| RGC Ref.: A-HKBU201/1      |
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# 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<br>ARNA  |
| Unit / Department / Institution             | Department of Chemistry,<br>Hong Kong Baptist<br>University | Institut Européen de Chimie et<br>Biologie, INSERM U1212 -<br>Université de Bordeaux |
| Contact Information                         | edmondma@hkbu.edu.hk  | jean-louis.mergny@inserm.fr  |

## 3. Project Duration

|                         | Original   | Revised          | Date of RGC/<br>Institution Approval<br>(must be quoted) |
|-------------------------|------------|------------------|--|
| Project Start date      | 01/01/2013 |                  | 01/01/2013   |
| Project Completion date | 31/12/2016 | And and a second | 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

#### NR/RGC 8 (Revised 10/15)

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

3. ....

| Date of approval from the RG | C: <u>N/A</u> |                      |  |
|------------------------------|---------------|----------------------|--|
| Reasons for the change:      |               | ***                  |  |
| <u> </u>                     |               | 2024 (See) 3022 (See |  |
| <i>1.</i>                    | . *           |                      |  |

#### 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),

#### IR/RGC 8 (Revised 10/15)

hypoxia-inducible factor-1a (HIF-1a), 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.

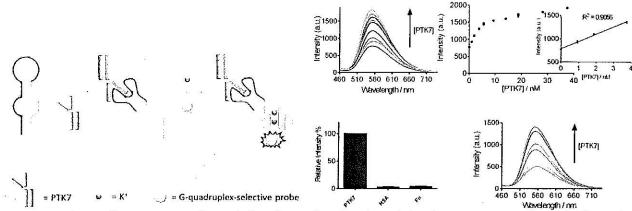


Fig. 2 Schematic representation of the G-quadruplex-based luminescence sensing platform for PTK7 detection and the luminescence spectra and the relationship between luminescence intensity of complex/G4-quadruplex system at  $\lambda = 556$  nm in response to various concentrations of PTK7.

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).

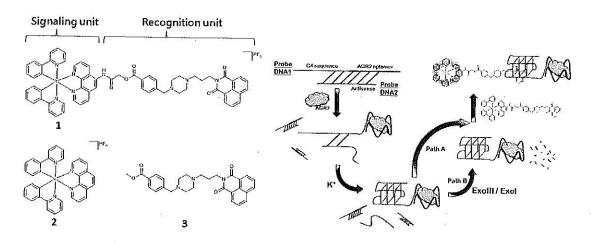


Fig. 3 (a) Chemical structure of Ir(III) complexes 1–2 and the G-quadruplex loop binder 3. (b) Schematic diagram showing the AGR2 sensing platform utilizing the DNA binder-linked Ir(III) complex 1.

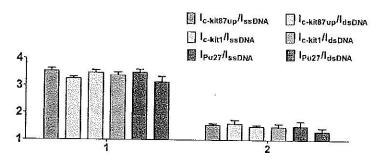


Fig. 4 Diagrammatic bar array representation of the luminescence enhancement selectivity ratio of complexes 1 and 2 upon the addition of c-kit87up, c-kit1 or Pu27 G-quadruplex over ssDNA or dsDNA.

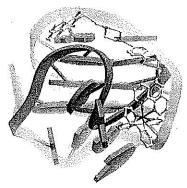


Fig. 5 Side view of the interactions of 1 with G-quadruplex structure in hypothetical molecular model. The G-quadruplex is depicted as a ribbon representation (green), while 1 is depicted as a space-filling representation showing carbon (beige), oxygen (red) and nitrogen (blue).

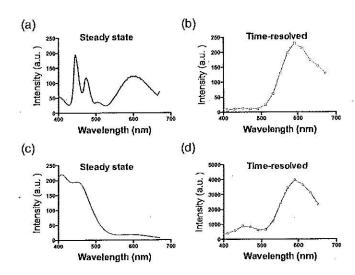


Fig. 6 Steady-state photoluminescence and TRES of 1 in the presence of (a, b) perylene, (c, d) coumarin.

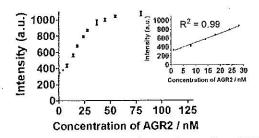


Fig. 7 Linear plot of the change in luminescence intensity at  $\lambda = 585$  nm vs. AGR2 concentration using the sensing mechanism path B in Fig. 3.

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 Publicat | ions        | Author(s)      | Title and                         | Submitted to  | Attached | Acknowledged   |
|-------------|---------------|-------------|-------------|----------------|-----------------------------------|---------------|----------|----------------|
| Year of     | Year of       | Under       | Under       | (bold the      | Journal/ Book                     |               |          | the support of |
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| 2015 |   |  | Lu, JL.<br>Mergny*,<br>CH.                                | "Luminesc ence switch-on detection of protein tyrosine kinase-7 using a G-quadrupl ex-selective probe" Che m. Sci., 2015,  | E  | Yes  | Yes |
| 2015 |   |  | Wong,<br>CH.<br>Leung*, D.<br>-L. Ma*.                    | ce switch-on probe for terminal deoxynucle otidyl transferase (TdT) activity detection by using an iridium(III) -based i-motif probe" Che m. Commun., 2 015, 51, | No | Yes  | Yes |
| 2015 |   |  | S. Lin, B. He, C. Yang, CH . Leung*, JL. Mergny*, DL. Ma* | 9953.<br>"Luminesce<br>nce   |    | Yes  | Yes |

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|   |     |      |       |              | luminescent   |      |           |      |
|   |     |      |       |              | switch-on     |      |           |      |
|   |     |      |       |              | probe" Bios   |      | n 6       |      |
|   |     | ALC: |       |              |               |      |           |      |
|   |     |      |       |              | ens.          |      |           | 3    |
|   |     |      |       |              | Bioelectron   |      |           |      |
|   |     | 8    |       |              | ., 2016, 79,  |      |           |      |
|   |     |      |       |              | 41.           | is . |           |      |
| 2016  |     | 8    |       | L. Lu*, Z.   | "A versatile  | No   | Yes       | Yes  |
|   |     |      |       | Mao, TS.     | nanomachi     | -    | yest Mark | 150F |
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|   |     |      |       |              | sensitive     |      |           |      |
|   |     |      |       | <b>.</b>     | detection of  | (6)  |           |      |
|   |     |      | à     | -L. Ma*      | platelet-deri |      |           |      |
|   |     |      | NAT   |              | ved growth    |      | g         |      |
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|   |     |      |       |              | utilizing a   |      |           |      |
|   |     |      |       |              | G-quadrupl    |      | 1         |      |
|   |     |      | vas X |              |               |      |           |      |
|   |     |      |       |              | ex-selective  |      |           |      |
| ,   | ) N |      | 125   |              | iridium(III)  |      |           |      |
|   |     |      |       |              | complex" B    |      |           |      |
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| 2016 |   |            |    | M.   | "Developm    | No           | Yes  | Yes  |
|      |   |            |    | Wang, W.   | ent of an    |              |      |      |
|      |   |            |    | Wang,  | iridium(III) |              |      |      |
|      |   |            |    | TS. Kang,  | complex as   |              |      |      |
|      |   |            |    | СН.  | a            |              |      |      |
|      |   |            |    | Leung*, D.   | G-quadrupl   |              |      | 5    |
| 175  |   |            |    | -L. Ma*  | ex probe     |              |      |      |
|      |   | Ī          |    |  | and its      |              |      |      |
|      |   |            | ļ  |  | application  |              |      |      |
|      | 88                                      |            |    |  | for the      |              | 10   |      |
|      |   |            |    |  | G-quadrupl   |              |      |      |
|      | ļ<br>Ē                                  |            |    |  | ex-based     |              |      |      |
| -    | 1                                       |            | ¥  | 35   | 1            |              | 55   | *    |
|      |   |            |    |  | luminescent  |              |      |      |
| 100  |   |            |    |  | detection of | 1            |      | •    |
|      |   | §          |    |  | picomolar    |              |      |      |
|      | ļ                                       |            |    |  | insulin" An  | <b> </b><br> |      |      |
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|      |   |            |    |  | Chem.,2016   |              |      |      |
|      | 5.45                                    | 50.80      |    |  | , 88, 981.   |              |      |      |
| 2016 |   | No. 9 (12) |    | S. Lin, L.   | "A           | No           | Yes  | Yes  |
|      |   |            |    | Lu, JB.  | G-quadrupl   |              |      |      |
|      |   | 8          |    | Liu, C.  | ex-selective |              | 7    | 6    |
| *    | ļ                                       |            | D. | Liu, TS.   | luminescent  |              |      |      |
|      |   |            |    | Kang, C.   | iridium(III) |              |      |      |
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|      |   |            |    | Yang, CH   |              |              |      |      |
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|      |   |            |    | -L. Ma*  | application  |              | 2    |      |
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|      | 20                                      |            |    |  | Subjects, 20 |              |      |      |
|      |   |            |    |  | 16, DOI:10.  |              |      |      |
|      |   |            |    | el .   | 1016/j.bbag  |              |      |      |
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|      |   |            |    | . Kang, JJ.  |              |              |      |      |
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| L. Ma*. complex as catalyst" Bi osens. Bioelectron 2016, 86, 454.  S. Lin, TS. G-quadrupl ex-selective luminescent witch-on detection of thymine DNA glycosylase activity" Bi osens. Bioelectron 2016, 86, 849.  S. Lin, L. Lu, W. Biolectron 2016, 86, 849.  S. Lin, L. Lu, TS. Kang, JL. of an iridium(III) cH. Leung*, D. Leung*, D. Leung*, D. Leung*, D. Leung*, D. L. Ma*. G-quadrupl ex DNA and its application in luminescent switch-on detection of Siglec-5" A nad. Chem.  |  |            |  |      | The state of the s |                   |
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| detection of Siglec-5" A nal. Chem.,   |  |            |  |      |  | assesses          |
| Siglec-5" A nal. Chem.,  |  |            |  |      |  | Applementa        |
| nal. Chem.,  |  |            |  |      |  | ĺ                 |
|  |  |            |  |      |  | 20                |
| 2016 88  |  |            |  |      |  |                   |
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| 10290.   |  |            |  |      |  |                   |

| 2016   |   | 387-92230-0 |   | L. Lu, W.  | "Iridium(III | No | Yes           | Yes |
|--------|---|-------------|---|------------|--------------|----|---------------|-----|
|        |   | *           |   | Wang, C.   | ) complexes  | j  | 3             |     |
|        |   |             | ~ | Yang, TS.  | with         |    |               |     |
|        |   |             |   | Kang, CH   | 1,10-Phena   |    |               |     |
|        |   |             |   |            | nthroline-b  |    |               |     |
|        |   |             |   | Leung*, D. | ased         | *  |               |     |
|        |   |             |   |            | N^N ligand   |    |               |     |
|        |   |             |   |            | s            |    |               |     |
|        |   |             |   |            | as highly se |    | S             |     |
|        |   |             |   |            | lective lumi |    |               |     |
|        |   |             |   |            | nescent      |    |               |     |
|        |   |             |   |            | G-quadrupl   |    |               | ]   |
|        |   |             |   | 100 M      | ex probes    | 0  |               |     |
|        |   |             |   |            | and applicat |    | 0             |     |
|        |   |             |   |            | ion          |    |               |     |
|        |   | 3           |   |            | for switch-o |    |               |     |
|        |   |             |   |            | n ribonucle  |    |               |     |
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| 100 mg |   | 8,          |   |            | " J. Mater.  |    |               |     |
|        |   |             |   |            | Chem.        |    |               |     |
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|        |   |             |   |            | 6791.        |    | 672 16,675 14 |     |

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/<br>Place              |  | Conference Name  | Submitted to RGC (indicate the year ending of the relevant progress report) | Attached<br>to this<br>report<br>(Yes or No) | this Joint | Accessible from the institutional repository (Yes or No) |
|-----------------------------------|--|--|---|--|------------|--|
| July/2013/<br>Singapore           | Luminescent<br>G-quadruplex-based<br>probes                      | 4 <sup>th</sup> International<br>Meeting on<br>G-quadruplex<br>Nucleic Acids | No  | No   | Yes        | Yes  |
| Octber/2013<br>/Beijing,<br>China | Label-free<br>luminescent<br>oligonucleotide-base<br>d probes    | Conference and   | No  | No   | Yes        | Yes  |
| August/2014<br>/Zurich            | Label-free DNA-based biosensing with luminescent metal complexes | The European Biological Inorganic Chemistry Conference (EuroBIC) 12          | No  | No   | Yes        | Yes  |

## √R/RGC 8 (Revised 10/15)

| Octber/2015 | Label-Free          | The 16 <sup>th</sup> Beijing | No | No | Yes       | Yes |     |
|-------------|---------------------|------------------------------|----|----|-----------|-----|-----|
| /Beijing,   | Luminescent         | Conference and               |    |    |           |     |     |
| China       | Oligonucleotide-Bas | Exhibition on                | 5  |    |           |     |     |
|             | ed Probe for Enzyme | Instrumental                 |    |    |           | 6 6 | 122 |
|             | Activity Detection  | Analysis (BCEIA)             | *2 |    | Universal |     |     |

## 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<br>submission/<br>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.