(Revised Jan 10)

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(please insert ref. above)

NSFC/RGC Joint Research Scheme
Annual Progress / Mid-term Report

Report for the period ending 31 Dec 2016

Part A: The Project and Investigator(s)

1. Project Title

Development of Multi-modality AIE Nanoprobes for Targeted Detection of Drug Resistant Gene AXL in Lung Cancer and their Preclinical Application

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Dr. Wing Yip Lam*	Prof. Zhenfeng Zhang
Post	Research Assistant Professor	Professor
Unit / Department / Institution	Department of Chemistry/The Hong Kong University of Science and Technology	Sun Yat-sen University Cancer Center
Co-investigator(s) *(with title)*	Prof. Ben Zhong Tang Dr. Yuning Hong	N/A

*The PI has been changed to Dr. Wing Yip Lam effective from 1 Aug 2015 (approval from RGc dd 15 Oct 2015 refers)

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01-01-2015	N/A	N/A
Project Completion date	31-12-2018	N/A	N/A
Duration *(in month)*	48	N/A	N/A
Deadline for submission of Joint Completion Report	31-12-2019	N/A	N/A

Part B: Report on Project Progress

5. Project Objectives

5.1 Objectives as per original application

1. To develop new multi-modality CT/MRI-fluorescence materials for preclinical application;
2. To create new multi-modality contrast agents with AIE characteristics through innovative structural design and facile fabrication routes;
3. To decorate the materials with effective ligands for targeted detection of the expression of drug-resistant gene AXL in lung cancer and explore their application for in vivo molecular imaging

5.2 Revised Objectives

No revision was made

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3.

6. Research Activities

(please state the scope of investigation undertaken; results achieved; problems encountered; deviations from the original plan and the reasons for doing so etc.)

This is the first progress report for this project

6.1 Research activities in relation to the project objectives that were carried out up to the last Progress Report

N/A

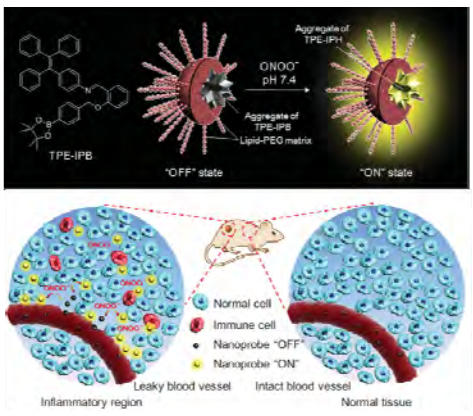
6.2 Areas addressed and results expected during this reporting period *(as stated at 6.5 of the last Progress Report)*

N/A

6.3 Research activities and collaboration in relation to the project objectives that were carried out during this reporting period *(please also list out dates, duration and purpose of visit(s) and activities carried out on the trips made)*

Luminogens with aggregation-induced emission (AIE) characteristics are promising materials for constructing efficient organic light-emitting diodes, their clinical applications and capability for sensitive detection of chemicals and biomolecules have not yet been exploited. Molecular imaging by integrating the intriguing AIE fluorescence with other modalities may generate new tools for earlier diagnosis of diseases.

The elevated generation of peroxynitrite ($ONOO^-$) is a key feature of acute and chronic inflammation as well as an indicative omen of many major diseases such as cancer, cardiopathy, diabetes, and Alzheimer's disease. In this reporting period, we synthesized a fluorescence light-up nanoprobe based on a new and distinctive imine-functionalized tetraphenylethene derivative, namely TPE-IPB, for highly specific detection of elevated $ONOO^-$ generation and high-contrast imaging of inflammation region in vivo. Formulation of TPE-IPB utilizing lipid-PEG matrix yields nanoprobes (TPE-IPB-PEG), which are non-fluorescent in aqueous solution but emit intense yellow fluorescence after reacted with $ONOO^-$ at pH 7.4. The in vivo studies reveal that the emission of TPE-IPB-PEG is switched on selectively in the inflammatory region with elevated generation of $ONOO^-$. The high specificity of the nanoprobe toward elevated $ONOO^-$ production also allows us to precisely and noninvasively monitor in vivo therapeutic efficacy of anti-inflammatory agents. To the best of our knowledge, this is the first report on the fluorescent probes enabling in vivo tracking of therapeutic effect of anti-inflammatory agents.



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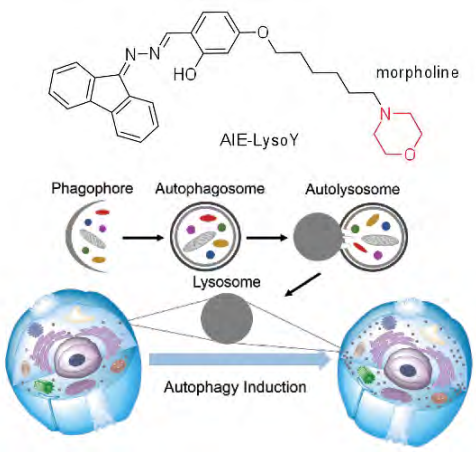
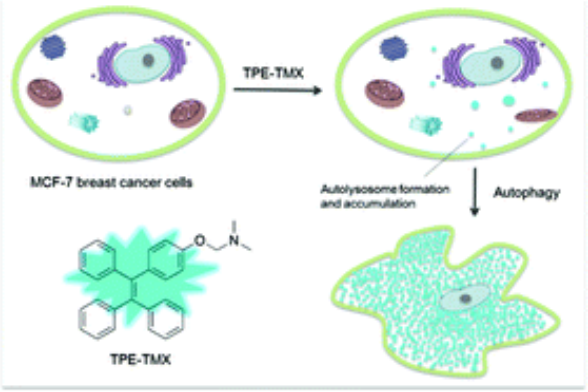


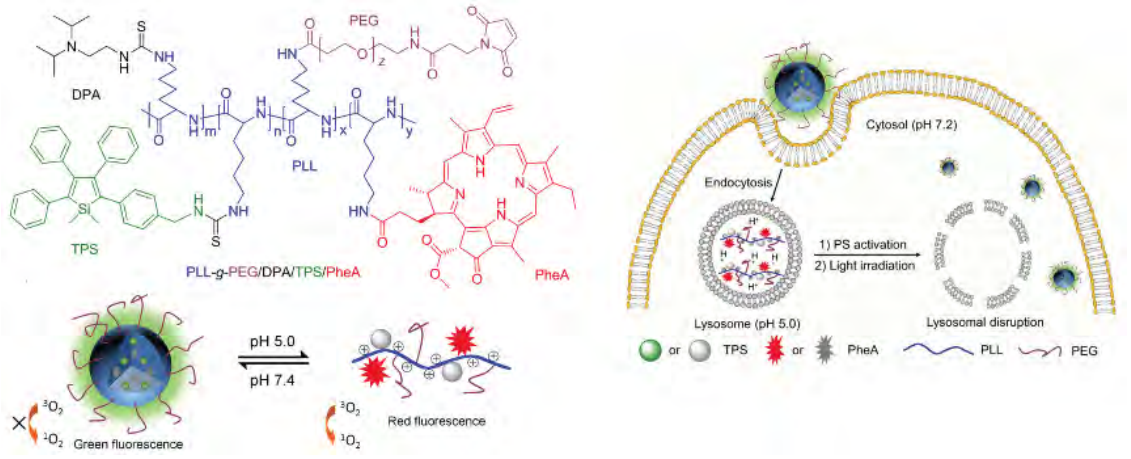
Autophagy has become a hot research topic owing to its close relationship with cancers, aging, longevity, and neurodegenerative diseases, such as Danon disease, Pompe disease, etc. Lysosome is known as the leading player of autophagy. Investigating lysosomal activities is thus indispensable to in-depth investigation of autophagy, through which cells dissemble unnecessary or dysfunctional cellular components in a regulated manner. Recently, we prepared an AIE-active lysosome-specific probe with excited-state intramolecular proton transfer (ESIPT) characteristics, namely AIE-LysoY. Guided by its morpholine functionality, AIE-LysoY selectively accumulates in and lights up the lysosome of cells. Thanks to the collective effect of AIE and ESIPT properties, AIE-LysoY can visualize the lysosome in HeLa cells with superior resolution and contrast. It also enjoys the advantages of large Stokes shift, simple operation, varied incubation concentration and time, excellent photostability and high specificity, which enables it to locate the lysosomes accurately and provide more insight on lysosomal-related intracellular activities such as autophagy.

Tamoxifen (TMX) is pharmacologically classified as a selective modulator of estrogen receptor (ER) for treating breast cancer. It is commonly used as chemotherapeutic agent for curing ~70% patients with ER+ breast cancer. Yet, only a few studies have been carried out to investigate how TMX distributes and functions at the cellular level. Recently, we modified TMX by replacing its ethyl group with a phenyl group and explored the capability of the resulting fluorogen (TPE-TMX) as a theranostic agent for imaging and treatment of breast cancer. Thanks to its easy synthesis, superior photostability and long-term tracing feature, TPE-TMX has been successfully applied for in situ and in vitro tracking of drug distribution and cancer therapeutic efficacy in living cells. Experimental results show that TPE-TMX acts as an estrogen competitor that binds to ER to form a drug–ER complex. As a result, less estrogen is able to bind with ER. With the shortage of the estrogen–ER complex, the cells become unhealthy. This may activate the process of cell death, leading to an increased number of autolysosomes and hence autophagy and cell ablation. Thus, the present work is anticipated to open a new avenue for pharmacokinetics and pharmacodynamics of drug development.

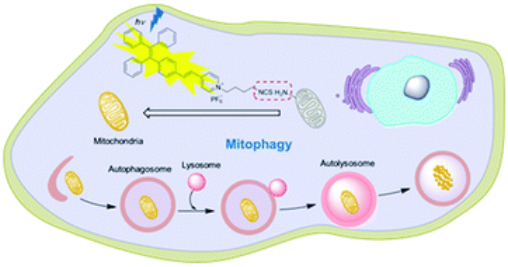


Integrated diagnosis and therapy systems that can offer traceable cancer therapy are in high demand for personalized medicine. In this reporting period, we developed a pH-responsive polymeric probe containing tetraphenylsilole (TPS) with aggregation-induced emission characteristic and pheophorbide A (PheA) photosensitizer (PS) with aggregation-caused quenching effect for self-tracking, selective cancer cell imaging, activated photodynamic ablation of cancer cells with limited side effects, and in situ prediction of the therapeutic response. At physiological conditions (pH 7.4), the probe self-assembles into nanoparticles (NPs), which show weak fluorescence of PheA with low phototoxicity but strong green fluorescence from TPS for probe self-tracking. Upon uptake by cancer cells and entrapment in lysosomes (pH 5.0), the NPs disassemble to yield weak emission of TPS but strong red

fluorescence of PheA with restored phototoxicity for PS activation monitoring. Upon light irradiation, the generated reactive oxygen species can cause lysosomal disruption to trigger cell apoptosis. Meanwhile, the probe leaks to the cytoplasm (pH 7.2), where the TPS fluorescence is restored for in situ visualization of the therapeutic response. The probe design thus represents a novel strategy for traceable cancer therapy. The combination of imaging and therapeutic functionalities in a single probe for simultaneous targeted cancer imaging, therapy, and monitoring of therapeutic response would be highly beneficial for personalized cancer treatment.



We also synthesized a new AIE fluorogen (TPE-Cy-NCS) functionalized with an isothiocyanate moiety and examined its application in mitochondrion imaging and mitophagy monitoring. TPE-Py-NCS can stain the mitochondria of live cells specifically with superior photostability. Due to the formation of strong and stable chemical bonds with the mitochondrial proteins, its emission is preserved after treatment with an uncoupling agent or organic solvent in fixed cells, enabling its usage for real-time monitoring of the mitophagy process.



Instead of normal electronic communication via email and phone, a student from the mainland collaborator has joined the Hong Kong research team to develop strategies for achieving the research goals, to exchange ideas and research outputs, and to solve problems that may encounter during the course of the proposed research project.

6.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	Addressed *(please tick)*	Percentage achieved *(please estimate)*
1.	√	60
2.	√	55
3.	√	50

6.5 Areas to be addressed and results expected in the next reporting period

Most of the AIE luminogens developed so far exhibit blue or green emission. Few of them show strong light emission in the red spectral region. To expand their biological applications, it is necessary to extend their emission color to the red spectral region because luminogens with

longer-wavelength emission can be excited using low-energy irradiation, thus minimizing photo-damage to the live cells. They can also circumvent the spectral overlap with auto-fluorescence of biosubstrates and show high penetration depth, which is an advantage for *in vivo* and deep tissue imaging. We will devote effort to develop these molecules. Different substituents will be incorporated into their molecular structures to endow the resulting materials with desirable solubility, targeting ability and functionality for different applications. It is expected that new water-soluble AIE luminogens with longer-wavelength emission and wide biological applications will be generated in the next reporting period.

Part C: Research Output to date

7. 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 funding support from this scheme by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and 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 this Joint Research Scheme *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*					
2016				Situ, B.; Chen, S.; Zhao, E.; Leung, C. W. T.; Chen, Y.; Hong, Y.; Lam, J. W. Y.; Wen, Z.; Liu, W.; Zhang, W.; Zheng, L.;* Tang, B. Z*	"Real-Time Imaging of Cell Behaviors in Living Organisms by a Mitochondria Targeting AIE Fluorogen" *Advanced Functional Materials* **2016**, *26*, 7132–7138.	31 Dec 2016	Yes	Yes
2016				Song, Z.; Mao, D.; Sung, S. H. P.; Kwok, R. T. K.; Lam, J. W. Y.; Kong, D.; Ding, D.;* Tang, B. Z*	"Activatable Fluorescent Nanoprobe with Aggregation-Induced Emission Characteristics for Selective In Vivo Imaging of Elevated Peroxynitrite Generation" *Advanced Materials* **2016**, *28*, 7249–7256	31 Dec 2016	Yes	Yes

2016				Gu, X.; Zhao, E.; Zhao, T.; Kang, M.; Gui, C.; Lam, J. W. Y.; Du, S.; Loy, M. M. T.; Tang, B. Z*	"A Mitochondrion-Specific Photoactivatable Fluorescence Turn-on AIE-based Bioprobe for Localization Super-Resolution Microscope" *Advanced Materials* **2016**, *28*, 5064–5071.	31 Dec 2016	Yes	Yes
2016				Chen, S.; Wang, H.; Hong, Y.; Tang, B. Z*	"Fabrication of Fluorescent Nanoparticles Based on AIE Luminogens (AIE Dots) and Their Applications in Bioimaging" *Materials Horizons* **2016**, *3*, 283–293.	31 Dec 2016	Yes	Yes
2016				Leung, C. W. T.; Wang, Z.; Zhao, E.; Hong, Y.; Chen, S.; Kwok, R. T. K.; Leung, A. C. S.; Wen, R.; Li, B.; Lam, J. W. Y.; Tang, B. Z*	"A Lysosome-Targeting AIEgen for Autophagy Visualization" *Advanced Healthcare Materials* **2016**, *5*, 427–431.	31 Dec 2016	Yes	Yes
2016				Song, Z.; Zhang, W.; Jiang, M.; Sung, H. H. Y.; Kwok, R. T. K.; Nie, H.; Williams, I. D.; Liu, B.;* Tang, B. Z*	"Synthesis of Imidazole-Based AIEgens with Wide Color Tunability and Exploration of Their Biological Applications" *Advanced Functional Materials* **2016**, *26*, 824–832.	31 Dec 2016	Yes	Yes

2016				Zhao, Y.; Kwok, R. T. K.; Lam, J. W. Y.; Tang, B. Z*	"A Highly Fluorescent AIE-active Theranostic Agent with Anti-Tumor Activity to Specific Cancer Cells" *Nanoscale* **2016**, *8*, 12520–12523.	31 Dec 2016	Yes	Yes
2015				Gu, X.; Zhao, E.; Lam, J. W. Y.; Peng, Q.; Xie, Y.; Zhang, Y.; Wong, K. S.; Sung, H. H. Y.; Williams, I. D.; Tang, B. Z*	"Mitochondrion-Specific Live-Cell Bioprobe Operated in a Fluorescence Turn-On Manner and a Well-Designed Photoactivatable Mechanism" *Advanced Materials* **2015**, *27*, 7093–7100.	31 Dec 2016	Yes	Yes
2015				Yuan, Y.; Kwok, R. T. K.; Tang, B. Z.;* Liu, B*	"Smart Probe for Tracing Cancer Therapy: Selective Cancer Cell Detection, Image-Guided Ablation, and Prediction of Therapeutic Response in Situ" *Small* **2015**, *11*, 4682–4690.	31 Dec 2016	Yes	Yes

8. Recognized International conference(s) in which paper(s) related to this research project was/were delivered *(please attach a copy of each delivered paper.)*

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)*
N/A	N/A	N/A	N/A	N/A	N/A

9. 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
Zhang Pengfei	Doctor of Philosophy	1 Sept 2015	31 Aug 2018
Zhang Haoke	Doctor of Philosophy	1 Sept 2015	31 Aug 2018

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