DOCTORAL THESIS

Exploring label-free G-quadruplex-based luminescent sensing platform for the detection of biomolecules and metal ions

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Abstract

G-quadruplexes represent a versatile sensing platform for the construction of label-free molecular detection assays due to their diverse structures that can be selectively recognized by G-quadruplex-specific luminescent probes. In this thesis, we have explored the applications of the label-free G-quadruplex-based luminescent detection platforms for the detection of biomolecules and metal ions.

Chapter 1 provides an overview of the principles and recent developments of the field of luminescent oligonucleotide-based probes, and highlighting in particular the use of the "label-free" strategy for the construction of simple and inexpensive sensing platforms.

Chapter 2 introduces the basic experiments performed during the course of this thesis, including UV/Vis absorption spectroscopy, luminescence spectroscopy, nuclear magnetic resonance, mass spectrometry circular dichroism spectroscopy and G-quadruplex fluorescent intercalator displacement assay.

Chapter 3 describes a G-quadruplex-based switch-on luminescence assay for the detection of gene deletion using iridium(III) complex **1** as a G-quadruplex-selective probe. Our method is based on the formation of a split G-quadruplex upon hybridization of two critically designed quadruplex-forming sequences with the mutant DNA sequence, resulting in a "switch-on" luminescence response.

Chapter 4 describes a label-free, oligonucleotide-based, switch-on luminescence detection method for T4 polynucleotide kinase activity using a G-quadruplex-selective luminescent iridium(III) complex **2**. The application of the assay for screening potential T4 PNK inhibitors is also demonstrated. To our knowledge, this is the first metal-based assay for PNK activity that has been reported in the literature.

Chapter 5 describes a label-free oligonucleotide-based luminescence switch-on assay for the selective detection of sub-nanomolar Pb^{2+} ions in aqueous solution and real water samples. Iridium(III) complex **1** was employed as a G-quadruplex-specific luminescent probe and a guanine-rich DNA sequence (PS2.M, 5′-$GTG_3TAG_3CG_3T_2G_2$-3′) was employed as recognition unit for Pb^{2+} ions. The assay could detect Pb^{2+} ions in aqueous media with a limit of detection of 600 pM, and also exhibited good selectivity for Pb^{2+} ions over other heavy metal ions. Furthermore, the application of the assay for the detection of Pb^{2+} ions in spiked river water samples was demonstrated.

Chapter 6 describes a label-free G-quadruplex-based luminescent switch-on assay for the selective detection of micromolar histidine in aqueous solution. Iridium(III) complex **8** was employed as a G-quadruplex-specific luminescent probe while a guanine-rich oligonucleotide (Pu27, 5′-$TG_4AG_3TG_4AG_3TG_4A_2G_2$-3′)/cupric

ion (Cu^{2+}) ensemble was employed as a recognition unit for histidine. The assay could detect down to 1 μM of histidine in aqueous media, and also exhibited good selectivity for histidine over other amino acids with the use of the cysteine-masking agent *N*-ethylmaleimide. Furthermore, the application of the assay for the detection of histidine in diluted urine samples was demonstrated.

Chapter 7 summarizes the work that was conducted in this thesis, and the future outlook of G-quadruplex-based sensing is presented.

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