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Full Length Research Paper

Aptamer biosensor for *Streptococcus hemolyticus* detection based on fluorescence quenching by gold nanoparticles

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This study describes a nanodiagnostic method using specific aptamer and gold nanoparticles (AuNPs) to detect *Streptococcus hemolyticus*. In this assay, fluorescein (FAM) labeled single-stranded DNA (ssDNA) probe was hybridized with the complement aptamer sequences which immobilized on the surface of AuNPs, and the fluorescence was quenched by AuNPs. However, when the aptamer DNA specifically combined with *S. hemolyticus*, the quenched fluorescence was released. Therefore, fluorescent intensity could reflect the *S. hemolyticus* existence. When *S. hemolyticus* was tested through different bacteria dilutions, the linear analysis was used to calculate minimal *S. hemolyticus* CFU, which is 33 CFU/ml. Hence, this test provided methodological exploration for rapid and sensitive identification of *S. hemolyticus* using aptamer and AuNPs. This rapid, sensitive, stable and novel method could be applied widely in the clinical laboratory and the food supervisory department in the future.

Key words: Aptamer, fluorescence, gold nanoparticles, *Streptococcus hemolyticus*.

INTRODUCTION

Aptamers are short, synthetic nucleic acid molecules that are created using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodology (Song et al., 2011; Nutiu and Li, 2005). The bacteria served as the target instead of single-stranded DNA (Hamula et al., 2011). The aptamers evolved from SELEX have wide range of targets, which included molecules, complex membrane proteins and even whole cells (Shangguan et al., 2006; Ulrich et al., 2002; Gopinath et al., 2006). A large number of experiments have confirmed that the aptamers have high specificity and affinity to a type of protein or cells. However, it is not easy to obtain the specific aptamers because of the long selection cycles.

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Meanwhile, gold nanoparticles (AuNPs) have been widely applied in biological research. One of the most important advantages of AuNPs are efficiently quenching almost all fluorophores (Zhang et al., 2010). Wang and his colleagues (2008) developed a thrombin biosensor which was based on combining gold nanoparticles as fluorescence quencher. Moreover, in the study of Zhang et al. (2010), a method that relied on aptamers and AuNPs to detect adenosine, potassium ion and cocaine at the same time was designed. Furthermore, in the past few years, potential colorimetric detection methods that relied on AuNPs have been used in nanobiotechnology for detection of cell, protein or DNA (Medley et al., 2008; Song et al., 2011; Zhao et al., 2008). Unfortunately, bacteria as an important test target in real life has never been reported combined with fluorescence quencher method.

Streptococcus hemolyticus is a bacterium often found in

Table 1. Sequences of oligonucleotides used in this work.

Name	Sequences
Complementary DNA	5'-CGTGTGCATCTGAAAAAAAAAA-SH 3'
Target DNA (Hamula et al., 2011)	5'-FAM-CAGATGCACACGCTGAAGAAACTGAGGTCGTAGGTTTTCTTCGGG-3'

the throat, saliva and food. It can cause serious illnesses, including pharyngitis, endocarditis, acute glomerulonephritis, scarlet fever, *Streptococcal* toxic shock syndrome and invasive systemic infections (Cunningham, 2000). Current detection methods mainly rely on culture and gene amplification, which either require at minimum 6 to 8 h overnight incubation or 3 days identification and high clean environment (Leung et al., 2006; Kocoglu et al., 2006). Thus, the lengthy detecting time and high hardware requirement cause patients unnecessary suffering and higher financial stress for the hospitals. Therefore, it is urgent to develop more fast methods for detecting *S. hemolyticus.*

In this article, we designed a unique nano gold probe method in which the carboxyfluorescein (FAM) signal combined with the aptamers could be quenched using AuNPs to measure the strength of fluorescence signal linked to the amount of bacteria. The results demonstrate a rapid and novel method for identifying *S. hemolyticus* in the laboratory and the potential application in future test.

MATERIALS AND METHODS

Reagents

Two synthetic oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co., Ltd (Shanghai, China), and the sequences are shown in Table 1. The sequences include the target DNA labeled with FAM and the *S. hemolyticus* aptamer sequences (Hamula et al., 2011). NaH_2PO_4, NaCl and Na_2HPO_4 were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China) and used without further purification. Double distilled water was used throughout the experiment.

Strains and culture medium

All positive control strains and negative control strains are as follows: *beta- hemolytic streptococcus* (CMCC(B)32210), *Enterobacter sakazakii* (ATCC 29004), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (CMCC(B)50115), *Staphylococcus aureus* (ATCC6538). All strains were maintained in semisolid agar (Guangdong Huankai Microbial Sci. & Tech, Co., Ltd., Guangdong, China) at −4°C and then cultured to check for purity in brain heart infusion (BHI) broth or blood agar plate (Guangdong Huankai Microbial Sci. & Tech, Co., Ltd., Guangdong. China).

Preparation of experimental suspension

All works were carried out in a biosafety cabinet. Aseptic technique was used during the whole process. All bacterial waste was sterilized at temperature of 121°C for 20 min prior to disposal. In brief, *S. hemolyticus*, *E. sakazakii*, *P. aeruginosa*, *S. typhimurium* and *S. aureus* were grown in BHI broth overnight at 37°C with shaking for 24 h. Then 1 ml of *S. hemolyticus* was serially diluted by 10-fold with 7.5% NaCl solution from a range of 10^0 to 10^{-8} and the final dilutions were grown in blood agar plate to count the cell density. The residual culture medium from dilution was separated individually by centrifuge with 10000 rpm for 10 min a time. The other negative strains suspension was grown in the BHI broth at 37°C or 24 h with shaking as same with *S. hemolyticus*, but no accounting.

Synthesis of gold nanoparticles

AuNPs were synthesized according to the literature (Grabar et al., 1995). Briefly, the trisodium citrate solution (38.8 mM, 50 ml) was added to the boiling $HAuCl_4$ solution (1 mM, 250 ml) and the solution was allowed to boil with stirring for 15 min. The color of the solution changed from pale yellow to deep red within 5 min, and the solution was allowed to cool down to room temperature. The prepared AuNPs were stored at 4°C before being used. Finally, the concentration of AuNPs were estimated and detected by transmission electron microscope (TEM, JEM-1230, JEOL, Japan).

Preparation of complementary DNA-coated gold nanoparticles

The complementary DNA-coated AuNPs were prepared according to the method reported by Jin et al. (2003). Briefly, 0.5 OD (260 nm) thiolated complementary DNA was added to 1 ml 10 nM AuNPs. Then 16 h later, 2 M NaCl solution was slowly added to the mixture and the solution was set aside for 8 h. Afterward, 0.1 M NaCl was added to the solution and set aside for another 8 h before 0.2 M NaCl was added for an additional 8 h. Finally, the mixture was salted with 0.3 M NaCl. After this salt aging process, the solution was centrifuged at 12 000 rpm for 15 min. The red precipitate obtained was then washed with 10 mM phosphate (pH 7.0, 0.3 M NaCl) three times. After centrifugation, the colloid was re-suspended in 10 mM phosphate (pH 7.0) buffer (0.3 M NaCl). The immobilized complementary DNA-coated AuNPs were stored in the refrigerator at 4°C. Subsequently, 1 µM target DNA was added to the solution obtained. The hybridization was completed after 30 min and the mixture was centrifuged at 12 000 rpm for 15 min. The final red precipitate was re-dispersed in the 10 mM phosphate (pH 7.0) buffer (0.3 M NaCl).

Detection conditions

Phosphate buffered saline (PBS) solutions (10 mM phosphate (pH 7.0); buffer (2 M NaCl); 10 mM phosphate (pH 7.0); buffer (0.3 M NaCl); 10 mM phosphate (pH 7.0)) were prepared using PHSJ-5 pH meter (Shanghai REX Instrument Factory, China). In brief, 60 µL aptamer-coated nanoparticles probes were mixed with *S. hemolyticus* of different concentrations. Then the solution was incubated for 30 min at 25°C. Finally, the fluorescence emission

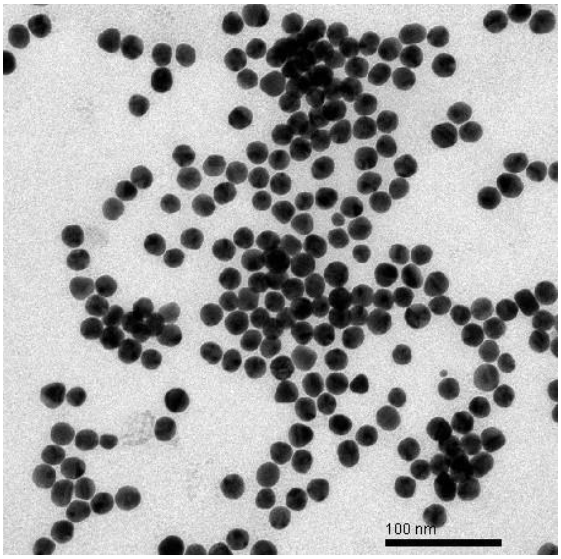

Figure 1. Transmission electron microscope image of the prepared AuNPs.

spectrum was measured using fluorospectrophotometer (F7000 Hitachi Japan).

RESULTS AND DISCUSSION

In this study, we have designed an aptamer-based AuNPs system to detect *S. hemolyticus.* Thiolated complementary DNA was immobilized on AuNPs and hybridized with the FAM-labeled aptamer so that the fluorescence of FAM was quenched. When the target bacteria were added, the binding between target bacteria and aptamer caused the FAM far from AuNPs surface and subsequently revealed fluorescence. If the aptamer has no binding with target bacteria or experienced low binding energy, the FAM fluorescence will still be close to AuNPs, which results in FAM quenching still. Traditionally, *S. hemolyticus* culture requires over 3 days, which is too long for diagnosing. This new feasibility method could shorten the detection process.

Characterization of gold nanoparticles

First, the AuNPs were characterized by TEM (Figure 1). In total, 150 AuNPs were randomly selected for counting and the size distribution of AuNPs was measured by Nano Measurer 1.2. The average diameter was 17.94 ± 0.1nm.

Sensing mechanism

5'-Thiolated complementary sequences were first assembled at the surface of AuNPs through Au-S bond, which could hybridize with target DNA, 5'-FAM- *S. hemolyticus* aptamer. This hybridization brought the fluorescent dye FAM to the proximity of the AuNPs surface where the fluorescence signal was significantly quenched (Figure 2). As was expected, the stronger binding ability between *S. hemolyticus* and aptamer destroyed the duplexes between aptamer and complementary DNA, thus leading to the release of 5'-FAM-*S. hemolyticus* aptamer and the emission of fluorescent dye FAM was observed. This indicated that the fluorescence on-off may provide quantitative measurement of *S. hemolyticus.*

Fluorescence measurement

In Figure 3, significant fluorescence signal was observed when FAM-aptamer was excited at the maximal excitation wavelength of 490 nm. After the reaction with AuNPs, the fluorescence was quenched dramatically. However, the fluorescence intensity at 515 nm in the presence of 10^8 *S. hemolyticus* was 2.5 fold higher than that without *S. hemolyticus*. The observed quenching phenomenon largely originated from the ultrahigh fluorescence quenching ability of gold nanoparticles (Dulkeith et al., 2005). When the *S. hemolyticus* concentration increased from 10^2 to 10^7 CFU/ml, the fluorescence intensity increased gradually at 515 nm (Figure 4).

Negative control experiment

To test the selectivity of the designed aptamer probe, other pathogenic bacterium such as *S. aureus, E.*

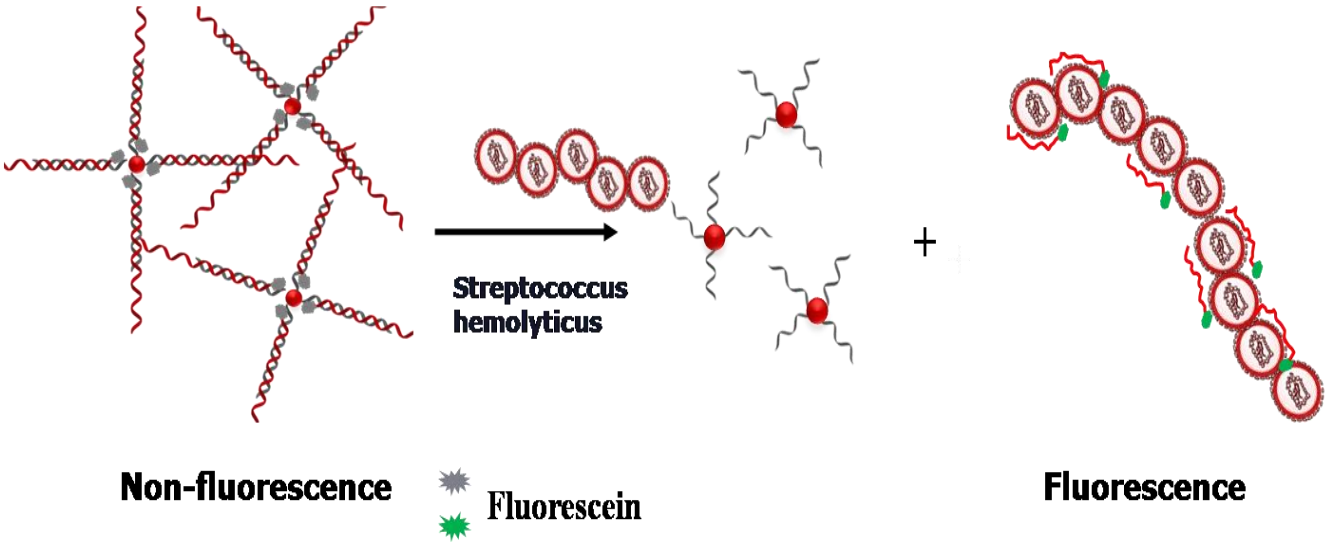

Figure 2. Scheme of *Streptococcus hemolyticus* detection using aptamer-based AuNPs. When the *S. hemolyticus* combines with aptamer labeled FAM, the complementary DNA labeled with gold nanoparticles separates with the aptamer and releases FAM fluorescence.

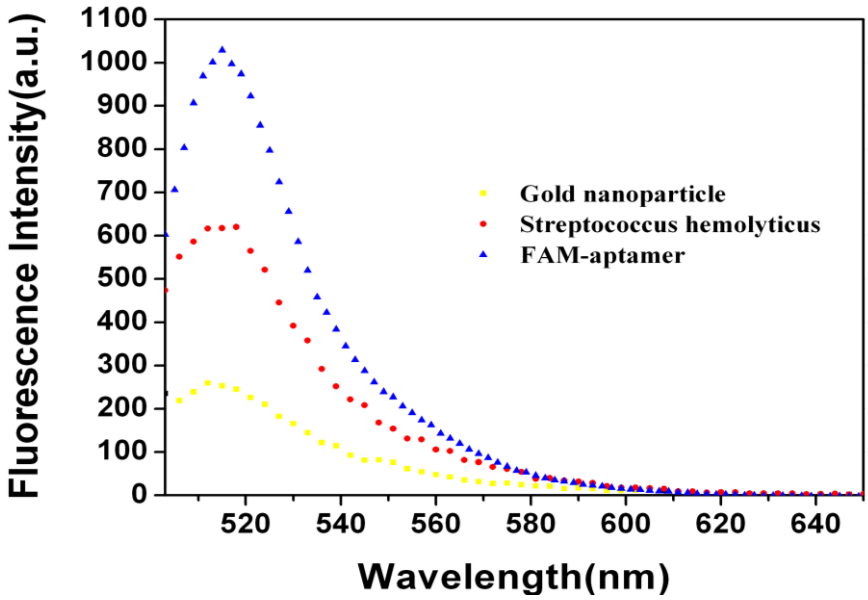

Figure 3. Fluorescence emission spectra of target DNA and AuNPs. Excitation wavelength was at 490 nm. FAM-Aptamer has strong fluorescence. When complementary DNA labeled with gold nanoparticles were added into FAM-Aptamer mixture, fluorescence was immediately quenched. Finally, the *S. hemolyticus* was added to the mixture and fluorescence was recovered.

sakazakii, P. aeruginosa, and *Salmonella* were tested. As shown in Figure 5, *S. hemolyticus* may result in the increase of fluorescence intensity at 515 nm. However, other bacterium could induce a little fluorescence change. This demonstrated that aptamer-based AuNPs are greatly selective for *S. hemolyticus* compared with other negative controls.

Detection limit

The calibration curve was constructed by plotting the

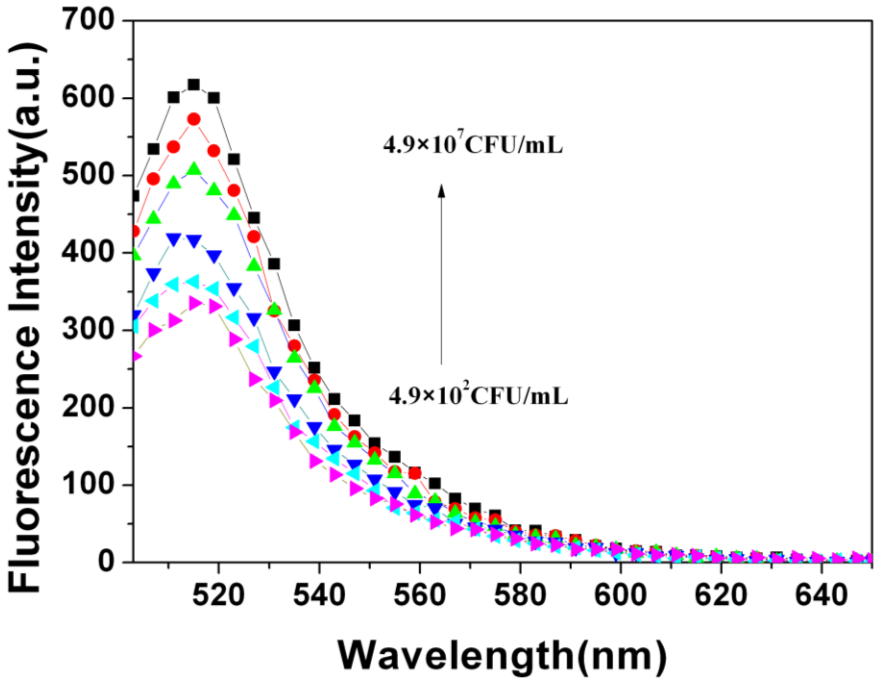

Figure 4. Fluorescence emission spectra of AuNPs with different concentration of *Streptococcus hemolyticus*. With the increasing *S. hemolyticus* concentration, the fluorescence signal is enhanced.

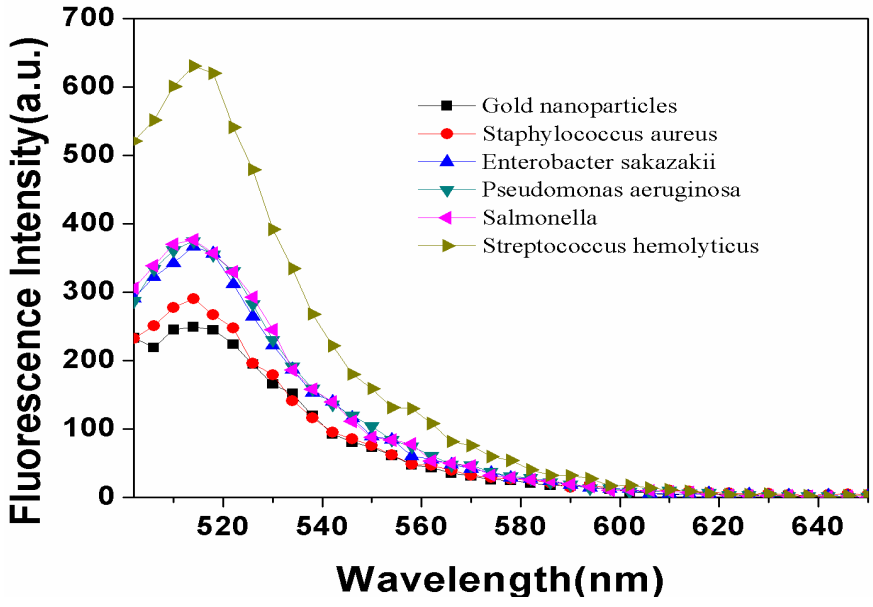

Figure 5. Fluorescence emission spectra of AuNPs with different bacterium. *Streptococcus hemolyticus* has the strongest ability in fluorescence enhancement compared with other negative control strains.

maximum of the fluorescence intensity against Log_{10} *S. hemolyticus* concentration (Figure 6). Lower nano-molar levels of *S. hemolyticus* could be easily quantified in a linear range of at least 4.9×10^4 to 4.9×10^7 CFU/ml, with a calculation detection limit as low as 33 CFU/ml (according to the definition by IUPAC (CDL = 3Sb/m)).

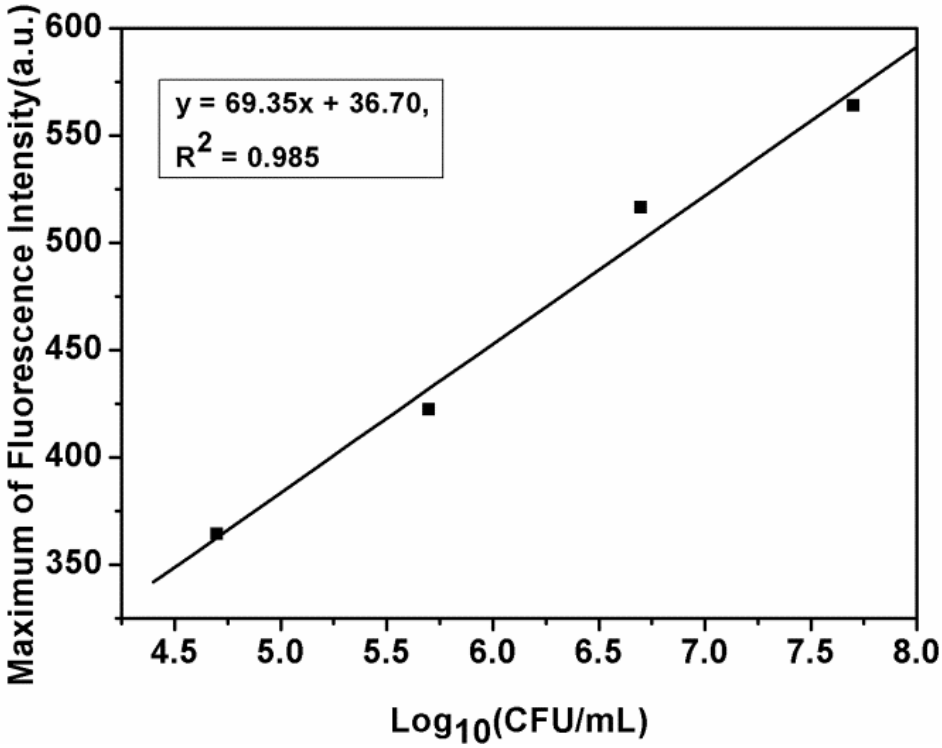

Figure 6. Calibration curve for *Streptococcus hemolyticus* with aptamer-based AuNPs. The calibration curve was constructed to calculate detection limit by plotting the maximum of the fluorescence intensity against Log_{10} *S. hemolyticus* concentration.

Conclusion

A highly sensitive and selective assay based on unique fluorescence quenching property of AuNPs and the high specificity of aptamer has been developed for the detection of *S. hemolyticus.* In this essay, FAM-aptamer is in close proximity to the surface of AuNPs through the hybridization of complement ssDNA, in which the fluorescence signal is quenched. In the presence of *S. hemolyticus*, the aptamer-target binding separates the duplex, releases the FAM-aptamer and restores the fluorescence. This nanodiagnostic method has great sensitivity and selectivity to *S. hemolyticus* compared with other negative controls, which led to a little fluorescence enhancement. More attention will be focused on testing the described method in other bacteria and virus and further apply the method in more realistic and complex situations. Thus, AuNPs probe may have a potential application in real world detection.

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REFERENCES

Cunningham MW (2000). Pathogenesis of Group A *Streptococcal* Infections. Clin. Microbiol. Rev. 13:470-511.

Dulkeith E, Ringler M, Klar TA, Feldmann J, Muñoz Javier A, Parak WJ (2005). Gold Nanoparticles Quench Fluorescence by Phase Induced Radiative Rate Suppression. Nano Lett. 5:585-589.

Gopinath S C B, Misono T S, Kawasaki K, Mizuno T, Imai M, Odagiri T, Kumar P K R (2006). An RNA aptamer that distinguishes between closely related human influenza viruses and inhibits haemagglutinin-mediated membrane fusion. J. Gen. Virol., 87: 479-487.

Grabar K C, Freeman R G, Hommer M B, Natan M J (1995). Preparation and Characterization of Au Colloid Monolayers. Anal Chem., 67: 735-743.

Hamula CLA, Le XC, Li X-F (2011). DNA Aptamers Binding to Multiple Prevalent M-Types of *Streptococcus pyogenes*. Anal Chem. 83:3640-3647.

Jin RC, Wu GS, Li Z, Mirkin CA, Schatz GC (2003). What controls the melting properties of DNA-linked gold nanoparticles assemblies. J. Am. Chem. Soc. 125:1643-1654.

Kocoglu E, Karabay O, Yılmaz F, Ekerbicer H (2006). The impact of incubating the throat culture for 72 h on the diagnosis of group A beta-hemolytic streptococci. Auris nasus larynx 33:311-313.

Leung AKC, Newman R, Kumar A, Davies HD (2006). Rapid antigen detection testing in diagnosing group A β-hemolytic streptococcal pharyngitis. Expert Rev. Mol. Diagn. 6:761-766

Medley CD, Smith JE, Tang Z, Wu Y, Bamrungsap S, Tan W (2008). Gold Nanoparticles-Based Colorimetric Assay for the Direct Detection of Cancerous Cells. Anal. Chem. 80:1067-1072.

Nutiu R, Li Y (2005). Aptamers with fluorescence-signaling properties. Methods 37:16-25.

Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, Mallikaratchy P, Sefah K, Yang CJ, Tan W (2006). Aptamers evolved from live cells as effective molecular probes for cancer study. P. Natl. Acad. Sci. 103:11838-11843.

Song K-M, Cho M, Jo H, Min K, Jeon SH, Kim T, Han MS, Ku JK, Ban C (2011). Gold nanoparticles-based colorimetric detection of kanamycin using a DNA aptamer. Anal Biochem. 415:175-181.

Ulrich H, Magdesian MH, Alves M JM, Colli W (2002). *In Vitro* Selection of RNA Aptamers That Bind to Cell Adhesion Receptors of *Trypanosoma cruzi* and Inhibit Cell Invasion. J. Biol. Chem. 277:20756-20762.

Wang W, Chen C, Qian M, Zhao XS (2008). Aptamer biosensor for protein detection using gold nanoparticles. Anal. Biochem. 373:213-219.

Zhang J, Wang L, Zhang H, Boey F, Song S, Fan C (2010). Aptamer-Based Multicolor Fluorescent Gold Nanoprobes for Multiplex Detection in Homogeneous Solution. Small 6:201-204.

Zhao W, Brook MA, Li Y (2008). Design of Gold Nanoparticles-Based Colorimetric Biosensing Assays. ChemBioChem 9:2363-2371.