

## **G-quadruplex microspheres-based optical RNA biosensor for arthropod-borne virus pathogen detection: A proof-of-concept with dengue serotype 2**

### **ABSTRACT**

Dengue virus (DENV) is a positive-sense single-stranded RNA virus and that the detection of viral RNA itself is highly desirable, which can be achieved by using RNA biosensor diagnostic method. Herein, acrylic micropolymer-based optical RNA biosensor was developed by binding anionic copper(II) phthalocyanine (CPC) planar aromatic ligand to the G-quadruplex DNA probe via end-stacking with  $\pi$ -system of the guanine (G) quartet, and a blue coloration was developed on the G-quadruplex microspheres. Hybridization of G-quadruplex DNA probe with target DENV serotype 2 (DENV2) RNA unfolded the G-quadruplex, and rendering release of the CPC planar optical label, causing discoloration of the G-quadruplex microbiosensor. Optical characterization of the RNA biosensor was performed by means of fiber optic reflectance spectrophotometer at maximum reflectance wavelength of 774 nm. The reflectance response enhancement of the RNA-responsive G-quadruplex-based reflectometric biosensor was linearly proportional to the target oligo DENV2 RNA concentration in the range of 2 zM–2  $\mu$ M, with a 0.447 zM limit of detection and a rapid response time of 30 min. Heightening in the reflectance signal based on structural transition of G-quadruplex in response to target RNA was successfully implemented in real-time DENV2 detection in non-invasive human fluid samples (i.e. saliva and urine) under informed consent.

**Keyword:** Dengue virus; G-quadruplex DNA; Microspheres; Optical biosensor; RNA biosensor