RGC Ref.: N_CUHK443/12 NSFC Ref.:
(please insert ref. above)

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

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

Part A: The Project and Investigator(s)

1. Project Title

Development of Novel Organoboron Chromophores for Two-Photon Cell Imaging

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Dennis K. P. Ng	Prof. Guoqiang Yang
Post	Professor	Research Professor, Director of Laboratory
Unit / Department / Institution	Department of Chemistry, The Chinese University of Hong Kong	CAS Key Laboratory of Photochemistry, Institute of Chemistry, Chinese Academy of Sciences
Contact Information	E-mail: dkpn@cuhk.edu.hk	E-mail: gqyang@iccas.ac.cn
Co-investigator(s) *(with title and institution)*	Prof. Pui-Chi Lo Department of Biomedical Sciences, City University of Hong Kong	Prof. Shuangqing Wang CAS Key Laboratory of Photochemistry, Institute of Chemistry, Chinese Academy of Sciences

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/13		
Project Completion date	31/12/16		
Duration *(in month)*	48		

Deadline for Submission of Completion Report	31/12/17		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To design, synthesize, and characterize a series of two-photon-absorbing organoboron compounds, including BODIPYs and aza BODIPYs with emphasis on the amphiphilic analogues.

2. To study the photophysical properties of these compounds, focusing on their two-photon absorption and emission properties, and singlet oxygen generation efficiency, and attempting to reveal the structure-property relationships.

3. To evaluate the potential of these compounds in two-photon cell imaging, particularly their subcellular localization property, and as efficient photosensitizers for two-photon PDT.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____
_____

1.
2.
3. ....

6. Research Outcome

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

Two series of BODIPY derivatives bearing a D-π-D type quadrupolar structure were first prepared in which two 4-(diphenylamino)phenylethynyl or 4-(dimethylamino)phenylethynyl motifs were introduced to the 2- and 6-positions of the BODIPY core through Sonogashira cross-coupling reactions (*J. Mater. Chem. C*, in preparation). These compounds were fully characterized with various spectroscopic methods, and their linear optical absorption and fluorescence properties were investigated in detail in toluene. The series containing two dimethylamino donors exhibited a red-shifted Q-band absorption and fluorescence emission, but their fluorescence quantum yields and lifetimes were significantly lower and shorter compared with the diphenylamino-substituted counterparts. Electrochemical properties of the series of distyryl BODIPYs were also studied by cyclic voltammetry. It was found that the introduction of these electron donors, particularly the dimethylamino group, resulted in lowering of the first

oxidation potential, indicating their strong charge-transfer ability. The TPA properties of these compounds were also investigated by a two-photon fluorescence excitation method in toluene. The TPA cross-sections reached the values of 105-1314 GM in the near-infrared range (900-1100 nm) and 45-617 GM in the telecommunication range (1100-1400 nm). The series containing two diphenylamino electron-donating groups generally exhibited higher TPA cross-sections than the dimethylamino-substituted series with values up to 1314 GM (at 1050 nm) and 617 GM (at 1411 nm) for the distyryl analogue.

Another series of π-extended BODIPY dimers with a dialkynyl diketopyrrolopyrrole or benzothiadiazole bridge were also synthesized by copper-free palladium-catalyzed Sonogashira coupling reactions, in which the BODIPY moieties were connected with strong electron-donating 4-(diphenylamino)phenylethynyl or 9-(4-ethynylphenyl)carbazole groups at the terminals (*J. Mater. Chem. C*, in preparation). As these bridging units are electron deficient, these compounds possess a unique D-π-A-π-D structure. These fully conjugated π systems displayed significantly red-shifted and intense Q-band absorptions and fluorescence emissions compared to the BODIPY monomer. The TPA cross-sections were generally higher for the carbazole-containing derivatives, and for the diketopyrrolopyrrole- and benzothiadiazole-linked dimers, the values reached 2053 GM and 2913 GM, respectively, at 890 nm in tetrahydrofuran. The TPA cross-sections were generally lower in toluene and chloroform. The BODIPY monomer showed negligible TPA properties, suggesting that the remarkably high cross-sections of these dimers were due to their unique D-π-A-π-D structure.

All the above organoboron compounds had limited solubility in aqueous media. To enable the compounds to be used for biomedical applications, OEG chains were introduced to the π skeleton of these dyes. Two advanced OEG-substituted BODIPY-based photosensitizers were synthesized and characterized (*J. Med. Chem.*, submitted). With a glibenclamide moiety, these compounds could localize in the ER of HeLa and HepG2 cells. The BODIPY π skeleton was conjugated with two styryl or carbazolevinyl groups, which could substantially red-shift the Q-band absorption and fluorescence emission, and impart TPA property to the chromophores up to 453 GM at 1010 nm (for the carbazolevinyl derivative). Upon irradiation, these photosensitizers caused photocytotoxicity to these two cell lines with IC_{50} values down to 0.09 μM, for which the cell death was triggered mainly by ER stress as shown by a series of ER stress studies.

Apart from these BODIPY derivatives, a series of novel PABDP derivatives were designed and synthesized for subcellular imaging (*Chem. Eur. J.*, in preparation). Having a number of OEG substituents, these compounds exhibited good solubility and remained emissive in aqueous media with a fluorescence quantum yield of ca. 0.4. The TPA properties of these compounds were also investigated by a two-photon fluorescence excitation method in deionized water. The TPA cross-sections (100-500 GM) in the infrared range (900-1100 nm) were remarkable for chromophores without strong electron-donating groups. Interestingly, there was a one-photon forbidden but two-photon allowed absorption band at ca. 500 nm. This TPA band could be attributed to the $S_0 \rightarrow S_2$ transition, which was supported by theoretical calculations. With a view to directing the dyes to the mitochondria or lysosomes of cells, a triphenylphosphonium or morpholine moiety was introduced, respectively. However, despite all the compounds were membrane permeable and could accumulate inside the cells, only the triphenylphosphonium-substituted derivative showed high affinity toward the lysosomes. Fluorescence cellular images could be obtained under both one-photon and two-photon excitation conditions.

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

This study has shown that organoboron compounds with appropriate modifications could function as efficient TPA materials that are suitable for biomedical applications. The BODIPY dimers with a D-π-A-π-D structure are particularly promising which exhibit TPA cross-sections up to ca. 3000 GM at 890 nm. The several reactive sites at the BODIPY units can be modified with special functional units, such as hydrophilic groups to improve the water solubility, chemo-reactive moieties to detect biologically important species, and tumor-targeting ligands to achieve targeted PDT for cancer. These biomedical applications are of much current interest, and it is believed that these novel functional dyes with superior TPA properties could contribute to the development of these fields. For the ER-localized distyryl BODIPYs, they are highly promising photosensitizers not just because of their high in vitro potency, but also due to their specific localization property at ER that can trigger ER stress and immunity. This immunotherapeutic action can greatly enhance the efficacy of traditional PDT. It is believed that further studies along these directions can enrich the chemistry and extend the applications of this interesting class of compounds.

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)

With the advance of laser technology, simultaneous absorption of two photons can be readily observed. This two-photon absorption process involves radiation of longer wavelengths compared with the normal one-photon absorption. This feature enables deeper light penetration into scattering media and higher spatial resolution, which are particularly useful for biomedical applications. As a result, there has been considerable interest in the development of efficient two-photon-absorbing materials. The aim of this project is to develop novel organoboron chromophores that exhibit high two-photon absorption cross-sections and can be used for two-photon cell imaging and photodynamic therapy. We have designed, synthesized, and characterized several series of such derivatives with different electronic skeletons and functional groups, and measured their one-photon and two-photon absorption and fluorescence properties. The use of these compounds as fluorescent probes to image cancer cells and specific subcellular organelles, and as photosensitizers to eradicate cancer cells upon illumination have also been demonstrated. The results show that with appropriate chemical modifications, these compounds can exhibit remarkably high two-photon absorption cross-sections (up to ca. 3000 GM at 890 nm), label specific subcellular organelles (e.g. endoplasmic reticulum), and cause cell death by specific pathways.

Part C: Research Output

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

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications	Author(s)	Title and Journal/	Submitted	Attached	Acknowled	Accessible

Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Book *(with the volume, pages and other necessary publishing details specified)*	to RGC *(indicate the year ending of the relevant progress report)*	to this report *(Yes or No)*	ged the support of this Joint Research Scheme *(Yes or No)*	from the institution al repository *(Yes or No)*
		√		**Y. Zhou,** Y.-K. Cheung, C. Ma, S. Zhao, **P.-C. Lo,** W.-P. Fong, K. S. Wong, and **D. K. P. Ng***	Endoplasmic Reticulum-Localized Two-Photon-Absorbing Boron Dipyrromethenes as Advanced Photosensitizers for Photodynamic Therapy, *J. Med. Chem.*, submitted.	No	Yes	Yes	No
			√	**Y. Zhou,** C. Ma, Q. Wang, **P.-C. Lo,*** K. S. Wong, T. Kinoshita, and **D. K. P. Ng***	Pyrrolopyrrole Aza Boron Dipyrromethene Based Two-Photon Fluorescent Probes for Subcellular Imaging, *Chem. Eur. J.*, in preparation.	No	Yes	Yes	No
			√	**W.-J. Shi, Y. Zhou,** C. Ma, K. S. Wong, N. Gao, Q.-H. Xu, and **D. K. P. Ng***	Synthesis and Two-Photon Absorption Properties of Novel D-π-A-π-D Boron Dipyrromethene Dimers Linked with a Dialkynyl Diketopyrrolopyrrole or Benzothiadiazole Unit. *J. Mater. Chem. C*, in preparation.	No	Yes	Yes	No

			√	**W.-J. Shi,** G. Wicks, A. Rebane, and **D. K. P. Ng***	Quadrupolar BODIPY Derivatives with Strong Two-Photon Absorptions Extending to the Telecommunication Wavelengths. *J. Mater. Chem. C*, in preparation.	No	Yes	Yes	No

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
07/2016/Nanjing, China	Development of Novel Near-infrared Fluorescent Probes for Subcellular Imaging	The 9th International Conference on Porphyrins and Phthalocyanines	No	Yes	Yes	No
12/2016/Auckland, New Zealand	Development of Novel Two-photon Fluorescent Probes for Subcellular Imaging	The 8th Asian Biological Inorganic Chemistry Conference	No	Yes	Yes	No
05/2017/Hong Kong, China	Endoplasmic Reticulum-Targeting BODIPYs for Two-Photon Bioimaging and Photodynamic Therapy	The 24th Symposium on Chemistry Postgraduate Research in Hong Kong	No	Yes	Yes	No
07/2017/Hong Kong, China	Boron-Containing Functional Dyes for Biomedical Applications	The 16th International Meeting on Boron Chemistry	No	Yes	Yes	No

07/2017/Hong Kong, China	Endoplasmic Reticulum-Targeting BODIPYs for Two-Photon Bioimaging and Photodynamic Therapy	The 16th International Meeting on Boron Chemistry	No	Yes	Yes	No
12/2017/Hong Kong, China	Endoplasmic Reticulum-Localized Two-Photon-Absorbing BODIPY-Based Photosensitizers	The 4th Japan-Taiwan-Singapore-Hong Kong Quadrilateral Symposium on Coordination Chemistry	No	Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Wenjing Shi	PhD	01/08/2009	July 2013
Haigang Yu	MPhil	01/08/2012	Sep 2014
Yimin Zhou	PhD	01/08/2014	July 2018 (expected)

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

The TPA measurements were in collaboration with Prof. Aleksander Rebane of Montana State University, Prof. Qinghua Xu of the National University of Singapore, and Prof. Kam Sing Wong of the Hong Kong University of Science and Technology. The computational studies were in collaboration of Prof. Takumi Kinoshita of the University of Tokyo.