RGC Ref.: A-HKBU201/12
(please insert ref. above)

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

(Please attach a copy of the completion report submitted to the ANR by the French researcher)

Part A: The Project and Investigator(s)

1. Project Title (ANR Acronym)

Development of Point-of-Care Diagnostic Tools Based on the Conformational Switch of Oligonucleotides (OligoSwitch)

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

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Dr. Ma Dik-lung	Dr. Mergny Jean-Louis
Post	Associate Professor	Directeur - Laboratoire ARNA
Unit / Department / Institution	Department of Chemistry, Hong Kong Baptist University	Institut Européen de Chimie et Biologie, INSERM U1212 - Université de Bordeaux
Contact Information	edmondma@hkbu.edu.hk	jean-louis.mergny@inserm.fr

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2013		01/01/2013
Project Completion date	31/12/2016		31/12/2016
Duration *(in month)*	48		48

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1) To develop and optimize the luminescent oligonucleotide-based detection methodologies for protein biomarkers and mutant DNA using novel transition metal complexes or fluorescently-labeled oligonucleotides. A range of biomarkers and gene deletion products will be tested to evaluate the feasibility of the proposed strategies, and the experimental parameters of the assay will be rigorously and iteratively optimized to enhance the sensitivity and response time of the assays.

2) To analyse and confirm the conformational change of DNA/RNA sequences in the presence of its cognate targets by biophysical methods including UV-melting, circular

dichroism spectroscopy, gel mobility shift assays and surface plasmon resonance spectroscopy.

3) To synthesize novel platinum(II), iridium(III) and rhodium(III) transition metal complexes as selective luminescent probes for monitoring the structure-switching response of the target-sensitive oligonucleotides.

4) To characterize the physical properties, photophysical properties and selectivities of the novel transition metal complexes towards different oligonucleotide conformations using physical (mass spectrometry), spectroscopic (nuclear magnetic resonance, photoluminescence and UV/visible absorption) biological and biophysical assays. Based on these results, the physical properties, photophysical properties and selectivities of the metal complexes will be further optimized by tuning the organic ligands around the metal center.

5) To systematically compare and evaluate the optimized DNA and RNA-based assays, as well as the different signal transducers (luminescent metal complexes, organic dyes, and fluorescently-labeled oligonucleotides), with regards to the sensitivity, selectivity and response time of the assay.

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: ______________________________

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1.
2.
3. ....

6. Research Outcome

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

The utilization of luminescent transition metal complexes for various applications has witnessed tremendous growth over the past several decades, particularly as luminescent probes, for photochemical applications, or for constructing organic optoelectronics. Metal complexes have a several salient advantages which make them capable as attractive alternatives to organic fluorophores for use in luminescent sensing applications. Meanwhile, the progression of a disease in a person is often accompanied by changes in various physiological parameters in the human body. These signals, known as "biomarkers", can be described as gauges of ordinary biological processes, pathological processes, physiological responses to therapeutic intervention or any other measurable diagnostic indicator for evaluating the risk or the existence of a disease. In this project, we have developed a rapid, sensitive and reliable method for the detection of gene deletion, which could be further applied as a universal methodology for the detection of any mutant DNA. This work was published in the peer-reviewed journal *Biosensors and Bioelectronics* (*Biosens. Bioelectron.*, **2015**, *70*, 338). In addition, several label-free G-quadruplex-based luminescent switch-on platforms for biomarker detection were also developed in this project, including detection platforms for targets such as protein tyrosine kinase-7 (PTK-7), interferon-gamma (IFN-γ), anterior gradient homolog 2 (AGR2), hypoxia-inducible factor-1α (HIF-1α), human platelet-derived growth factor BB (PDGF-BB), VEGF165, insulin, among other examples. These works have been published in highly-rated peer-reviewed journals, including *Chemical Science*, *Chemical Communications*, *Analytical Chemistry* and *Biosensors and Bioelectronics* (listed in Part C).

As part of this project, a series of luminescent Ir(III) complexes were synthesised and evaluated for their ability to act as luminescent G-quadruplex-selective probes (Fig. 1). Complexes exhibiting high luminescence for G-quadruplex DNA compared to dsDNA and ssDNA were employed to construct a label-free G-quadruplex-based assay for PTK7 in aqueous solution. PTK7 is an important biomarker for a range of leukemias and solid tumors. In the presence of PTK7, the specific binding of the sgc8 aptamer sequence triggers a structural transition and releases the G-quadruplex-forming sequence. The formation of the nascent G-quadruplex structure is then detected by the G-quadruplex-selective iridium(III) complex with an enhanced luminescent response (Fig. 2). The selectivity of this detection platform for PTK7 over other proteins (human serum albumin (HSA), human plasma fibronectin purified protein (Fn)) was also evaluated, and the results showed that the luminescence response of the system for PTK7 was significantly stronger than that for five-fold excess concentrations of the other proteins (Fig. 2). The detection platform also functioned well in the presence of biological debris, demonstrating that this assay could potentially be further developed for the detection of PTK7 in biological samples.

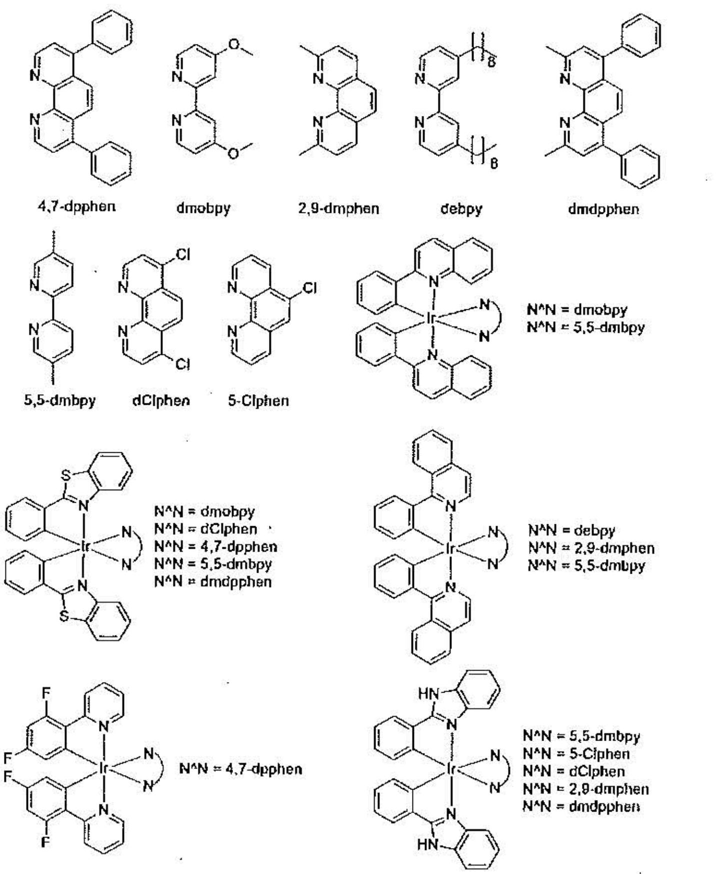

Fig. 1 Chemical structures of cyclometallated iridium(III) complexes synthesized in this project.

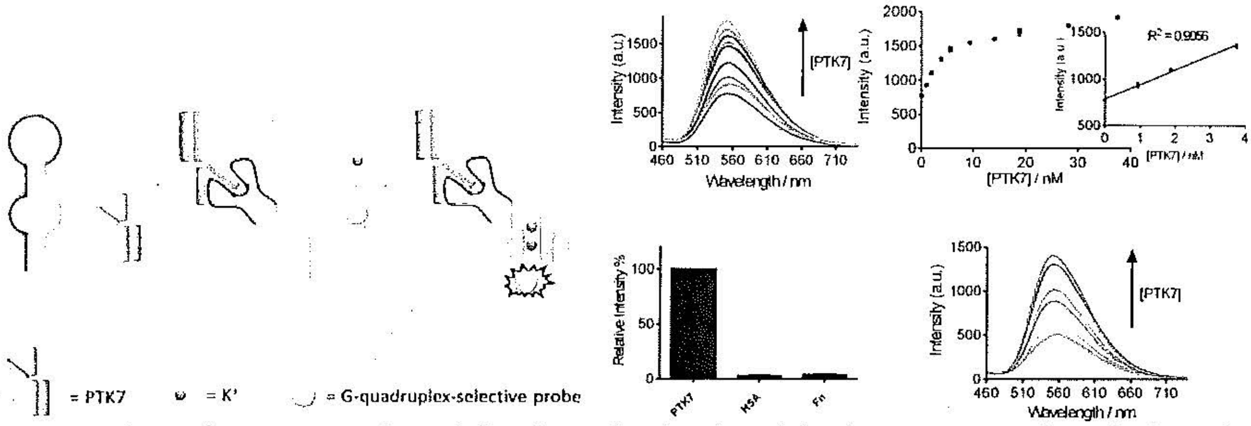

Fig. 2 Schematic representation of the G-quadruplex-based luminescence sensing platform for PTK7 detection and the luminescence spectra and the relationship between luminescence intensity of complex/G4-quadruplex system at $\lambda = 556$ nm in response to various concentrations of PTK7.

This project also led to synthesis of a highly selective G-quadruplex probe **1** which was made by linking a known G-quadruplex groove binder, benzo[*de*]isoquinoline motif, to an Ir(III) complex. The conjugated complex **1** showed advantages of both the parent complex **2** and the groove binder **3** (Fig. 3). Notably, complex **1** exhibits superior affinity and selectivity for G-quadruplex DNA over other conformations of DNA or protein compared to the parent complex **2** (Fig. 4). Molecular modelling revealed a groove-binding mode between complex **1** and G-quadruplex (Fig. 5). Meanwhile, complex **1** also possesses the prominent advantages of transition metal complex probe including large Stokes shift and long lifetime phosphorescence. We successfully employed time-resolved emission spectra (TRES) measurements to demonstrate the detectability of long lifetime luminescence of **1** in the strong fluorescence media (Fig. 6). We then employed **1** to develop a G-quadruplex-based sensing system for the detection of AGR2, a potential serum biomarker for cancer, as a "proof-of-principle" study (Fig. 3 and Fig. 7).

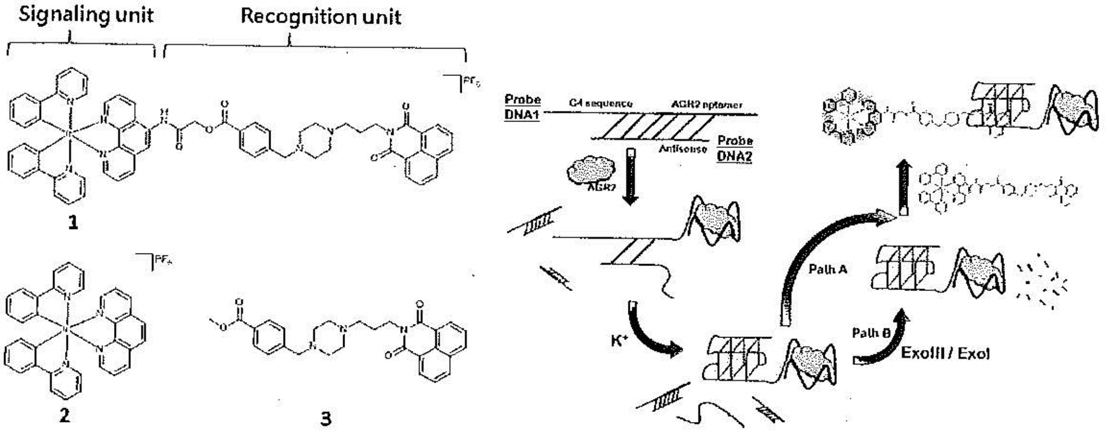

Fig. 3 (a) Chemical structure of Ir(III) complexes **1–2** and the G-quadruplex loop binder **3**. (b) Schematic diagram showing the AGR2 sensing platform utilizing the DNA binder-linked Ir(III) complex **1**.

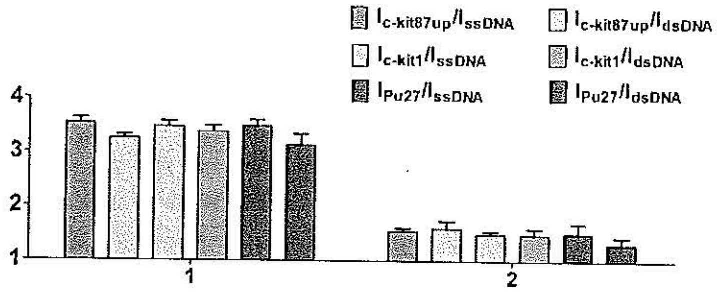

Fig. 4 Diagrammatic bar array representation of the luminescence enhancement selectivity ratio of complexes **1** and **2** upon the addition of c-kit87up, c-kit1 or Pu27 G-quadruplex over ssDNA or dsDNA.

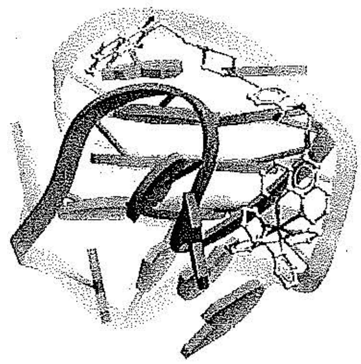

Fig. 5 Side view of the interactions of **1** with G-quadruplex structure in hypothetical molecular model. The G-quadruplex is depicted as a ribbon representation (green), while **1** is depicted as a space-filling representation showing carbon (beige), oxygen (red) and nitrogen (blue).

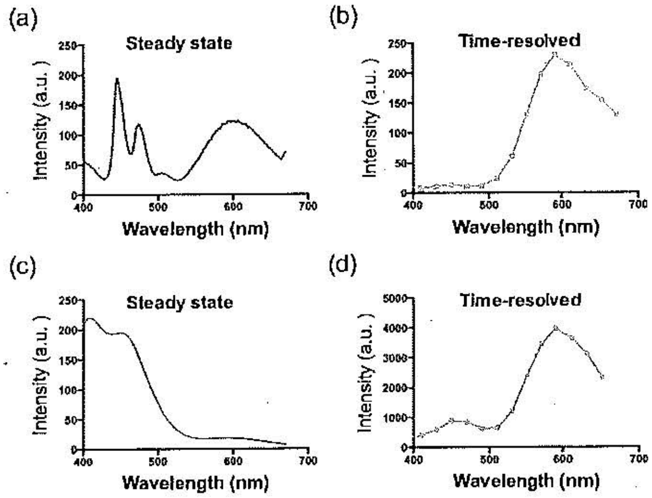

Fig. 6 Steady-state photoluminescence and TRES of **1** in the presence of (a, b) perylene, (c, d) coumarin.

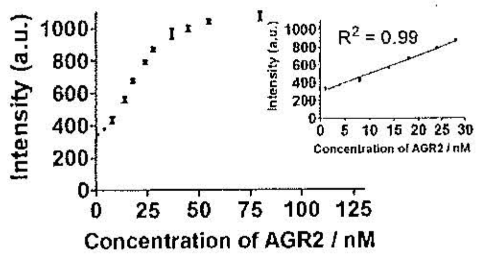

Fig. 7 Linear plot of the change in luminescence intensity at λ = 585 nm vs. AGR2 concentration using the sensing mechanism path B in Fig. 3.

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

This project led to the development of a range of DNA-based sensors for biomarkers and DNA detection utilizing metal complexes. In these assays, the metal complexes play a purely optical role, and their structural recognition properties are of great importance. Towards the future, we anticipate that increasing efforts will be devoted towards developing metal-based probes capable of sensing protein biomarkers in real samples. While a number of detection platforms in this project were able to function in diluted cell extract or cell serum, the application of metal complexes in unadulterated samples will require further research and optimization. Sample pre-treatment protocols may be necessary to remove chemical or biological species that are likely to interfere with the mechanism of the assay. Furthermore, in order to gain greater acceptance by the clinical community, validation of the metal-based detection assays in real samples will be required with comparison to current gold-standard benchmarks.

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)*

In this project, we have developed a rapid, sensitive and reliable method for the detection of gene deletion, which is a universal methodology that can be applied for the detection of any mutant DNA. In addition, several label-free G-quadruplex-based luminescent switch-on platforms for biomarker detection were also developed in this project, including platforms for targets such as protein tyrosine kinase-7 (PTK-7), interferon-gamma (IFN-γ), anterior gradient homolog 2 (AGR2), hypoxia-inducible factor-1α (HIF-1α), human platelet-derived growth factor BB (PDGF-BB), VEGF165, insulin, among other examples. A number of detection platforms in this project were able to function in diluted cell extract or cell serum. In the future, we anticipate that increasing efforts will be devoted towards developing metal-based probes capable of sensing protein biomarkers in real samples.

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) *(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)*					
2014				**L. Lu, D. S.-H. Chan,** D.W.J. Kwong, **H.-Z. He*, C.-H. Leung*, D.-L. Ma*.**	"Detection of nicking endonuclease activity using a G-quadruplex-selective luminescent switch-on probe" *Chem. Sci.*, **2014**, 5, 4561.	No	Yes	Yes

2015				**D.-L. Ma*, L. Lu, S. Lin, B. He, C.-H. Leung.**	"A G-triplex luminescent switch-on probe for the detection of Mung Bean nuclease activity" *J. Mater. Chem. B*, **2015**, *3*, 348.	No	Yes	Yes
2015				**M. Wang, B. He, C.-H. Leung, J.-L. Mergny*, D.-L. Ma***	"Label-free luminescent detection of LMP1 gene deletion using an intermolecular G-quadruplex-based switch-on probe" *Biosens. Bioelectron.*, **2015**, *70*, 338.	No	Yes	Yes
2015				**K.-H. Leung, H.-Z. He, B. He, H.-J. Zhong, S. Lin**, Y.-T. Wang, **D.-L. Ma*, C.-H. Leung*.**	"Label-free luminescence switch-on detection of hepatitis C virus NS3 helicase activity using a G-quadruplex-selective probe" *Chem. Sci.*, **2015**, *6*, 2166.	No	Yes	Yes

2015				**S. Lin, W. Gao, Z. Tian, C. Yang, L. Lu, J.-L. Mergny*, C.-H. Leung*, D.-L. Ma***	"Luminescence switch-on detection of protein tyrosine kinase-7 using a G-quadruplex-selective probe" *Chem. Sci.*, **2015**, *6*, 4284.	No	Yes	Yes
2015				**L. Lu, M. Wang, L.-J. Liu,** C.-Y. Wong, **C.-H. Leung*, D.-L. Ma*.**	"A luminescence switch-on probe for terminal deoxynucleotidyl transferase (TdT) activity detection by using an iridium(III)-based i-motif probe" *Chem. Commun.*, **2015**, *51*, 9953.	No	Yes	Yes
2015				**S. Lin, B. He, C. Yang, C.-H. Leung*, J.-L. Mergny*, D.-L. Ma***	"Luminescence switch-on assay of interferon-gamma using a G-quadruplex-selective iridium(III) complex" *Chem. Commun.*, **2015**, *51*, 16033.	No	Yes	Yes

2015				**K.-H. Leung, B. He, C. Yang, C.-H. Leung***, H.-M. D. Wang*, **D.-L. Ma***.	"Development of an aptamer-based sensing platform for metal ions, proteins and small molecules through terminal deoxynucleotidyl transferase-induced G-quadruplex formation" *ACS Appl. Mater. Interfaces*, **2015**, *7*, 24046.	No	Yes	Yes
2015				**L. Lu, H.-J. Zhong, B. He, C.-H. Leung*, D.-L. Ma***.	"Development of a luminescent G-quadruplex-selective iridium(III) complex for the label-free detection of adenosine" *Sci. Rep.*, **2015**, 6, 19368.	No	Yes	Yes
2016				**M. Wang, Z. Mao, T.-S. Kang, C.-Y. Wong, J.-L. Mergny*, C.-H. Leung* and D.-L. Ma***	"Conjugating a groove-binding motif to Ir(III) complex for the enhancement of G-quadruplex probe behavior" *Chem. Sci.*, **2016**, *7*, 2516.	No	Yes	Yes

2016				**L. Lu, M. Wang, Z. Mao, T.-S. Kang, X.-P. Chen, J.-J. Lu, C.-H. Leung, D.-L. Ma***	"A novel dinuclear iridium(III) complex as a G-quadruplex-selective probe for the luminescent switch-on detection of transcription factor HIF-1α" *Sci. Rep.*, ***2016***, *6, 22458.*	No	Yes	Yes
2016				**X. Lin, K.-H. Leung, L. Lin, L. Lin, S. Lin, C.-H. Leung, D.-L. Ma***	"Determination of cell metabolite $VEGF_{165}$ and dynamic analysis of protein-DNA interactions by combination of microfluidic technique and luminescent switch-on probe" *Biosens. Bioelectron.*, **2016**, *79*, 41.	No	Yes	Yes
2016				**L. Lu*, Z. Mao, T.-S. Kang, C.-H. Leung*, D.-L. Ma***	"A versatile nanomachine for the sensitive detection of platelet-derived growth factor-BB utilizing a G-quadruplex-selective iridium(III) complex" *Biosens. Bioelectron.*, **2016**, *85*, 300.	No	Yes	Yes

2016				**M. Wang, W. Wang, T.-S. Kang, C.-H. Leung*, D.-L. Ma***	"Development of an iridium(III) complex as a G-quadruplex probe and its application for the G-quadruplex-based luminescent detection of picomolar insulin" *Anal. Chem.*,**2016**, *88*, 981.	No	Yes	Yes
2016				**S. Lin, L. Lu, J.-B. Liu, C. Liu, T.-S. Kang, C. Yang, C.-H .Leung*, D.-L. Ma***	"A G-quadruplex-selective luminescent iridium(III) complex and its application by long lifetime" *Biochim. Biophys. Acta Gen. Subjects,* **2016**, DOI:10.1016/j.bbagen.2016.08.022..	No	Yes	Yes
2016				**L. Lu, W. Wang, M. Wang, T.-S. Kang,** J.-J. Lu, X.-P. Chen, Q.-B. Han, **C.-H. Leung, D.-L. Ma*.**	"A luminescent G-quadruplex-selective iridium(III) complex for the label-free detection of lysozyme" *J. Mater. Chem. B,* **2016**, *4*, 2407.	No	Yes	Yes

2016				**X. Miao, W. Wang, T.-S. Kang,** J. Liu, K.-K. Shiu, **C.-H. Leung, D.-L. Ma*.**	"Ultrasensitive electrochemical detection of miRNA-21 by using an iridium(III) complex as catalyst" *Biosens. Bioelectron*., **2016**, *86*, 454.	No	Yes	Yes
2016				**S. Lin, T.-S. Kang, L. Lu, W. Wang, D.-L. Ma *, C.-H. Leung*.**	"A G-quadruplex-selective luminescent probe with an anchor tail for the switch-on detection of thymine DNA glycosylase activity" *Biosens. Bioelectron*., **2016**, *86*, 849.	No	Yes	Yes
2016				**S. Lin, L. Lu, T.-S. Kang, J.-L. Mergny*, C.-H. Leung*, D.-L. Ma*.**	"The interaction of an iridium(III) complex with G-quadruplex DNA and its application in luminescent switch-on detection of Siglec-5" *Anal. Chem.*, **2016**, *88*, 10290.	No	Yes	Yes

2016				**L. Lu, W. Wang, C. Yang, T.-S. Kang, C.-H. Leung*, D.-L. Ma*.**	"Iridium(III) complexes with 1,10-Phenanthroline-based N^N ligands as highly selective luminescent G-quadruplex probes and application for switch-on ribonuclease H detection" *J. Mater. Chem. B*, **2016**, *4*, 6791.	No	Yes	Yes

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)*
July/2013/ Singapore	Luminescent G-quadruplex-based probes	4th International Meeting on G-quadruplex Nucleic Acids	No	No	Yes	Yes
Octber/2013 /Beijing, China	Label-free luminescent oligonucleotide-based probes	The 15th Beijing Conference and Exhibition on Instrumental Analysis	No	No	Yes	Yes
August/2014 /Zurich	Label-free DNA-based biosensing with luminescent metal complexes	The European Biological Inorganic Chemistry Conference (EuroBIC) 12	No	No	Yes	Yes

Octber/2015 /Beijing, China	Label-Free Luminescent Oligonucleotide-Based Probe for Enzyme Activity Detection	The 16th Beijing Conference and Exhibition on Instrumental Analysis (BCEIA)	No	No	Yes	Yes

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
He Hong-Zhang	PhD	16 Sep 2011	15 Sep 2014
Leung Ka-Ho	PhD	01 Jan 2012	31 Dec 2014
Lu Lihua	PhD	01 Dec 2012	30 Nov 2015
Wang Modi	PhD	16 Sep 2013	15 Sep 2016

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

In this project, we have also collaborated with Dr. Chung-Hang Leung from University of Macau and Prof. Jin-Ming Lin from Tsinghua University for the development of the diagnostic tools based on the conformational switch of oligonucleotides and the luminescence signals of iridium(III) complexes.