mr. Leo Li is currently jointly supervised by Prof. Yang and Prof. Yao;
Miss Chunyuan Zhang is currently jointly supervised by Prof. Yang and Prof. Lin;
Ms. Pengyun Liu and Mr. Zongchang Yang were jointly supervised by Prof. Yang and Prof. Ko;
Mr. Kwok Hei Yau was jointly supervised by Prof. Yang and Prof. Vanhoutte.
Dr. Alex Chan was jointly supervised by Prof. Tse and Prof. Yang.

This innovative collaborative research project has provided training to 6 postgraduate students/postdoctoral fellow in an interdisciplinary environment, built up an extensive collaborative research network in drug discovery in Hong Kong (HKU and CUHK) and around the world (with Prof. Richard C. Boucher of the Cystic Fibrosis and Pulmonary Disease Research Center at University of North Carolina, Chapel Hill, USA) by combining the expertise in chemistry, physiology, and pharmacology, and contributed to the development of "Medical Services" and "Innovation and Technology", which are two of the six key industries for Hong Kong. The outcome of the collaborative research has been published in leading international journals of chemistry, biology and medicine. In

addition, two international patents have been awarded for synthetic ion channels and their effect on membrane potentials, and licensed to ProQR Therapeutics for further development of drugs for the treatment of cystic fibrosis. Therefore, this research project has great impact not only in basic research on chemistry and biology of ion channels but also on biotechnology and pharmaceutical industries.

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)

Biological ion channels are a kind of protein micropore in the cell membrane which are responsible for communicating chemically and electrically with the extracellular environment. The ability to mimic ion channels would enhance understanding of how ion transported in nature, and provide strategies for the treatment of diseases caused by the dysfunction of ion channels. However, so far, numerous synthetic ion channels are too big to be drug-like. Therefore, it is of great importance to search for small molecules that mimic the functions of nature ion channels, at the same time, have better stability and bioavailability.

In this collaborative research project, we have developed some new self-assembly transporters possessing important functions, such as the potassium channel, chloride/bicarbonate exchanger and sodium/chloride cotransporter. Those ion transporters have shown some applications in different biological models, like cystic fibrosis, hypertension and arrhythmia. Those ion channel-forming molecules have low molecular weights (around 500), can be readily synthesized, and their pharmacological properties can be readily modified for therapeutic applications. These preliminary studies have opened up brand-new opportunities for the treatment of ion channel-related human diseases.

Moreover, we have discovered some liner oligopeptides able to from Na^+ and Cl^- channels. To the best of our knowledge, they are the shortest channel-forming peptides. This discovery may give us some clue to uncover the structure of primitive ion channels and to explore the origin of life.

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.)

The Latest Status of Publications				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 *(indicate the year ending of the relevant progress report)*	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				B. Ma, H.-Y. Zha, N. Li, D. Yang, G. Lin*	Effect of Structural Modification of α-Aminoxy Peptides on Their Intestinal Absorption and Transport Mechanism. *Mol. Pharmaceutics* **2011**, *8*, 1073–1082.	2012	No	No**
2012				B. Ma, C. Yin, D. Yang, G. Lin*.	Effect of Structural Modification on the Gastrointestinal Stability and Hepatic Metabolism of α-Aminoxy Peptides. *Amino Acids*, **2012**, *43*, 2073–2085.	2012	No	Yes
2012				F. Chen, B. Ma, Z.-C. Yang, G. Lin*, D. Yang*.	Extraordinary Metabolic Stability of Peptides Containing α-Aminoxy Acids. *Amino Acids* **2012**, *43*, 499–503.	2012	No	Yes
2012				B. Shen, X. Li, F. Wang, X.-Q. Yao*, D. Yang*.	A Synthetic Chloride Channel Restores Chloride Conductance in Human Cystic Fibrosis Epithelial Cells. *PLoS ONE*, **2012**, *7(4)*, e34694.	2012	Yes	Yes
2012				B. Ma, S. Chai, N. Li, K. K.W. To, W. L. T. Kan, D. Yang, G. Lin*	Reversal of P-Glycoprotein-Mediated Multidrug Resistance by a Synthetic α-Aminoxy Peptidomimetic. *Int. J. Pharm.* **2012**, *424*, 33–39.	2012	No	No**
2012				K.-H. Yau, J. C.-W. Mak, S. W.-S. Leung, D. Yang, P.M. Vanhoutte*	A Synthetic Chloride Channel Relaxes Airway Smooth Muscle of the Rat. *PLoS ONE*, **2012**, *7(9)*, e45340.	2012	No	Yes
2014				H.-Y. Zha, B. Shen, K.-H. Yau,	A Small Synthetic Molecule Forms Selective Potassium		Yes	Yes

				S.-T. Li, X.-Q. Yao*, D. Yang*	Channels to Regulate Cell Membrane Potential and Blood Vessel Tone. *Org. Biol. Chem.* **2014**, *12*, 8174–8179.			
			2014	Z.-C. Yang, P.-Y. Liu, L. Li, X.-Q. Yao*, D. Yang*	Short Peptides of α-Amino Acids as Synthetic Ion Channels		Yes	Yes
			2014	X. Li, B. Shen, N.-Y. Zhu, H.-Y. Zha, W. Han, Y.-D. Wu, X.-Q. Yao, D. Yang*	A Synthetic Chloride Channel Formed by Self-Assembling Small Molecules.		No	Yes
			2014	H.Y. Zha, K.H. Yau, B. Shen, X.Q. Yao, W.H. Ko, D. Yang*	A Small-molecule Based Synthetic Chloride Channel Mediates Chloride Transport across Liposomal Membrane and Cultured Airway Epithelia		No	Yes
			2014	P.-Y. Liu, S.-T. Li, X.-Q. Yao, W.-H. Ko, D. Yang*	Synthetic Small Molecules Mediate Transport of Both Cations and Anions		No	Yes
			2014	P.-Y. Liu, S.-T. Li, W.-H. Ko, X.-Q. Yao, D. Yang*	A Synthetic Small Molecule Functioning as Chloride-Bicarbonate Dual-Transporter		No	Yes
			2014	C.-Y. Zhang, B. Ma, D. Yang*, G. Lin*	Pharmacological Studies of Small Molecules as Synthetic Ion Channels		No	Yes

** The authors forgot to mention this RGC CRF grant number in publications.

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)*
Mar. 2011; Anaheim, USA	A Small Synthetic Molecule Forms K^+ Channels to Regulate Membrane Potential of Living Cells	The 241st American Chemical Society National Meeting	2012	Yes	Yes

Mar. 2011; Anaheim, USA	Synthetic Chloride Channels	The symposium entitled "A Celebration of International Organic Chemistry" at the 241st American Chemical Society National Meeting	2012	No	Yes
Jun. 2011; Seoul, South Korea	Using Synthetic Organic Chemistry to Probe Biological Mechanisms	1st Korea Forum on Organic Chemistry	2012	No	Yes
Jun. 2011; Montréal, Canada	Self-Assembled Synthetic Ion Channels	Biological and Medicinal Chemistry Symposium on Peptides at the 94th Canadian Chemistry Conference	2012	No	Yes
Sept. 2011; Guangzhou, China	Pharmacokinetics, disposition and metabolism of a novel α-aminoxy peptide and its analogue in rat	2011 ISSX/CSSX Workshop	2012	No	Yes
Dec. 2011; Hong Kong	Using Synthetic Organic Chemistry to Probe Biological Mechanisms	CUHK Workshop on "Novel Functional Materials for Biological Applications"	2012	No	Yes
Feb. 2012; Hanoi, Vietnam	In the Search of Miracle Drugs	The 1st Asian Chemical Biology Initiative Meeting	2012	No*	Yes
Apr. 2012; Hong Kong	Using Aminoxy Acids as Novel Building Blocks for Peptidomimetics	The 2012 New Journal of Chemistry Symposium "New Directions in Chemistry"	2012	No*	Yes
Aug. 2012; Hefei, China	Using Aminoxy Acids as Novel Building Blocks for Peptidomimetics	Summer School of Science of Chiral Chemistry & Chiral Materials	2012	No*	Yes
Oct., 2012; Zurich, Switzerland	Using Small Organic Molecules to Probe Biological Mechanisms	7th Annual Dorothy Crowfoot Hodgkin Symposium	2012	No	Yes
Apr. 2013; Seoul, South Korea	Self-Assembled Synthetic Ion Channels: Design, Characterization and Biomedical Applications	Korean Chemical Society-Accounts of Chemical Research Symposium	2014	Yes	Yes
Jul. 2013; Crystal City, Virginia, USA	Self-Assembled Synthetic Ion Channels and Their Biomedical Applications	The 8th International Symposium on Macrocyclic and Supramolecular Chemistry	2014	Yes	Yes
Oct. 2013; Shanghai, China	Synthetic Ion Channels	The 1st JOC/OL Symposium	2014	Yes	Yes
Dec. 2013; Kumamoto, Japan	Self-Assembled Synthetic Ion Channels and Biomedical Applications	The International Kick-off Symposium for Integrated Science for Molecular Chirality in Biology and Chemistry	2014	Yes	Yes
Nov. 2013; Jinan, China	Self-Assembled Synthetic Ion Channels: Design, Characterization and Biomedical Applications	The 2013 National Symposium for Medicinal Chemistry	2014	Yes	Yes

Oct. 2013; Chongqing, China	Self-Assembled Synthetic Ion Channels: Design, Characterization and Biomedical Applications	The 8th National Symposium for Organic Chemistry	2014	Yes	Yes
Dec. 2014; Singapore	Self-Assembled Synthetic Ion Channels	The 8th Singapore International Chemical Conference	2014	Yes	Yes
Dec. 2014; Kuala Lumpur, Malaysia	Self-Assembled Synthetic Ion Channels	The 9th International Conference on Cutting-Edge Organic Chemistry in Asia	2014	Yes	Yes

* No abstract submission was required for those invited lectures.

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
Kwok Hei Yau	Ph.D.	Sept. 1, 2008	Aug. 2011
Hui-Yan ZHA	Ph.D.	Sept. 1, 2007	May, 2012
Peng-Yun Liu	Ph.D.	Jan. 1, 2009	April, 2014
Zong-Chang Yang	Ph.D.	Sept. 1, 2009	May, 2014
Chun-Yuan Zhang	Ph.D.	Sept. 1, 2011	
Shing-To Li	Ph.D.	Jan. 1, 2012	
Fangfang Shen	Ph.D.	Sept. 1, 2012	

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

Several international patents have been awarded on synthetic ion channels and their effect on membrane potentials:

X. Li, D. Yang. Synthetic Chloride Channels. U.S. Patent No. US 8,378,138, issued on Feb. 18, **2013**.

X. Li, D. Yang. Synthetic Chloride Channels. Japanese Patent No. JP 5586961, issued on Aug. 1, **2014**.

X. Li, D. Yang. Synthetic Chloride Channels. Chinese Patent 2007 8 0051510.X, issued on Aug. 13, **2014**.

X. Li, D. Yang, B. Shen, X.-Q. Yao. Method of Modulating Membrane Potential of a Cell. Chinese Patent ZL 2008 8 0127474.5, issued on Aug. 27, **2014**.

Our work on synthetic chloride channels has attracted attention from pharmaceutical/biotech industry. ProQR Therapeutics, a Dutch biotech company focused on cystic fibrosis treatment, has licensed our patents on synthetic ion channels to develop drugs for the treatment of cystic fibrosis.

International collaborations with Prof. Richard C. Boucher of the Cystic Fibrosis and Pulmonary Disease Research Center at University of North Carolina (Chapel Hill, USA) and Prof. Guoshun Wang at Louisiana State University Health Sciences Center (New Orleans, USA) have been set up to test the potential of our synthetic ion channels in model studies of cystic fibrosis.

Our work on the synthetic chloride ion channels has been presented by Prof. Yang as invited seminars at the following institutions:

Fudan University (Oct. 2013; Shanghai, China)

Hong Kong Polytechnic University (Jan. 2013; Hong Kong)

Nanjing University (Nov. 2012; Nanjing, China)

Peking University (Oct. 2011; Beijing, China)

Yonsei University (Jun. 2011; Seoul, South Korea)

Prof. D. Yang was invited to write an account on the synthetic ion channel work in *Accounts of Chemical Research* (published by ACS with impact factor 21.640).

For the work on synthetic chloride channels, Mr. Bin Ma, a postgraduate student in Co-PI Prof. Lin's lab, won the Best Presentation Award at the 2011 ISSX/CSSX Workshop (Sept. 2011; Guangzhou, China).