DOCTORAL THESIS

Luminescent iridium(III) complex-based sensing assays for the disease-related biomolecule and the endogenously generated small molecule

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Date of Award:
2020

ABSTRACT

Transition metal complex, especially iridium(III) complex, possesses substantial advantages in sensing applications. To date, a series of iridium(III) complex-based chemosensors and biosensors have been constructed for the detection of a wide range of biologically important analytes, including ions, small molecules, amino acids, peptides and proteins. **Chapter 1** provides an overview of the general synthetic routes and properties of iridium(III) complexes. General strategies for the development of iridium(III) complex-based chemosensors and the utilization of iridium(III) complex as a DNA probe in biosensors are also reviewed.

Chapter 2 describes the application of an iridium(III) complex as a switch "on-off-on" chemosensor for the detection of both exogenously supplied and endogenously generated sulfide ion *in vitro*, *in cellulo* and *in vivo*. The optimized probe (**1–Fe^{3+}**), which coordinates Fe^{3+} ion to an iridium(III) complex, could achieve a limit of detection (LOD) of sulfide ion down to 2.9 μM and establish a linear detection range from 10 to 1500 μM. While **1–Fe^{3+}** did not show any luminescence response *in vitro* under a high concentration of thiols, it exhibited a significant luminescence enhancement when the concentration of thiols was perturbed *in cellulo* and *in vivo*. This phenomenon can be explained by the presence of cystathionine gamma-lyase (CGL) and cystathionine beta synthase (CBS) *in cellulo* that could

catalyze the conversion of the hydrogen sulfide (H_2S) precursors, including cysteine and glutathione (GSH), into H_2S. The results of this work have been published in a peer-reviewed scientific journal (*Biosens. Bioelectron*, **2017**, *94*, 575).

Chapter 3 discusses the adaptation of an oligonucleotide-based Vascular endothelial growth factor 165 ($VEGF_{165}$) biosensor on a portable microfluidic device. An iridium(III) complex with an extensive conjugation system was used as a long-lived and red-emitting G-quadruplex probe. The polypropylene (PP)-based suspended-droplet microfluidic chip allows easy sample introduction, flexible sample volume range and valve-free manipulation of a stepwise reaction. We successfully assembled all the required components, including a ultraviolet (UV) lamp, a filter, a rotatable sample holder and a detector, into a portable box. The device could achieve a LOD of $VEGF_{165}$ down to the picomolar level, which is comparable to the results of a conventional fluorometer. The results of this work have been published in a peer-reviewed scientific journal (*Dalton Trans.*, **2019**, *48*, 9824)

The integration of graphene oxide nanomaterial to an oligonucleotide-based isothermal signal amplification system is presented in **Chapter 4**. Strand displacement amplification (SDA) could substantially amplify the signal from the target Hepatitis B virus (HBV) gene, while the electron accepting graphene oxide could effectively quench the emission of iridium(III) complex and enlarged the

luminescence fold-enhancement of the system. The system could achieve a LOD for the HBV gene down to the picomolar level and was selective for the wild-type HBV gene over the single-base mutated HBV gene. The operation mechanism and the important rules for the formation of a stable split G-quadruple are detailed in this chapter. The results of this work have been submitted to a peer-reviewed scientific journal.

In **Chapter 5**, the adaptation of SDA on an exonuclease III-assisted amplification (EXO) as a quadruple-cycle phosphorescence amplification system is reported. A systematic three-round structural optimization campaign was performed for the first time to iteratively improve the G-quadruplex selectivity of a large pool of iridium(III) complex. The proof-of-principle application of a self-assembly tetrahedron nanostructure (TNS)-based aptasensor was demonstrated using a cancer biomarker mucin 1 as the target analyte. This TNS-based aptasensor revealed a 57% higher luminescence enhancement compared to the conventional dsDNA aptasensing approach. The results of this work will be submitted to a peer-reviewed scientific journal upon completion of mechanism studies.

Chapter 6 summarizes the properties, advantages, improvements and potentials for the developed iridium(III) complex-based sensors.

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