Improving Colorimetric Assays through Protein Enzyme-Assisted Gold Nanoparticle Amplification

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CONSPECTUS

The discovery of the DNA-mediated assembly of gold nanoparticles was a great moment in the history of science; this understanding and chemical control enabled the rational design of functional nanomaterials as novel probes in biodetection. In contrast with conventional probes such as organic dyes, gold nanoparticles exhibit high photostability and unique size-dependent optical properties. Because of their high extinction coefficients and strong distance-dependent optical properties, these nanoparticles have emerged over the past decade as a promising platform for rapid, highly sensitive colorimetric assays that allow for the visual detection of low concentrations of metal ions, small molecules, and biomacromolecules. These discoveries have deepened our knowledge of biological phenomena and facilitated the development of many new diagnostic and therapeutic tools. Despite these many advances and continued research efforts, current nanoparticle-based colorimetric detection systems still suffer from several drawbacks, such as limited sensitivity and selectivity.

This Account describes the recent development of colorimetric assays based on protein enzyme-assisted gold nanoparticle amplification. The benefits of such detection systems include significantly improved detection sensitivity and selectivity. First, we discuss the general design of enzyme-modified nanoparticle systems in colorimetric assays. We show that a quantitative understanding of the unique properties of different enzymes is paramount for effective biological assays. We then examine the assays for nucleic acid detection based on different types of enzymes, including endonucleases, ligases, and polymerases. For each of these assays, we identify the underlying principles that contribute to the enhanced detection capability of nanoparticle systems and illustrate them with selected examples. Furthermore, we demonstrate that the combination of gold nanoparticles and specific enzymes can probe enzyme dynamics and function with high specificity, offering substantial advantages in both sensitivity and specificity over conventional detection methods. The screening of nuclease, methyltransferase, protease, and kinase activities can be colorimetrically performed in a straightforward manner.

Finally, we discuss examples of colorimetric assays for metal ions and small molecules that constitute important advances toward visual monitoring of enzyme catalytic functions and gene expression. Although these enzyme-assisted assay methods hold great promise for myriad applications in biomedicine and bioimaging, the application of the described techniques in vivo faces formidable challenges. In addition, researchers do not fully understand the interactions of gold nanoparticles with enzyme molecules. This understanding will require the development of new techniques to probe enzyme-substrate dynamics at the particle interface with higher spatial resolution and chemical specificity.

Introduction

Today people frequently think of metal nanoparticles as relatively modern fields of knowledge. In its broadest terms, however, metal nanoparticles are not new: they have existed widely in the natural world, for millions of years, created by living things or volcanic eruptions. Indeed, people have exploited the properties of metal nanoparticles for centuries. For instance, gold and silver nanoparticles...
Gold nanoparticles (AuNPs) have perhaps been the most widely studied metal nanoparticle system over the past decade.\textsuperscript{2–12} These metal nanoparticles are typically characterized by a strong plasmon resonance absorption band in the visible spectrum that gives rise to intensely colored solutions.\textsuperscript{3} They can be prepared in a broad range of diameters ranging from 2 to 250 nm with high levels of precision and uniformity. The resulting nanoparticles are readily functionalizable with small molecules or biological macromolecules containing different types of functional groups such as thiol, phosphate, and amine. The facile and effective surface modification strategies for AuNPs have driven interest in the development of miniaturized detection technologies that are more robust, higher throughput, and lower cost than conventional methods.

A particular branch of AuNP research is the development of high-sensitivity colorimetric assays for biological molecules without the need for time-consuming techniques and expensive instrumentation.\textsuperscript{3} The colorimetric assays are generally based on an absorbance shift of colloid particles that can be modified through changes in the size and shape of the particles or in their local environment. For colorimetric detection of DNA sequences, there are two major classes of AuNP-based assays. The first class utilizes a three-component sandwich assay format (Figure 1a).\textsuperscript{12} In this type of assays, two sets of AuNPs are used, each bearing a distinct, noncDNA sequence immobilized at the 5’-and 3’-terminus, respectively. If a perfectly matched target DNA is added to the nanoparticle solution, the target would serve as a linker strand and recognize the sequences immobilized on the two sets of the particles, leading to formation of large particle aggregates. As a result, a red-to-blue color change is observed, indicating a bathochromic shift of the plasmon band. The aggregation is a reversible process because of the reversible nature of DNA hybridization. The second class of the colorimetric assay relies on the difference in binding properties of a single-stranded DNA (ssDNA) and a double-stranded DNA (dsDNA) toward unmodified AuNPs (Figure 1b).\textsuperscript{13} Under optimized conditions, a ssDNA binds to unmodified nanoparticles and effectively stabilizes them against salt-induced aggregation, but a dsDNA does not. The essential difference arises because the ssDNA and dsDNA adopt different geometry conformations. The ssDNA can uncoil to expose its bases for stabilizing the particle, while the duplex structure does not permit the uncoiling.\textsuperscript{14} A notable benefit of this method is the elimination of covalent functionalization of particles needed in the three-component sandwich assay format.\textsuperscript{15,16}

Despite enormous advances made in the AuNP-based colorimetric assays for biomolecules, a number of constraints associated with conventional prototype designs still exist, such as limited sensitivity and selectivity. For example, the limit of colorimetric detection for a short DNA sequence by conventional sandwich assays (using 14 nm nanoparticles) is about 10 nM, which is much higher than the detection limit (typically below 1 nM) required in many diagnostic assays.\textsuperscript{2} When the target concentration is below 10 nM, the solution color change is generally not discernible to the naked eye due to weak interparticle plasmon coupling caused by insufficient particle aggregates.\textsuperscript{2,4} Another notable constraint is that conventional DNA assays need stringent control over melting temperatures for detection of a single-base mismatched target. It should also be noted that conventional assays are limited to short DNA fragments. To meet these challenges, some efforts have turned to enzyme-assisted nanoparticle amplification as a potential solution.\textsuperscript{7}

In this Account, we would like to demonstrate that the incorporation of enzymes into the design of nanoparticle-based detection systems can substantially improve assay sensitivity and selectivity for a variety of analytes. We focus on the recent progress made in this field, with particular emphasis on cases of nucleic acid detection, enzyme activity screening, and metal ion sensing. We also discuss general design principles underlying the signal amplification and the possibilities and limitations of these methods.
Enzymes

Most enzymes are proteins that perform specific catalytic functions and control the pathway of organic transformations. A remarkable characteristic of enzymes is their catalytic power that may accelerate chemical reactions by factors of a million. Among many types of enzymes, the enzymes involved in the actions of nucleic acids and peptides are powerful biological tools. Table 1 summarizes commonly used enzymes, grouped according to function, for AuNP-based colorimetric assays.

Nucleases, which include exonucleases and endonucleases, are DNA-manipulation enzymes capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acids. Exonucleases cleave DNA molecules from the ends, while endonucleases cleave the phosphodiester bond within a polynucleotide chain. Restriction enzymes are endonucleases that cleave only DNA molecules in which they recognize particular subunits. By using restriction endonucleases with different functions, different amplification strategies can be developed based on the three-component sandwich colorimetric assay. For example, by utilizing restriction enzymes to cleave dsDNA, a signal amplification through linker-cleavage can be achieved (Figure 2, Strategy A). In this strategy, the target can be repeatedly used to cleave excess linker strands. Alternatively, the signal could be amplified through nicking endonuclease-induced accumulation of the linker DNA. As shown in Figure 2 (Strategy B), the elongated DNA fragment can serve as a linker strand after the cleavage of ssDNA by nicking endonuclease. After the cleavage, the duplex formed by the target and template DNA can be recycled. It should be noted that the reaction temperature is critical for retaining catalytic cycles in both strategies.

DNA ligase is another important type of enzymes that catalyze the formation of the phosphodiester bonds by joining nucleic acid molecules via DNA or RNA as substrate. The requirement of complementarities between the substrate and nucleic acid molecules provides the possibility of single-nucleotide polymorphism (SNP) detection. An early example using Tth DNA ligase for SNP detection was demonstrated by Li and co-workers. Other than the enzymes for DNA manipulation, proteases and protein kinases that use peptides (or polypeptides) as substrates could also be used in AuNP-based colorimetric assays.

Detection for DNA

The detection of DNA sequences has attracted much attention over the past few years owing to its importance in many aspects of molecular genetics. Although commercial techniques based on polymerase chain reaction (PCR) and fluorophore staining could meet the requirement of sensitivity and selectivity, they suffer from several drawbacks, that include tedious handling procedures, easy contamination, high costs, and inaccessibility of on-the-spot sample analysis. The advantages of nanoparticle probes in DNA analysis with improved detection sensitivity and shortened detection time at a substantially lower cost clearly represent the promise of nanoparticle-based technology. The combination of enzyme amplification and functional nanoparticles presents a newer and more novel method that could improve the detection of DNA molecules of interest.

**Nanoparticle-Coupled Isothermal DNA Amplification.**

Niemz and co-workers have pioneered a rapid method for

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**Table 1. Commonly Used Enzymes in AuNP-Based Colorimetric Assays**

<table>
<thead>
<tr>
<th>enzyme</th>
<th>function</th>
<th>examples</th>
<th>substrate</th>
<th>sensing targets</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuclease</td>
<td>hydrolytic cleavage</td>
<td>BsaBI restriction endonuclease, NLAIIw nicking endonuclease, exonuclease III, RNase H, EcoRI</td>
<td>DNA/RNA</td>
<td>DNA, enzyme, metal ion</td>
<td>20, 25, 27, 40, 47</td>
</tr>
<tr>
<td>polymerase</td>
<td>polymerization of nucleotides</td>
<td>Phi29 DNA polymerase, Bst DNA polymerase, T4 DNA ligase, Tth DNA ligase, EcoRI methylase, Dam MTase</td>
<td>DNA</td>
<td>DNA</td>
<td>19, 21</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>connecting DNA</td>
<td>DNA ligase</td>
<td>DNA</td>
<td>DNA</td>
<td>21, 22, 29</td>
</tr>
<tr>
<td>methyltransferase</td>
<td>transferring methyl groups</td>
<td>EcoRI methylase, Dam MTase</td>
<td>DNA</td>
<td>enzyme</td>
<td>23, 35</td>
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<tr>
<td>protease</td>
<td>hydrolytic cleavage</td>
<td>thrombin, trypsin</td>
<td>peptide</td>
<td>enzyme</td>
<td>23, 41</td>
</tr>
<tr>
<td>protein kinase</td>
<td>transferring phosphate groups</td>
<td>cAMP dependent protein kinase A, v-Src kinase</td>
<td>peptide</td>
<td>enzyme</td>
<td>24, 44</td>
</tr>
</tbody>
</table>

**FIGURE 2.** General strategies for endonuclease- and polymerase-based DNA amplification through use of different restriction endonucleases.
Enzyme Assisted Gold NP Colorimetric Assays

Detecting short (10–20 base pairs) DNA sequences, based on the combination of a novel isothermal amplification method with oligonucleotide-modified AuNPs. By taking advantage of an exponential nucleic acid amplification reaction (>10^6 folds) through use of strand extension and single strand nicking, their method permits detection of a DNA sequence at 100 fM concentration in less than 10 min total assay time. Li and co-workers have demonstrated an alternative isothermal amplification method that involves using thermophilic DNA polymerase and a restriction endonuclease. However, it is worth noting that with a nicking or restriction endonuclease the reaction time has to be controlled as a prolonged period of time allows new DNA strands to be synthesized by thermophilic DNA polymerase even without a template or a primer under isothermal conditions.

NEANA Approach and Exo III-assisted Signal Amplification. Recently, our group has reported a simple, efficient NEANA method for enhanced detection of DNA sequences using a nicking enzyme and DNA-modified AuNPs. Through this dual-mode amplification, a detection limit of 10 pM for ssDNA sequences of different lengths (36mer, 48mer, and 80mer) with single-base discrimination is achievable within hours. Despite the advantage over conventional assays, the NEANA approach has a relatively limited scope for detection of random DNA sequences, because of the requirement of a specific sequence for target DNA to be recognized by the nicking enzyme. To overcome the limitation, we have further combined the rolling circle amplification with the NEANA approach, which enables the detection of arbitrary DNA sequences down to 1 pM.

Exonuclease-Based Amplification. Recently, Yang and co-workers have shown that replacing nicking endonuclease with exonuclease III (Exo III) enables a universally adaptable system for detection of arbitrary DNA sequences. A distinctive feature of Exo III is that its function does not require a specific recognition site. In addition, Exo III specifically digests double-stranded DNA sequences from recessed or blunt-ended 3'-hydroxyl termini. By combining the Exo III enzyme and AuNPs, Yang and co-workers demonstrated colorimetric DNA detection with high sensitivity (down to 15 pM) and single-base mismatch selectivity.

Thrombin-Based Amplification. Apart from those methods based on cross-linking of DNA-modified AuNPs, the colorimetric detection of DNA sequences can also be achieved using a noncross-linking approach developed by Huang and co-workers. They found that the spatial distance between a AuNP and thrombin, a serine protease involved in a multitude of biological processes, has a strong influence on the catalytic activity of thrombin. This effect can be harnessed to develop complementary colorimetric techniques for DNA assays. As shown in Figure 4, DNA-modified AuNPs are first mixed with the thrombin and a thrombin-binding aptamer. Upon addition of fibrinogen-modified AuNPs, the thrombin is able to cleave the fibrinogen into many fragments, resulting in the formation of particle aggregates. However, in the presence of a complementary target DNA, the thrombin and AuNPs form a sandwich structure with the target DNA. Owing to the steric hindrance...
and the electrostatic charge of the particles, the biological activity of the thrombin is substantially suppressed.

**Ligase- and Nuclease-Assisted Detection for SNPs.** SNPs have been linked to many pathogenic and genetic diseases associated with a change in the sequence of particular genes. Early examples of SNP detection are mainly based on the single-base mismatch-induced difference in local chemical environment and thermodynamic properties of dsDNA, such as melting temperature.12 Despite their usefulness in screening for SNPs, these detection methods are often costly and complex to operate, and lack of multiplex capability. Furthermore, they do not have adequate selectivity to discriminate SNPs with melting temperatures subtly different from those of the DNA sequences without point mutations.

To address the substantial challenges of developing systems for SNP typing, a convenient chip-based approach involving nanoparticle-coupled DNA-templated ligation reactions was invented with the design rationale of combining multiplex detection capability and ultrahigh selectivity (Figure 5a).29 In contrast to conventional methods, this approach eliminates the need for costly instrumentation and complex stringency wash processes while enabling the semiquantification of target concentrations. A notable demonstration of this approach is multiplex detection of SNPs at different positions adjacent to the nicking site (Figure 5b). The results showed strong position-dependent effects of sequence mismatches on the DNA-templated ligation reactions. Remarkably, this method allows for identifying a point mutation site which is four nucleotide bases away from the nicking site.

Alternatively, a single-strand-specific nuclease could also be coupled with AuNPs for detection of SNPs. Mung bean nuclease derived from mung beans has been used to probe irregular structural sites in ssDNA molecules and specifically cleave the molecules in a stepwise manner to yield 5’-phosphoryl-terminated products. Notably, this nuclease prefers digestion of ssDNA over dsDNA by 30,000-fold. On the basis of this attribute available to mung bean nuclease, Chen and co-workers demonstrated a three-step strategy for colorimetrically detecting the point mutation of oligonucleotides.30 They concluded that the position of the mutation up to 4 base-pairs from the 5’-end of a 40-base-pair sequence can be detected through use of a combination of the single-strand-specific nuclease and particle amplification. It should be noted that mung bean nuclease requires use of Zn2+ and a solution at relatively high salt concentration.

Liu et al. have recently reported an interesting strategy, based on the combination of a single-strand-specific nuclease and AuNPs, for label-free colorimetric detection of SNPs.31 This method takes advantage of different binding affinities of the nucleoside monophosphate (dNMP), ssDNA, and dsDNA toward unmodified AuNPs. Specifically, the dNMP formed by digesting ssDNA via a structure-selective nuclease provides much more stability than the ssDNA and dsDNA of the same base-composition and concentration. One notable drawback of this approach is its low sensitivity (∼5 nM) and inability to differentiate the position of the point mutations. Additionally, this method is limited to assays in purified ssDNA target, as impurities such as RNA, protein, and enzyme may cause interference with the colorimetric detection.

**Screening for Enzyme Activity**

Elevated enzyme activity is often linked to uncontrolled cell division, angiogenesis, and metastasis that cause the growth of malignant cells. Concerning the prevention, diagnosis, and treatment of cancers, of particular interest is to develop methods that enable rapid monitoring of enzyme activity. Conventional methods for measuring the activity of enzymes typically require laborious experimental procedures and the use of radio- or fluorophore-labeled substrates, which inevitably present detection constraints such as photobleaching and autofluorescence.32 As was discussed previously, the use of enzymes has proven to improve the sensitivity and selectivity of nanoparticle-based assays.
Conversely, AuNPs can be utilized to measure enzyme dynamics and function with high specificity.

**DNA as the Enzyme Substrate.** The first reported screening of enzyme activity using AuNPs as colorimetric indicators was reported by Mirkin and co-workers (Figure 6a). In a demonstration of the technique, the researchers used a ssDNA to link DNA-functionalized nanoparticles and measured the activity of a deoxyribonuclease I (DNase I) that cuts the DNA at a specific site. Upon addition of the enzyme, the color of the solution gradually changed from purple to red, resulting from the release of single nanoparticles through the DNase I-catalyzed hydrolysis of nucleic acids. This same system could also be extended to evaluate the efficiency of inhibitors of DNase I (Figure 6b and c). The relative degree of DNase I inhibition can be easily differentiated through visual inspection.

AuNP-based colorimetric assays could be further developed for both endonuclease and methyltransferase activity screening. Type II endonucleases typically recognize short and palindromic sequences that are equal to their reverse complements. By taking advantage of this unique feature, Qu and colleagues designed an enzyme-responsive system having only one type of ssDNA-modified AuNPs.33 A parallel development of colorimetric assays for DNase I was reported by Li and co-workers on the basis of different stabilities of DNA-modified AuNPs triggered by a specific enzymatic cleavage reaction (Figure 7).34 They found that, before enzymatic cleavage, DNA-modified nanoparticles were stable at high salt concentrations. However, upon cleavage of the DNA strands at the same salt concentration by DNase I, a distinct red-to-purple color change of the solution due to formation of particle aggregates was rapidly observed. In similar assay formats, the screening of methyltransferase and telomerase activities has been achieved using nanoparticles coupled with a methylation-sensitive restriction endonuclease and ssDNA primer, respectively.35,36

An intriguing study by Shen et al. demonstrated that changing the length of ssDNA sequences yields different particle dispersion profiles on unmodified AuNPs, with superior colloidal stability observed for short ssDNAs against salt-induced aggregation (Figure 8a).37 Different from traditional unmodified AuNP assays highlighted in Figure 1b, this approach relies on the fact that short ssDNAs typically adsorb onto AuNPs at a faster rate than long ssDNAs. Alternatively, the effect that dNMPs stabilize AuNPs better than ssDNAs and dsDNAs can be utilized to detect nuclease activity and selectivity.38 Positively charged AuNPs could also be employed for nuclease activity screening due to the...
electrostatic attraction between positively charged nanoparticles and polyanionic DNAs.\textsuperscript{39}

**RNA as the Enzyme Substrate.** Recently, utilizing RNA as an enzyme substrate, together with unmodified AuNPs, has also been demonstrated by our group for enzyme activity screening (Figure 8b).\textsuperscript{40} In this study, we found that a DNA–RNA duplex could not stabilize the unmodified nanoparticles at a certain salt concentration. Upon addition of an active HIV-1 RNase H enzyme, the RNA strands were specifically cleaved into fragments. Importantly, the resulting RNA fragments and ssDNAs could stabilize the nanoparticles against salt-induced aggregation. In contrast, without the enzyme, particle aggregation occurred with a concomitant color change.

**Peptide as the Enzyme Substrate.** The peptide substrate can be coupled to unmodified AuNPs and used to measure the activity of certain enzymes, such as proteases, in colorimetric assays. The strategy for monitoring protease activity through the cleavage of peptide bonds is mainly based on different interactions between the peptide and nanoparticles before and after the enzymatic reaction. The use of peptide as the enzyme substrate for nanoparticle-based colorimetric assays was first reported by Guarise and co-workers in 2006.\textsuperscript{23} Since then, many groups have pursued the development of enzyme substrates, including gelatin,\textsuperscript{41} fibrinogen,\textsuperscript{42} and Arg\textsubscript{65},\textsuperscript{43} for protease sensing. It should be noted that these assays are designed for measuring the activity of a purified enzyme. In cell culture media or cell lysates, there are often contaminating proteolytic enzymes which may be present to cleave the substrate. Additional control experiments using more specific reagents such as antibodies and inhibitors are thus required to cross-check whether the activity is due to the enzyme of interest.

Brust’s group demonstrated that the activity of kinases could also be colorimetrically monitored using AuNPs modified with kinase-specific peptides.\textsuperscript{24} They carried out a reaction using γ-biotin-ATP as a cosubstrate, resulting in the biotinylation of the nanoparticles. Subsequent addition of another set of avidin-modified nanoparticles led to a pronounced color change of the solution due to aggregation of nanoparticles via biotin–avidin binding. However, in the presence of a kinase inhibitor, no detectable color change occurred. Encouraged by these results, Stevens et al. reported a simple, one-step approach for colorimetric kinase detection, using one set of kinase-substrate-peptide-coated nanoparticles and a second set of complementary antiphosphotyrosine-antibody-modified nanoparticles.\textsuperscript{44} Another viable approach to simplifying colorimetric kinase assays is to directly use citrate-coated nanoparticles and specially designed cationic peptides.\textsuperscript{45}

The removal of phosphate group from peptides by hydrolysis has been used for alkaline phosphatase screening, as was demonstrated by Tung and co-workers.\textsuperscript{46} They made a special design on a phosphorylated peptide by putting the positively charged guanidine group next to the negatively charged phosphate group. Thus, no noticeable colorimetric change was observed when they added the peptide to unmodified AuNPs, probably due to the minimized binding between the guanidine group and AuNPs. In contrast, the authors found particle aggregations if the phosphate group was removed by alkaline phosphatase.

**Detection for Other Targets**

Many types of enzymes that perform specific catalytic functions and regulate gene expression are found to contain metal ions. For example, in many hydrolytic enzymes, the active site contains Zn\textsuperscript{2+} ion. For this reason, certain metal ions can be readily detected through nanoparticle-assisted enzymatic reaction. For example, we have recently demonstrated that Mg\textsuperscript{2+} ion could be detected using EcoRI-modified AuNPs (Figure 9a).\textsuperscript{47} In this assay, a specifically designed dsDNA was employed which contains an EcoRI-recognition site and complementary sticky ends. The EcoRI could cleave the dsDNA in the presence of Mg\textsuperscript{2+} ion as a factor. This assay showed an excellent selectivity for Mg\textsuperscript{2+} ion among a variety of monovalent, divalent, and trivalent metal ions (Figure 9b).
Another strategy for metal ion detection is based on the use of an ion-specific aptamer and nicking endonuclease.48 The aptamer specifically binds to K$^+$ and blocks the cleavage of a linker strand DNA by the nicking enzyme. Upon the addition of DNA-modified AuNP probes, particle aggregation occurs. However, in the absence of K$^+$, the aptamer hybridizes with the linker strand DNA, leading to the cleavage of dsDNA. Thus, the nanoparticles remain dispersed in solution.

Some enzymes, such as alkaline phosphatase, β-lactamase, and acetylcholinesterase, have been used to catalyze the reaction of small molecules for enzyme activity screening.49–53 Not surprisingly, the combination of these enzymes and AuNPs can lead to design of novel colorimetric systems for detection of small molecules such as glucose.54

Furthermore, some small molecules could modulate the enzymatic reaction by specific interaction with enzymes or substrates. One representative example was reported by Ren and co-workers for screening of small ligands such as thiazole orange through G-quadruplex-assisted enzymatic manipulation of DNA-modified AuNPs.55 This strategy was based on the fact that the stabilization of G-quadruplex by the small ligands inhibits the enzymatic cleavage of nucleotides by exonuclease I. A similar strategy has also been used for detection of tyrosinamide and organophosphate pesticides, respectively, by using citrate- and lipoic acid-stabilized AuNPs.56,57

Target-controlled enzymatic reactions could be further developed for rapid colorimetric detection of sequence-specific DNA-binding proteins. For example, Ou et al. reported a specific and quantitative assay for DNA-binding proteins based on Exo III manipulation.58 This assay has a linear response range from 0 to 120 nM and a detection limit of 10 nM.

**Conclusion**

The colorimetric assay through protein enzyme-assisted AuNP amplification is a relatively new research area. In this Account, we have reviewed recent advances in this area, with particular focuses on the rational design of detection systems and applications for enzyme activity screening and detection of nucleic acids, metal ions, as well as small molecules. Despite the fact that it is premature at this stage to draw any sweeping generalization, the use of enzymatic reactions does significantly improve the detection sensitivity and selectivity of AuNP-based colorimetric assays for a variety of analytes. The advances of enzyme-assisted AuNP amplification may allow the subtle design of materials and platforms tailored for portable in vivo and real time molecular or ion sensing.59 The coupling of enzymes and nanoparticles could also provide a powerful approach to preparing well-defined and tunable nanostructures.60 As a separate note, the use of protein enzyme-assisted amplification can be readily extended to other particle systems, including semiconducting61 and lanthanide-doped nanoparticles.62,63

**REFERENCES**


**FOOTNOTES**

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The authors declare no competing financial interest.
Enzyme Assisted Gold NP Colorimetric Assays Xie et al.


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Xie et al.


