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Label-free fluorescent aptasensor exploiting spectral changes in DNA aptamer-dye complexes

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The fluorescent dye, SYBR Green I (SG), is widely used to stain double-stranded DNA (dsDNA) because its fluorescence is activated in the intercalated state. This dye can also be used in turn-on fluorescent apta-sensors that introduce dsDNA motifs by including complementary DNA strands. However, intercalating dyes have not been successful for simple single-stranded DNA (ssDNA) apta-mers, because target binding seldom changes the double stranded content sufficiently to produce a detectable change in fluorescence intensity. Herein, we examine a system where target binding does not alter the fluorescence intensity, but we find that a clear spectral shift that enables ratio-metric detection. For the well-known Adenosine Triphosphate (ATP) binding apta-mer, we have showed that the target binding induces a clear change in fluorescence spectrum of SG. Thus, taking the ratio of fluorescence intensity at 564 nm to 523 nm (F564/F523) enables us to resolve a binding isotherm. Circular dichroism, UV-vis absorption and fluorescence spectroscopies were comprehensively applied to study the sensor's performance and the underlying mechanism of spectra shift and distortion. We find that the performance of this sensor is greatly influenced by dye per base ratios (dbrs). At a selected dbr=0.7, the sensor shows a preferable leaner range from 10 μM to 1 mM with a level of detection of 2.4 μM ([ATP]cell≥0.5 mM) and an apparent dissociation constant (Kd) of 12 μM, which is consistent with data reported in literature. In addition, the influence of intercalating organic dye molecules on the affinity of an apta-mer and consequently, the performance of the corresponding fluorescence apta-sensor was investigated and discussed for the first time. We expect this approach to be applicable to many other apta-mer systems, since secondary structure is usually scaffold by some degree of duplex regions available to host intercalating dyes.

Biography

Ye Liu has completed her Bachelor's and Master's degree in Applied Chemistry from Dalian University of Technology, China and then was accepted as a PhD student by Victoria University of Wellington, NZ, working with Associate Professor Justin M Hodgkiss in the Macdiarmid Institute. Her research interest during her master was NaYF4-based up-conversion nano/micro-materials and now is mainly focused on ssDNA-based apta-sensors.

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