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Biosensors Based on Gold Nano-Clusters

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Abstract. The world is entering an era of strong infectious diseases, and it is urgent to develop a new type of rapid, efficient detection system. This graph specifically illustrates the detection function of the gold nanoparticles, and the gold nanoparticles-based sensor. Gold-based nanoclusters (NCs) which has a small particle size, has a high degree of stability and no impact on the biological activity. Gold-based NCs has a surface plasma resonance effect and produces visible color, which can be detected by observing the color change using the agglomeration reaction of the detected substance with Gold-based NCs. In order to specifically detect certain biomolecules, some functional groups or small DNA molecular chains of nanolodin are usually modified on it to have a selective detection capability. In the review, we introduce a total of four nano-gold applications, for bacteria, viruses, DNA and cancer, also, respectively elaborating some recent Gold-based NCs detection applications.

Keywords: Gold Nanoparticles, Biology Detection, Virus, Bacteria, DNA

1. Introduction

With the outbreak of COVID-19 epidemic in the past two years, the popularity and demand for nucleic acid detection for confirmed cases indirectly promoted the research for all parts of medical detection.Nowadays, the detection systems of many hospitals or research institutes are faced with the problems of expensive price, slow detection development of new mutant strains, narrow function range of detection probes, and low stability.therefore It is urgent to develop rapid, low-cost and effective detection systems. Recently, many medical institutions are increasing the investment in various disease detection systems to cope with this era of rampant disease.

Au NCs is a particle in a metastable state containing the few number to hundred gold atoms, at a particle size of between 1.00 and 100 nm. Below the critical size for electronic energy quantization, in this state, the strong quantum constraint of free electron lead to discrete electronic states, so Au nanoclusters (Ag NCs) shows similar molecular properties, nanometer gold can react with biological activity and unique optical characteristics, Au NCs in the visible area has surface plasmon resonance (SPR) absorption, which illusates the near infrared (NIR) to the visible area demonstrating obvious fluorescence emission. Through this color change, gold nanoparticles can be used for detection,that is based on the surface plasmon resonance effect of nano-particles, therefore,the qualitative and quantitative detection can be realized by naked eye observing the system color change or ultraviolet visible spectrophotometry, named the colorimetric detection method. In many situation, the infrared absorption of biomolecules is weak. When infrared spectroscopy is used to represent and identify some biomolecules, the enhanced Raman scattering effect is usually needed by Au NCs. [1] The

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principle of Au NCs color change is that Au NCs can react with different substances, and there are two reaction mechanisms. 1) Au NCs is a kind of negative electric particles, which can react with the detected substances, and organically combine with it to produce agglomeration phenomenon. 2) Au NCs will directly combine with some single-chain molecules, changing the concentration of gold nanoparticles in the solution, resulting in a color change. This detection method has high extinction coefficient and high sensitivity. Due to the inertia and stability of its own gold atoms, Au nanoparticles can also play a stabilizing role as some electrochemical sensors. for example,Au NCs is highly biocompatible in mammalian systems, and is widely used in biomedicine. The combination of Au nanoparticles and biomolecules to produce nanobiological conjugates, fixing hundreds of active biomolecules and markers on nanomaterials (nanobiological conjugates), has been proven to raise the signal amplification which can response in a number of biosensor formats and amplify the electrode surface to improve its sensitivity. In some sensors, gold nanoparticles are also used as a catalyst to catalyze some chemical reactions. In order to make gold nanoparticles have specific detection effect, some functional group or small DNA chain always be modified on gold nanoparticles, the principle basing on functional group specific binding and basical complementary principle, because we want to detect selectively connection, named biological coupling. By this method, people can detect a variety of new bacteria or viruses, also applied detection in a large number of bacteria mixture of bacteria. In the following, several detection applications of Au NCs in the biological field will be introduced, for four parts of bacterial detection, virus detection, DNA detection and cancer detection.[2] In the bacterial detection, there is label-freed Au NCs detection, labeled detection, and Sensor array . Virus detection describes the detection function and therapeutic potential of Au NCs in Zika virus and SARS-CoV-2. The DNA assays specifically explain how the detection of the DNA uptake series were performed. Cancer detection introduces two detection systems based on Au NCs.

2. Applications of GOLD-BASED NCS

2.1. Bacteria detection

In the process of detecting bacterial cells, the photo luminescent gold-based NCs is usually used because of its specific fluorescence changes in the environment with bacterial cells . Sometimes detection in bacterial cells, we do not need to label-free gold-based NCs. Based on this, the method for specific detection of Escherichia coli O_{157}:H_7 proposed by Jia *et al.* [3], they proposed an immunofiltration strip method that can detect it rapidly and reduce the cost, which relies on the photothermal effect of gold nanoparticles to improve the sensitivity and effectiveness of detecting Escherichia coli O_{157}:H_7 (Figure1a). Gold-based NCs photothermal effect can not only amplify the electrical signal, but also determined according to the thermal contrasts and the concentration of Escherichia coli O_{157}:H_7 which conditions to provide the best effect, Furthermore, gold-based NCs photothermal effect can be harmless treatment for bacteria, which can be applied for actual production and will play a significant role. The upper limit for Escherichia coli O_{157}:H_7 is 1.95×10^4 CFU mL^{-1} (Figure1b, c), and the sensitivity is almost ten times than that of the normal process. In the future, for some water pollution, or some environmental safety, this method can not only provide an efficient and rapid detection, but also can eliminate bacterial cells at the same time.

Wen *et al.* [4] developed a novel detection nanoprobe for the viability of bacteria in water (Figure1d). This nanoprobe is a difunctional gold nanoprobe, which through the color change, namely the colorimetric method, to show the detection effect. Since the purpose is to detect the activity of the bacteria, the team combined the nanoprobe with the bacteria recognizing and the signal parts during the experiment, which can realize the detection extremely quickly. Based on the resulting color signal and bacterial vitality data, it is found that Gold-based NCs detectability can cover all bacterial content, and also can have the same utility for a variety of bacteria such as Staphylococcus aureus or Escherichia coli $O_{157}H_7$. At the end of the article, the team found that in the detection experiment of bacteria, a certain degree of recycling can be achieved, which will be an important advantage for the industrial detection of bacteria, and can realize recycling and low-cost detection.

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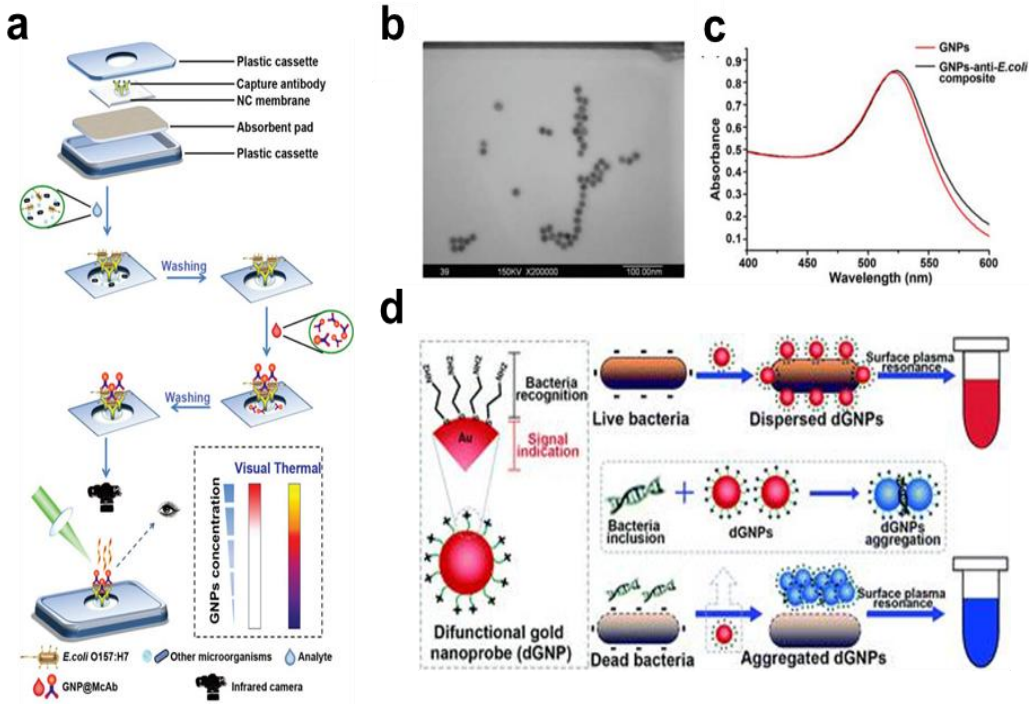


Figure 1. (a) Schematic diagram of the photothermal-immunofifiltration strip for E. coli O_{157}:H_7 detection. (b) TEM image of GNPs and (c) UV-visible spectra of the GNPs and GNP-anti-*E.coil* composite. (c)Rapid measurement of waterborne bacterial.

In some complex detection environments,always need to improve the selectivity of fluorescence Au NCs to detect the desired substances. The usual methods is to modify Au NCs, such as adding some functional groups, receptors. In the field of medical testing, the Wang's team have developed a functionalized gold nanomaterials (FGNM) detection material for acute intestinal obstruction (AIO) [5]. Its reaction mechanism is through by observing bacterial ectopic to show that FGNM has high sensitivity and effectiveness. In the report, the team adopted the design concept of the control experiment, dividing the 90 patients into three groups. The control group applied the tissue bacterial culture method, the experimental group used the prepared functionalized gold nanomaterials (FGNM) detector, and design a blank group to effectively and conveniently observe the experimental results (Figure2a). In the final data, it can be found that the group with the two methods showed a higher lymph node BT positive detection rate, while the functionalized gold nanomaterials can effectively target the serum interleukin, and the bacterial culture method has a higher seroconversion growth factor. Therefore, FGNM can be better than traditional methods in dealing with acute intestinal obstruction.

Bacterial cellulose nanocrystals (BCNCs) are a kind of cellulose nanomaterials which is tunable and biocompatible [6]. What's more, they show great behavior in the aspect of bio europrean union and can be used for biosensor applications. A report reveals that bacterial isolation can be used in BCNCS which is modified by A (con A) lectin and has added AuNPS to test unmarked surface-enhanced Ramen spectroscopy. Aggregated AuNP+bacteria +(con A+BCNC) conjugates produce SERS hot spot, which make escherichia Coli 8379 able to carry on SERS test at 103 CFU/ml level. Therefore, we can adopt optimized detection method to distinguish 19 common bacterial strains (**Figure2b**). Using SVM to analyze the date of large-scale SERS spectral in the nineteen bacterial strains is a machine learning technology based on optimization, which can be used as binary classifier. Furthermore, SVM classifier has a total high accuracy in indentifying bacterial strains correctly, which is 87.7%. In conclusion, this research shows the great potentiality, we can apply low-cost SERS biosensor based on nanocellulose and machine learning technology to large spectral dataset analysis.

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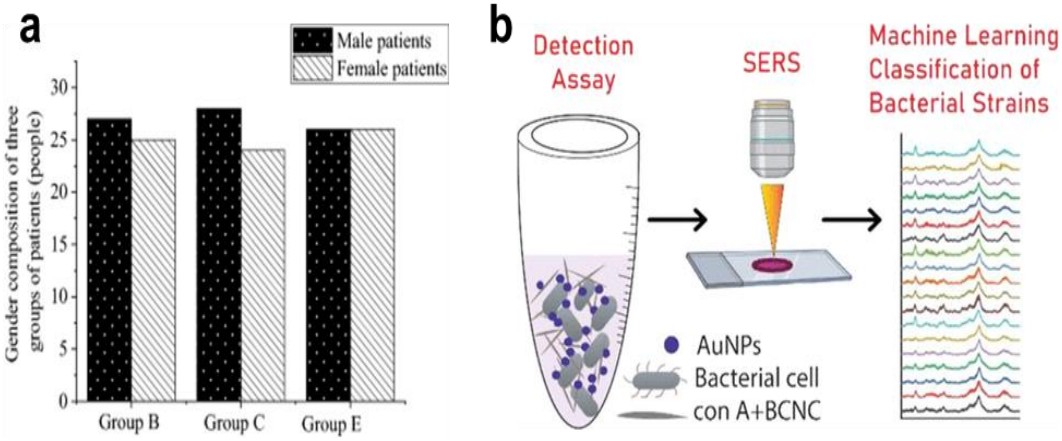


Figure 2. (a) Bacterial translocation detection in patients who with acute intestinal obstruction. (b) Lectin-modified bacterial cellulose nanocrystals embellished with AuPCs for selective detection of bacteria by surface-enhanced raman scattering coupled with machine learning.

Sensor array in order to realdtyize its complex functionalization and simultaneously detect a variety of bacteria, it is usually prepared into sensors together with other means of characterization. There will introduce the sensor application based on the brand new gold nanorod arrays.

Dizaji *et al.* [7] applied a novel structured gold nanorod arrays to improve the highly active platform in bacterial detection, which observes the bacterial species, activity and content through infrared spectroscopy. But conventional sensors are often difficult to observe in infrared spectra due to their low bacterial absorbance. In order to design new gold nanorod arrays, they used a physical vapor deposition to deposit nanoscale gold atoms onto the platform in SEIRA system at a certain deposition angle (Figure3), forming a three-dimensional anisotropic cylindrical gold nanorod structure. They used this modified platform to detect three different types of bacteria, and by comparing the infrared spectra from Staphylococcus aureu Escherichia coli,and Bacillus subtilis, they could see robust infrared spectra with signal-to-noise ratio and high reproducibility compared with other SEIRA systems such as aluminum foil as substrates. At the end of the experiment, they performed unsupervised (PCA, HCA) and supervised (SIMCA, LDA, and SVM classification) instrumnet analysis learning of the infrared spectrum of bacteria, showing that SEIRA with a gold nanorod modification platform of this new structure can identify almost all bacteria without labeling them.

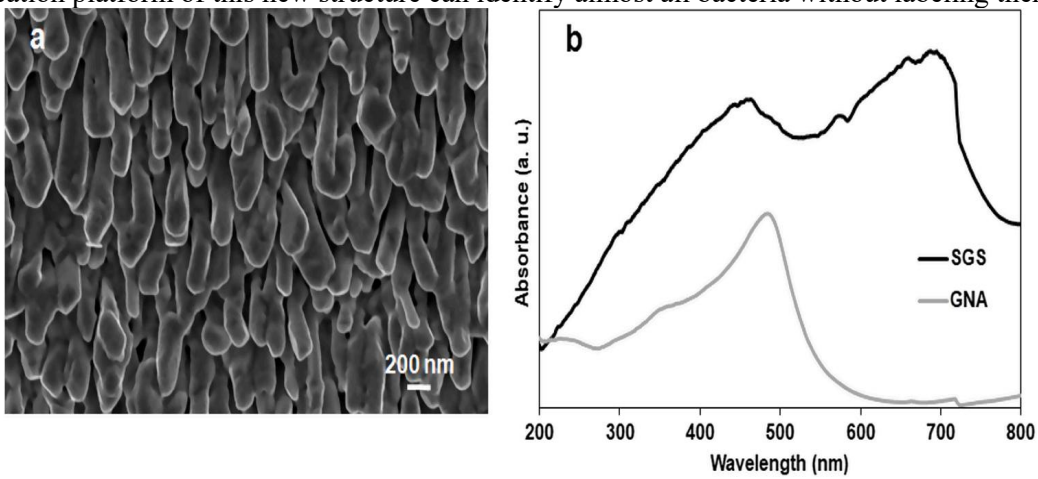


Figure 3. (a) Representative SEM images of GNA platforms. (b) UV–vis spectra of SGS, and GNA substrates.

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2.2. Virus detection

In virus detection, AuNPs can be used in the redox reaction with some metal ions, or by its electrical properties, to label and detect some viruses, or to use AuNPs deposit on sensors to enhance its electrical properties, which can amplify the detection signal. For example, for hydrogen peroxide detection, by changing electrochemical transduction functions to realize the detection with amplifing the signal of redox reactions and catalytic activity, which is known as the NP-metal enhancement amplification. AuNPs can catalyze the process of metal deposition, that can be used by enhancing the ratio with surface area and volume of AuNPs to improve the effectiveness and sensitivity of virus identification and detection. Furthermore, AuNPs in bioimmobilization can provide structural capabilities like exquisite scaffolds. Through this structural and signaling capability, AuNPs can detect viruses in many cases, and more sensitively and selectively of specific detection and recognition than other detection systems, Most importantly, AuNPs has good stability. In some optical signal transduction functions, such as color amplification, resonance light scattering and fluorescence quenching or enhancing, AuNPs-based Raman spectroscopy and dynamic light scattering play an important role. Overall, the colorimetric detection of viruses can be roughly divided into two situations: 1) AuNPs directly amplify the virus, and the color is more obvious to observe. 2) The agglomeration effect of AuNPs is used to color-show react to the virus.

The outbreak of COVID-19 and the growing number of confirmed people have posed unprecedented threats to people around the world. A rapid, effective detection and diagnostic tool is urgently needed to control the spread of the novel coronavirus. Recent many countries have developed a variety of detection methods for SARS-CoV-2, the traditional virus detection methods are revers-transcriptase polymersase chain reaction(RT-PCR)and Serology,The revers-transcriptase polymersase chain reaction (RT-PCR) can be amplied to detect and seperate viral genetic material that in isolated patient samples. The Serological assay contain enzyme-linked immunosorbent assay (ELISA) and Loop-Mediated isothermal amplification (LAMP). The enzyme-linked immunosorbent assay (ELISA) relies on the antibodies in the blood or saliva which are produced by the human natural defense system. the Serological assay is always used in the traditional detection, which the working principle is using specific antibodies in conjugation with a variety of signal reporting system such as fluorescent substance srs-CoV-2 virus, usually including chemiluminescent immunoassay (CIA), immunochromatographic test (ICT), immunostaining, immunofluorescence assay, single radial hemolysis, immunoblotting assay (IBA), particle agglutination.

Nassar *et al.* [8] developed an effective AuNPs-based virus detection method by constructing the nanogold spherical structure and conjugating the SA receptor of sialic acid to the Au NPs, that formed SA-Au NPs, allowing the plasmonic effect produced by binding with HA protein targets on the viral surface. Due to the complexity of this modified structure, one-pot synthesis method is usually used, when the color of initial solution of light yellow change to dark red, proving the preparation realize, and characterized by SEM and TEM to determine whether SA is already present in the structure of nanogold. The average particle size of the prepared SA-Au NPs was 30 ± 1 nm, the UV absorbance was 525 nm, and a negative zeta potential (30 ± 0.3 mV), the ARS-CoV-2 virus can bind to lung epithelial cell SA receptors via HA proteins on the viral surface to modify various host specificities, so that HA is used as a primary target for the probe. It indicated that patient swabs containing z, influenza B virus, and MERS virus can be detected and diagnosed by the colorimetric method. It is found that the carboxylic group in SA can affect HA binding in virus particles. When the virus particles are immersed in SA-Au NPs solution, HA binds to NP, making these NPs gather on the surface of virus. At the same time, the peak of the absorption spectrum changes, shortening the inter-particular distance toward the red shifting of SA-Au NPs, and the color will become deeper or even purple. But sometimes the tip of the detection swab is yellow-white, and that may be a transfer of viral particles that spread into the SA-Au NPs solution. The team is also exploring SARS-CoV-2 specific antibodies, which are in conjugation with AuNPs and can be used as a detection or therapeutic tool by UV-visible spectrophotometry. The absorbance of virus samples can be observed by UV-visible spectrophotometry, and they compared it with blank samples many times. As shown in Figure4,

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525nm is the absorption peak of blank samples, and the virus sample has a strong peak of 5a.u at 214nm, what's more, it has a very wide range of about 277nm. Generally, the absorption wavelength of nucleic acid is 260nm and the protein is 280nm. However as shown in the figure, it is too hard to determine whether the virus sample SA-Au NPs binds to the protein or the virus particles, So the research team will determine the which substance that really binds to SA-Au NPs by more confirmatory tests, such as analysis of the particle size, observing the internal structure and NPs surface which is modificated by electron microscopies.

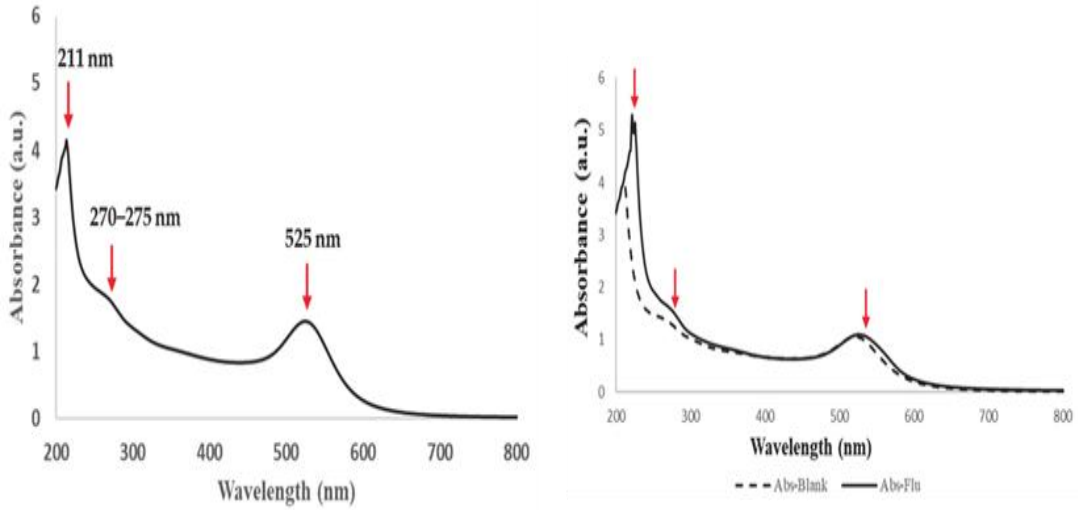


Figure 4. UV–Vis absorbance spectra of SA-Au NPs showing the SPR band at 525 nm.

Gold nanoparticles, developed by Alzate *et al* [9], developed Zika genetic material to detect electrochemical signals, which they linked to single-stranded DNA, named ssDNA and applied to nanobiological conjugates， In this process, no sample extraction or PCR amplification is required. The decorated nanoparticles have good stability and high sensitivity. Usually genosensor will be assembled to screen-printed gold electrode or carbon electrode, which was modified by layered gold nanostructure, which Ru3 + complex was used in as an electrochemical reporter. Through differential pulse voltammetry, called DPV based on the transient current density to observe the response of genosensor, found that it was linearly change, from the initial date of 10 to 600 fM and the range between 500 fM and 10 pM. In the layered gold nanostructure and screen-printed gold electrode have different detection limits. In the study, genetic sensors equipped with structured gold nanostructures were used to detect patients infected with the Zika virus, to amplify the electrical signals generated in response to the genetic material from the Zika virus. This gold nanoparticles have great advantages in the system of ultra-sensitive nanobiological conjugates, which enables the sensitive, efficient and stable detection of a variety of viruses through different decoration.

In the study, they chose three infected patients for ZIKV virus detecting by analyzing the DPV signal generated by nanobiologic conjugates in serum samples against patients without infection， so they can determine the presence of the virus[10]. The three serum samples (S1, S2, S3) showed different current density reactions from the blank group Figure 5a, and here they proposed a conjecture that the RNA load of each sample may cause such a change in the current density. They also used the paired t-test and the one-way ANOVA analysis methods, indicating that the difference in the DPV electrical signals correspondingly had a certain statistical significance of 99%. In Figure 5b, the team doped 1 PM into the synthetic target DNA, and tested the urine and saliva samples using electrochemical genetic sensors. The results showed that a large number of electrolytes existed in the urine, which promote the electron transfer process, and that the electron signals were more responsive than the samples of saliva,that's because the proteins in the saliva hindered it. Figure B shows that compared the bioconjugate to detect ZIKV virus with other native arboviruses, only in ZIKV virus

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exists statistical significance of electrical signals, and under the exist or not of the bioconjugate, they also make a control test, find that the gold nanoparticles modified bioconjugation sensor has a highly specific selection, can produce a signal response to a single virus under different physiological substrates.

Nano-biological conjugates used for the ultrasensitive detection of ZIKV. Figure 5a shew the DPV curves of three positive raw serum samples (S1, S2, and S3) from the patients who have ZIKV virus as the comparison to the negative no-target control (NC1). (B) Matrix effects and specificity: urine and saliva samples from healthy individuals were doped with one pM for ZIKV synthesis, and compared with 1 μM of synthetic DENV and CHIKV V genetic material in PBS buffer, and target-free NC1. Amplification capability: ZIKV signal is compared with a control with the target without nanobioconjugate (PC1) in 1 pM and the simple format, also with a control experiment by using nanobioconjugate but there is no (NC2). Error bars represent the standard deviation of the three independent analys metnods. The level of statistical significance was 99%, $p < 0.01$.

Schematic representation of the Electrochemical gene sensor platform assembly based on the concept of signal amplification by nanobioconjugates (Figure 5c). (a)Gene sensor platform placed among the SPCE / AABZ / au-linked capture probes and the signal probes derived from the nanobioconjugates in the presence of the target probe. Then, a complex based Ru^{3+} is coupled electrostatically, acting as a reporter for the electrochemical signal interrogated by DPV. (b) In the any situation that the target absent or not, the gene sensor is not assembled, so the resultant DPV signal is much smaller.

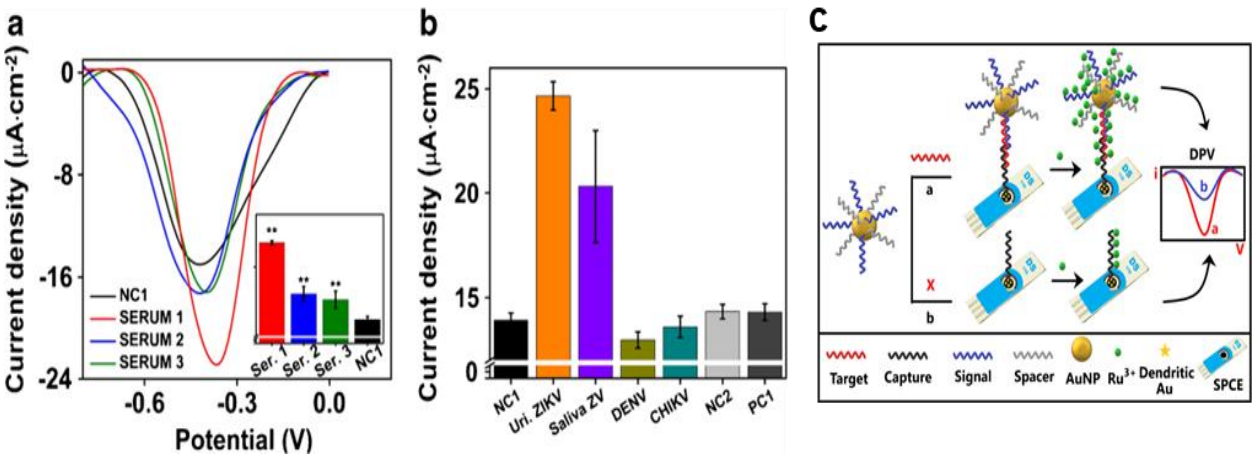


Figure 5. Nanobioconjugates as ultrasensitive detection of ZIKV.

2.3. Cancer detection

Nowadays, in order to observe the occurrence of cancer timely, many detection systems of cancer have adopted different methods. Fan *et al.*[11] have developed a gold nanostars (Au NSs)-based SERS substrate to detect cancer cells using surface-enhanced Raman scattering (SERS). When cells undergo gene mutations converted into cancer cells or cell apoptosis, intracellular β -galactosidase (SA- β -gal) will be expressed in large quantities, which can be efficiently detected by the content of this substance (Figure 6a). In the study, Fan *et al.* [11] prepared a monolayer gold surface on glass sheets, and assembled 4-mercaptophenyl boric acid (PMBA) molecules on it, and modified gold nanostars together with 4-mercaptophenylboric acid (PMBA) molecules. Decorated gold nanostars as lactose molecules linking the substrate to the SERS tag. When the intracellular β -galactosidase (SA-β-gal) contacts with functionalized Au-NF, the surface-enhanced Raman scattering SERS intensity was 1437 cm^{-1}, and the characteristic peak of 4,4 ′ -dimercaptoazobenzene (DMAB) formed by PATP by laser irradiation decreased, indicating intracellular β -galactosidase(SA- β -gal) activity. This gold

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nanoprobe structure improves the signal strength without losing the reproducibility, and has the sensitivity and stability in future cancer detection.

A kind of colorimetric analysis based on AuNPs has become a important research field applied to biomedical science [12]. This method can do a quick detection and can be visible with eyes. Using NaCl, we can conduct MD simulation on a truncated octahedron and study the interaction of the AuNP aggregation induction of citric acid coating on the surface that contains Au (111) and Au (100) further. What's more, they can prove this method through experimental devices. The team decorated anionic citrate on the surface to prevent AuNPs from aggregation. In the original detection reagent, the single-stranded DNA stabilizes the AuNPs with its negative charge due to the anti-DNA target, and the color does not change (Figure 6b). When the base complementary hybridization with the DNA target, the original anti-dna target fails its function, when the high concentration of sodium chloride induced AuNP aggregation, named the agglomeration effects, it can be observed that the solution changing from red to purple can be effectively detected within the range of 25–500 nM, and the results show a linear regression with a detection limit of 45nm. The result shows that the experimental result has great uniformity with computing result, which proves the validity of this method.

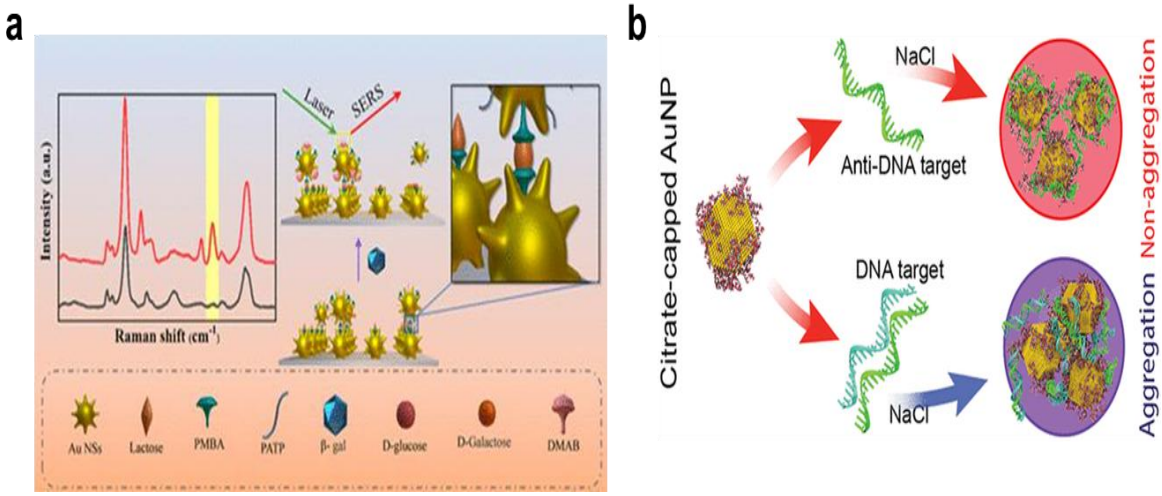


Figure 6. (a) Schematic diagram of DNA-Based Gold Nanoparticle Sensor for Bladder Cancer Detection (b)Schematic diagram of DNA-Based Gold Nanoparticle Sensor in Bladder Cancer Detection.

3. Conclusion

In summary, we have attempted a review of the recent efforts on gold-based detection in biology.AuPCs because of its high stability, biocompatibility, and sensitivity, have great potential in biomedical detection. people usually use the surface plasmon resonance effect of nanogarticles in the near infrared region, to observe the color redering of the solution. With the agglomeration effect of nanoparticles, the nanoparticles will bind with the measured biomolecules, producing the concentration change, then resulting to the color change of the solution. In the review, we also mentioned the methods that how to design to improve the selectivity of AuPCs detection, people specifically modified the AuPCs with some biology substances, such as light functional groups, DNA molecule chains and anions. We show an immunofiltration strip method for rapid detection of Escherichia coli in a bacterial detection process, and allows treatment of Escherichia coli according to the photothermal effect produced by AuPCs. For the activity detection of bacteria in water and the serum interleukin, a bi-functional probe that can realize the detection and recovery has been developed. To achieve more complex and multifunctional detection systems, new sensor structures based on nano-particles gold can be identified using infrared spectroscopy. In the virus detection process, for the COVID-19 and Zika virus, the specific protein of the virus is used to modify the surface of

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nanoparticles to form a system of biological conjugates. In DNA and cancer detection, its basic principle is the base complementary pairing principle, which is realized by the nanogold probe modified with the small DNA molecular chain. This efficient, stable, and highly sensitive detection probe is being adopted and used in many medical institutions, while driving the development of detection probes for a batch of inert molecules of the same properties, such as nano-silver. But at the same time, the development of the detection probe is faced with the problem of specificity and cost restriction. How to balance the two aspects determines whether the detection probe can be commercially available in medical biology. The global reserve of gold is considerable, and it is widely used in many medical small molecule robots or medical instruments, it indicates that the medical preparation of nanometer gold has been very mature, Furthermore, gold atoms is the most suitable metal material for the future development of emerging detection systems and sensors. Many scientific research institutions are actively developing AuPCs detection probes. Many researches shows that AuPCs is being used widely now.

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