Role of the Probe Sequence/Structure in Developing an Ultra-Efficient Label-Free COVID-19 Detection Method Based on Competitive Dual-Emission Ratiometric DNA-Templated Silver Nanoclusters as Single Fluorescent Probes

Molaabasi Fatemeh, Kefayat Amirhosein, Ghasemzadeh Abbas, Amandadi Mojdeh, Shamsipur Mojtaba, Alipour Mozhgan, Moosavifard Seyyed Ebrahim, Besharati Maryam, Hosseinkhani Saman, Sarrami-Forooshani Ramin

Abstract

We report the development of a label-, antibody-, enzyme-, and amplification-free ratiometric fluorescent biosensor for low-cost and rapid (less than 12 min) diagnosis of COVID-19 from isolated RNA samples. The biosensor is designed on the basis of cytosine-modified antisense oligonucleotides specific for either N gene or RdRP gene that can form silver nanoclusters (AgNCs) with both green and red emission on an oligonucleotide via a one-step synthesis process. The presence of the target RNA sequence of SARS-CoV-2 causes a dual-emission ratiometric signal transduction, resulting in a limit of detection of 0.30 to 10.0 nM and appropriate linear ranges with no need for any further amplification, fluorophore, or design with a special DNA fragment. With this strategy, five different ratiometric fluorescent probes are designed, and how the T/C ratio, the length of the stem region, and the number of cytosines in the loop structure and at the 3′ end of the cluster-stabilizing template can affect the biosensor sensitivity is investigated. Furthermore, the effect of graphene oxide (GO) on the ratiometric behavior of nanoclusters is demonstrated and the concentration-/time-dependent new competitive mechanism between aggregation-caused quenching (ACQ) and aggregation-induced emission enhancement (AIE) for the developed ssDNA-AgNCs/GO nanohybrids is proposed. Finally, the performance of the designed ratiometric biosensor has been validated using the RNA extract obtained from more than 150 clinical samples, and the results have been confirmed by the FDA-approved reverse transcription-polymerase chain reaction (RT-PCR) diagnostic method. The diagnostic sensitivity and specificity of the best probe is more than >90%, with an area under the receiver operating characteristic (ROC) curve of 0.978..

Keywords: Biosensing Techniques, COVID-19, DNA, Metal Nanoparticles, RNA