Utilization of unmodified gold nanoparticles in colorimetric detection

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This review begins with an overview of the appealing properties and various applications of gold nanoparticles, and briefly summarizes recent advances in using unmodified gold nanoparticles to detect different kinds of targets including nucleic acids, proteins, metal ions and small organic molecules. The key point to the unmodified gold nanoparticle-based visual detection assay is to control dispersion and aggregation of colloidal nanoparticles by targets of interest, which usually relies on affinities between gold nanoparticles and targets. The degree of dispersion or aggregation can be visualized through the change of the solution color or the precipitation of nanoparticles from the solution. Thus, the existence of the target molecules can be translated into optical signals and monitored by the naked eye conveniently. Finally, some future prospects of this research field are given.

gold nanoparticles, colorimetric detection, biosensor

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1 Introduction

Ultrasensitive detection has versatile applications in biochemical analysis, medical diagnosis and environment monitoring [1–5]. Colorimetric detection that provides naked-eye readout signals stands out in many detection protocols because it is simple, rapid, miniaturizable and suitable for real time on-site detection [6,7]. Gold nanoparticles (AuNPs) have been the most widely used probes for colorimetric detections during the past decade. AuNPs have myriad applications in catalysis, biological labeling, optoelectronic materials and information storage [8–13]. Also, they have excellent biocompatibility with sizes usually among 1–100 nm, comparable to many biomacromolecules such as proteins and nucleic acids. As a result, AuNPs can be applied to investigating the interactions between different kinds of biomacromolecules to provide clues for relevant physiological processes [14–21].

The analysis of molecules is an important aspect of the application of AuNPs [22]. Various analytical strategies based on AuNPs have been developed, including fluorescence enhancement or quenching, electrochemistry, colorimetry and surface-enhanced Raman spectroscopy. Among these approaches, colorimetry, mostly based on the surface plasmon resonant properties of AuNPs [23,24], is favored due to its simplicity. Surface Plasmon Resonance (SPR) originates from the interactions between incident light and conductive electrons on the surface of AuNPs. The intense absorbance peak appears in the UV-Vis spectroscopy when the wavelength of the incident light and the oscillating frequency are close and coupled [25,26]. The SPR peak is very sensitive to the interparticle distance and it undergoes an apparent redshift in the spectroscopy when AuNPs aggregate, inducing a visible color change of the
solution [27,28]. Effective control of the assembly/disassembly is the basis of colorimetric detection of AuNPs.

The controlled assembly of AuNPs using functional ligands was pioneered in 1996 [29,30]. Generally, these methods employed conjugated oligonucleotides on the surfaces of AuNPs and their hybridization with complementary DNA. Thereafter, researchers [31] reported the first colorimetric detection of target DNA by probe DNA functionalized AuNPs. The target DNA hybridized with the fully complementary probe DNA on nanoparticles, which led to the large-scale aggregation of AuNPs, bringing a visible color transition from red to purple [32–34]. During the following decades, colorimetric detection based on AuNPs towards various targets has been extensively investigated including DNA [35–37], proteins [38–40], small organic molecules [41–45], and ions, such as mercury(II) [46–48], lead(II) [49,50], copper(II) [51,52], calcium(II) [53], uranium(II) [54] and nitrite [55].

2 General principles for unmodified AuNP based colorimetric detection

Functionalized AuNPs for colorimetric detection, however, suffered from some drawbacks: differently functionalized AuNPs for different targets were required; separation of ligands for functionalizing AuNPs (such as DNA) could be costly; covalent conjugation of bioactive ligands onto the surface of AuNPs may partially sacrifice their reactivities. Unmodified AuNP may provide solutions to these problems. This set of strategies also relies on the target-triggered, distance-dependent color transition as an indicator like that in functionalized AuNPs, but the difference lies in inducing aggregation by removing protection from nanoparticles rather than by crosslinking them. Generally, the so-called “unmodified AuNPs” are prepared through the classical citrate-reduction process and are loosely capped by negatively charged citrates. As a result, they show high affinity to flexible, positively charged molecules but much less affinity to rigid, negatively charged ones. This difference in affinity could be transformed to the difference in colloidal stability of AuNPs in concentrated solutions of salts and finally converted to color change signals. In principle, any analyte with affinity towards unmodified AuNPs could be applied in this set of method.

3 Detection of DNA

Li et al. [56,57] reported the first study that utilized unmodified AuNPs to probe the target DNA in solution (Figure 1). They first mixed salt, single-stranded (ss) target DNA and unmodified AuNPs; no apparent color change could be observed. However, the color turned purple upon the addition of probe DNA whose sequence was fully complementary with the target DNA. This was quite simple, sensitive and selective. They reasoned that ss target DNA could uncoil sufficiently to expose its bases which contained nitrogen atoms with high affinity to AuNPs, whereas double-stranded (ds) DNA had a stable duplex geometry that always presented the negatively-charged phosphate backbone, as well as wrapped its bases inside the duplex [58]. Repulsion between the charged phosphate backbone of ds DNA and the capped citrates dominated the electrostatic interaction between the gold and ds DNA so that ds DNA would not be adsorbed. ss DNA was sufficiently flexible to partially uncoil its bases, so that it could be adsorbed onto the AuNPs and stabilized them even in the concentrated salt solution [59]. The stabilized AuNPs would not have any color transition whereas these mixed with ds DNA underwent fast aggregation, resulting in a visual readout. Many other groups reported similar results with tailored design for detecting DNA with different sequences [60–62].

4 Detection of proteins

Among various kinds of proteins, thrombin is an especially important enzyme in physiology and pathology because it is a coagulation factor in blood that has diverse effects in the coagulation cascade such as catalyzing fibrinogen to an active form that assembles into fibrin. Wei et al. [63] reported
a simple and sensitive approach for colorimetric sensing of thrombin using antithrombin aptamers and unmodified AuNPs (Figure 2). Aptamers are *in vitro* selected artificial nucleic acid receptors that possess high affinity and specificity toward their ligands. Incubation of antithrombin aptamers, unmodified AuNPs and thrombin resulted in the binding of aptamer-thrombin complexes and aggregated AuNPs, which exhibited the purple color, indicating the presence of thrombins.

Besides determining the presence/absence of enzymes, unmodified AuNPs could also screen the activity and selectivity of enzymes to their substrates. As a practical demonstration, the activity and selectivity of exonuclease can be measured simply by incubating the enzymes, substrate/non-substrate and unmodified AuNPs [64]. As for activity-determination, exonuclease can specifically cleave the rigid ds DNA substrate into a large quantity of tiny pieces of oligonucleotides or even nucleoside monophosphates, stabilizing the unmodified AuNPs against concentrated salt. The solution remained red, indicating the activity of the exonuclease. Likewise, the exonuclease was able to cut its substrate but did not affect the rigid structures of the nonsubstrates, and the difference of cut/uncut DNA was reflected by the color change of the unmodified AuNP colloidal solution.

In contrast, the cleavage events of nuclease substrates could also be sensed using active nuclease because the complete DNA strands and cleaved short DNA pieces showed different affinity to unmodified AuNPs [65]. This result is important because the DNA cleavage events play integral roles in DNA replication, repair and recombination.

5 Detection of metal ions

5.1 Mercury

Mercury is one of the most toxic elements that widely exist in the environment. It easily contaminates water, accumulates in the body through the enrichment of the food chain and causes heavy damage to many organs. Therefore, it is desirable to develop truly efficient monitoring tools for mercury in aqueous media [66]. During the past decade, the detection of mercury based on rationally functionalized AuNPs has been a continuously growing field [46–48]. However, unmodified AuNPs have only been actively applied in mercury detection recently. Independently, Li et al. [67] and Liu et al. [68] reported similar sensitive and selective analytical protocols using unmodified AuNPs and thymine-Hg2+-thymine complexes (Figure 3). The thymine-thymine mismatch could be effectively coordinated by mercury(II), causing the noncomplementary mismatch to fully hybridize [69,70]. Both of the two research teams mixed the oligonucleotides with rich, regularly arranged Thymine sequences 3′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT...
simultaneously induced the unmodified AuNPs to aggregate due to desorption of these protecting ligands. These stabilized AuNPs solution without the addition of mercury, however, had neither aggregation-induced color change nor fluorescence emission because the fluorescence was quenched by the surface of gold [72–75]. This strategy combined the simplicity of colorimetry and sensitivity of fluorescence detection to probe mercury. This principle had been similarly applied by many other groups [76–78].

5.2 Lead

Lead is another highly toxic metal and tends to accumulate in bodies. The complexes of lead are very stable against the environment or body and cannot be metabolized or degraded rapidly. Lead is also a carcinogen that yields various kinds of tumors [79].

Wang et al. [80] and Wei et al. [81] reported a method for visually sensing lead using lead-catalyzed DNAzyme cleavage and unmodified AuNPs, respectively (Figure 4). This sensing strategy is different from those mentioned above in that they used the catalytic activity of metal ions towards oligonucleotides rather than the binding activity of metal ions and oligonucleotides. This ds DNA is named DNAzyme because it shows specific responses to some di-valent metal ions [82]. In the concentrated salt solution, unmodified AuNPs aggregated because the hairpin structure of lead-specific DNAzymes could not help the AuNPs disperse. The addition of lead ions rapidly catalyzed the cleavage of DNAzyme, converting those rigid hairpin structures to flexible ss DNA. The as-formed ss DNA could be adsorbed onto the surface of unmodified AuNPs and re-dispersed them. The aggregation-to-dispersion transition yielded a purple-to-red color change, which was also easily observed by the naked eye.

6 Detection of small molecules

6.1 ATP

Adenosine-triphosphate (ATP), as the most important in vivo carrier for energy storage, matters much in a great many physiological and pathological processes. For instance, the ATP level undergoes a significant decrease while cell apoptosis or necrosis induces mitochondria damage. Thus the determination of ATP is highly critical [83].

Wang et al. [84] reported colorimetric detection of ATP by introducing unmodified AuNPs and ATP-specific aptamers (Figure 5). They initially hybridized the ATP-specific aptamer with its complementary sequence. The introduction of ATP, which showed stronger affinity with aptamers than the complementary sequence, led to the dehybridization of the as-formed duplex and the complementary sequence was displaced and released into the solution to stabilize the unmodified AuNPs, even in a concentrated salt solution. Other molecules had much less affinity to ATP-specific aptamers and could not dehybridize the duplex to liberate the complementary sequence to the flexible ss form; thus the unmodified AuNPs aggregated at the loss of protection. This strategy was special because it did not require the analyte to directly remove the protecting ligands from the nanoparticles but had a complementary sequence as an adjuvant. Not all the target-aptamer binding events caused sufficiently apparent conformation change that could be sensed by the unmodified AuNPs, but the hybridization-dehybridization events between the fully complementary duplexes in this assay could be certainly monitored by them. Thus this strategy could be easily expanded to be generic in principle by screening the target-specific aptamers with the SELEX process and its complementary sequences.

Figure 4 (Color online) Scheme of using lead-specific ds DNAzyme and unmodified AuNPs to detect Pb\(^{2+}\) (It was reproduced with permission from ref. [80]. Copyright Wiley-VCH Verlag GmbH & Co. (2008)).
6.2 Melamine

Melamine is a kind of chemical product commonly applied in plastic and fertilizer industry. It is only very recently that melamine has become an interesting analyte due to its contamination to infant formula. It was illegally incorporated into milk to fraudulently increase the apparent protein content as it has very high nitrogen content (66%) [85]. Excessive intake of melamine may cause serious health problems and the development of melamine sensor had been a great challenge [43].

Nitrogen atoms have great affinity to gold atoms, and the nitrogen-rich melamine must have excellent binding capability to AuNPs. The unmodified AuNP itself is a convenient colorimetric sensor for melamine [86]. Upon the addition of unmodified AuNPs in the sample, melamine rapidly coordinated with gold through both exocyclic amino groups and the three nitrogen hybrid ring and the AuNPs were crosslinked through melamine [87]. Unlike the salt-induced aggregation applied in most unmodified AuNP-based assays, this strategy was salt-free and directly responded to the target molecules, which is simple and sensitive; however, other compounds with multiple amino groups could interfere with this assay and thus compromise its selectivity.

Independently, Wei et al. [88] reported a similar strategy for the determination of melamine using unmodified AuNPs, but they improved this detection by using cyanuric acid as an adjuvant to eliminate the false positive results. Besides melamine, other structurally similar molecules containing rich nitrogen, such as adenine, also induced the aggregation of AuNPs but did not generate precipitates with cyanuric acid like melamine. This dual visual readout improved selectivity significantly. This assay was also salt-free and the introduction of salt in this system, however, adjusted the detection sensitivity and dynamic range to a desired concentration level, which was very important towards practical applications. Besides, facile precipitation and extraction steps of interferent casein enabled this assay to be applicable for real milk samples.

6.3 Cocaine

Cocaine (benzoylmethylecgonine) is a crystalline tropane alkaloid that is obtained from the leaves of the coca plant. It has abnormal exciting effects to the central nerve system [89]. Conventional methods using fluorescence or electrochemistry were usually labor-intensive, time-consuming and only had a relatively high limit of detection.

Zhang et al. [90] developed a fairly rapid and sensitive strategy towards colorimetric detection of cocaine by unmodified AuNPs and cocaine-specific aptamers (Figure 6). They rationally engineered the anticocaine aptamers by modification of the nonconserved regions and cleaved the engineered aptamers into two pieces, without apparent perturbation of their target-binding capabilities. The basic principle was similar with all others, but short pieces of aptamers showed much faster binding kinetics for oligonucleotides-AuNPs adsorption than longer counterparts, which is essential for on-site detection that requires rapid assays. Moreover, a cocaine molecule could bind two pieces of engineered aptamers rather than the original one, thus amplifying the efficiency of removing ligands from the surface of AuNPs, which improves the sensitivity of this assay. It is expected that more elaborate design and creation of the engineered aptamers could further improve the performance of this assay.

6.4 Cysteine

As the only thiol-containing one among the twenty amino acids that construct proteins, cysteine plays a pivotal role in many biological processes including redox reactions, protein folding, detoxification and metabolism, and it is also a crucial disease-related marker [91]. HPLC, electrochemistry and fluorescence have been developed to detect cysteine. These methods, however, are restricted by either complicated instrumentation or tedious operating procedures [92,93]. DNA-AuNP conjugates have been applied as a col-
orimetric sensor for cysteine and achieved a very low limit of detection [42]. Compared with their strategy, the utilization of unmodified AuNPs was more convenient.

Researchers used unmodified AuNPs and normal single stranded DNA to detect cysteine in aqueous media [94]. ss DNA was first adsorbed onto the AuNPs to prevent aggregation, even in a concentrated salt solution. When cysteine was added to the AuNPs/ss DNA solution, cysteine could bind to the gold surface via gold-sulfur interactions, replacing the relatively weak interaction between DNA and the gold surface. The cysteine-capped AuNPs had a significant decrease in tolerance to salt-induced aggregation. Accordingly, the color changed, indicating the existence of cysteines in the sample solution.

Alternatively, another unmodified-gold-nanoparticle-based cysteine sensing method was established using the coordination chemistry between cysteine and copper, as well as the Au-S chemistry between cysteine and gold [95]. The simultaneous introduction of cysteine and copper into the unmodified AuNP colloidal solution could result in the cross linking of AuNPs, causing a rapid, red-to-purple color change. Other amino acids without mercapto groups could not induce the aggregation of AuNPs, ensuring the high selectivity of this assay. In this protocol, copper acted as the aggregation-triggering agent and bound directly with the target and AuNPs, without the need of the displacement or releasing steps in common unmodified AuNP-based strategies, which was straightforward, rapid and effective.

Besides, for many other small molecules, such as hydrogen peroxides [96], DNA intercalators [97] can respond to the unmodified AuNP-based sensing systems. Moreover, other noble metal nanoparticles like unmodified silver nanoparticles [98–100] exhibited similar properties and could be applied in these systems as well, making this strategy more general towards various targets.

7 Multiplexed detection of different analytes

High sensitivity and selectivity of sensing protocols are undoubtedly required in multiplexed detection of different targets within one assay. Multiplexation is crucial because of its potential capability of obtaining more information within fewer samples. Also, in practical uses such as identification of diseases, simultaneous detection of multiple DNA/RNA and proteins is frequently desired. Unmodified AuNPs may have an advantage over rationally functionalized AuNPs in that they are not limited by specific ligands.

Recently, in combination with cationic water-soluble conjugated polymers, unmodified AuNPs were developed as a general sensor for multiple targets [101]. This approach was almost “universal” and applicable to a broad range of target molecules including DNA sequences, proteins, small organic molecules and metal ions (Figure 7). This strategy was special in that they found both the ss DNA and the ds, or otherwise folded DNA stabilized the unmodified AuNPs in the diluted salt solution, which was very different from other approaches summarized in this review. The cationic water-soluble conjugated polymers (here in their case [(9,9-bis(6'-N,N,N-trimethylammonium) hexyl) fluorene-alt-1,4-phenylene] bromide, abbreviated as PFP-Br), however, showed preferential binding capabilities to ss DNA to ds, or otherwise folded DNA [102]. The binding events deprived the ss DNA from the surface of the unmodified AuNPs, leading to their drastic aggregation, whereas there were almost no binding between the conjugated polymer and the ds, or otherwise folded DNA. Unlike all other assays reported in this review, the force that triggered aggregation of AuNPs was transferred from case-specific ligands to a conjugated polymer that had no direct interaction with the target, endowing this approach with potentially general applicability. The detection of proteins such as thrombin and DNA sequences, small molecules such as cocaine and ATP, and metal ions such as mercury and lead could be all incorporated in this assay. This was another example of a generic biosensor for multiplexed detection.

8 Conclusions

Utilization of unmodified AuNPs in colorimetric detection
inherits the simplicity of conventional AuNP-based sensing strategies because it can be read out by the naked eye, does not require the assistance of complicated instruments, and is not labor-intensive. Furthermore, the method avoids cumbersome modification and functionalized procedures for ligand-stabilized AuNPs, making them better for ready-to-use. These advantages make it useful for rapid, convenient on-site detection and most likely possess great prospects in the future.

However, there are still two intrinsic drawbacks for the use of unmodified AuNPs. Firstly, without the help from instruments, identification by the naked eye can at best provide a semi-quantitative result. The results can tell people a “Yes OR No” answer, but it is still hard for unmodified AuNPs to do a quantitative analysis. Secondly, the modification-free makes these AuNPs to some extent less tolerant to interference than ligand-stabilized AuNPs.

Considering these shortcomings, sensing platforms based on integrating unmodified AuNPs with lab-on-a-chip systems may realize their full potential for on-site applications. The on-site applications sometimes only require the qualitative or the semi-quantitative determination of the target, fitting the capabilities of unmodified AuNPs. Also, lab-on-a-chip-based miniaturized devices can offer sample pretreatment to eliminate possible interferences to circumvent the second drawback. The high-throughput property of the lab-on-a-chip is an excellent partner for the potential of multiplexing of unmodified AuNPs. It is believed that the combination of unmodified AuNPs and lab-on-a-chip systems may bring new prospects in biomedical analysis, medical diagnostics and environmental monitoring.

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