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A Review of Gold and Silver Nanoparticle-Based Colorimetric Sensing Assays

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ABSTRACT

The nanoparticle colorimetric-based methods have been extensively used for rapid detection, however there are few limitations which can be kept under control or avoided by understanding the crucial parameters involved in these reactions. This review addresses the main parameters
that influence colorimetric-based methods and provides a rational classification of the current approaches, by focusing particularly on gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs). The AgNP and AuNP-based colorimetric assays can be very efficient and sensitive especially for biomolecule identification and for metal ion detection in environmental screening. Specifically, this review highlights the detection of metal ions through their coordination with nanoparticle stabilizing ligands. The review also addresses various approaches based on label-free aptasensors to better understand their role as smart colorimetric sensing devices.

1. Introduction

The term nanoparticle (NP) defines any small object (from 1 to 100 nm) that behaves as a unit concerning its transport and properties. Indeed, as their properties change in function of their size, particles can be classified based on their diameter. Thus, ultrafine particles (or NPs) are particles with a diameter between 1 and 100 nm, whereas fine particles have a diameter between 100 and 2,500 nm. For tailored applications, NPs can be capped with a variety of different anionic and cationic ligands, from displaceable small molecules to polymer coatings. The choice of capping ligand depends on the NPs used that can range from conductive inks to biomedical tools.

Among the known nanoparticles, gold and silver NPs (AuNPs and AgNPs) have been widely studied because of their unique optical, electrical and photothermal properties. AuNPs and AgNPs show unique optical features in well-dispersed solutions, depending on their level of aggregation which is mostly determined by their specific surface plasmon resonance (SPR) profiles [1]. Metal-noble NPs are small enough to confine their electrons and produce quantum effects. This is a key parameter for naked-eye colorimetric sensing applications, because modifications of their surface charge are transformed into a visible color change. Furthermore, NPs also have a very high extinction coefficient that depends mainly on their size, shape and inter-particle distance. Such properties enable them to compete with analytical techniques, like absorbance or fluorescence spectroscopy. Colorimetric-based assays have been developed by exploiting the color changes associated with the aggregation of metal-noble NPs [2]. Due to their adaptability, high sensitivity, low cost and versatility (Figure 1), AgNP- and AuNP-based assays have been used for the detection of metal ions [3], small molecules [3b, 3f, 4], proteins [3f, 4z, 5], deoxyribonucleic acidDNA [3f, 5i, 6] and enzymes [5a. 6g. 7]. AuNPs are often used as sensing
elements to develop sensitive, selective, simple and label-free colorimetric assays [7h, 8]. Consequently, NP used as detection agents could be considered as a sort of “litmus test” for target molecules [5i, 9].

Figure 1: Possible colorimetric detection approaches for metals, ligands and macromolecules.

Although analytical methods, such as high-performance liquid chromatography, electrophoresis, voltammetry and fluorescence spectroscopy, are the preferred approaches for the detection of various macromolecules, sensitive, fast and high-throughput screening methods are still required [4x]. NP-based colorimetric methods are quick and user-friendly detection approaches that take advantage of various chemical mechanisms. For instance, such methods can be used for the rapid detection of influenza viruses through the binding between the influenza virus envelope protein hemagglutinin and sialic acid-stabilized AuNPs [10], or for high-throughput screening of endonuclease inhibitors [7c].

In this review, the colorimetric approaches that allows for the naked-eye detection of color changes through ultraviolet-visible (UV-Vis) absorption spectroscopy without fluorescence detection methods are addressed. Specifically, aptamer-based applications for the detection of metal ions, small ligands and biological macromolecules will be described. Accordingly, the methods have been subdivided on the analytes and the NP’s surface modifications and not on the basis of the used NPs, as the colorimetric performances of AgNPs or AuNPs are very similar. The applications involving aqueous/water-soluble stabilized NPs with no solid surface arrays, such as glass will be highlighted. To ensure that all key possibilities are fully explored, the
different sections herein have been divided as follows: detection of metals, small molecule
(cysteine, dopamine) and oligonucleotides (cross-linking).

2. Gold and Silver NP synthesis, morphology and properties
The most commonly used methods for the synthesis of AgNPs and AuNPs involve reducing
AgCl or AgNO₃ and HAuCl₄ with sodium citrate and sodium borohydride. The AuNP mixture is
boiled with vigorous stirring in a round bottom flask fitted with a reflux condenser for
approximately 10 min. Color change from yellow to wine red is observed within a few seconds
[4s, 4z, 11]. The AuNP solution concentration can be calculated following Beer’s law, using the
extinction coefficient of 2.7×10⁸ M⁻¹ cm⁻¹ at λ=520 nm [12]. For AgNPs, 1% tri-sodium citrate is
added to 0.3 mM silver nitrate solution, and the mixture stirred for 5 min. After a drop-by-drop
addition of 1 mM sodium borohydride solution in the dark, the resulting mixture is stirred at
room temperature for 2 h. The bright yellow AgNPs are then filtered through a Millipore syringe
(0.45 nm) to remove the precipitate [4d]. NPs are then characterized by diffusion light scattering
(DLS) to calculate their hydrodynamic radius in solution, or by transmission electron microscopy
(TEM) to describe their morphology (Fig. 2).
For colorimetric assays, AgNPs have some advantages compared with AuNPs. Specifically, AgNP extinction coefficients are higher than those of AuNPs of the same average size \cite{7h}, but with AuNPs being more popular. This could be explained by the fact that AgNPs functionalization usually leads to their chemical degradation and thereafter the AgNP surface can be easily oxidized, thus reducing their stability \cite{4f, 7h, 14}. Indeed, Manuco et al. reported that AuNPs are stable for more than 1 month at room temperature, whereas AgNPs only for about two weeks. This difference could be linked to the different reaction constants of thiolated gold and silver \cite{15}. The high extinction coefficients and distance-dependent optical properties of AuNPs accounts for the high sensitivity of AuNP-based colorimetric assays. Moreover, color changes can be easily observed by the naked eye, thus making them attractive for DNA-related colorimetric assays \cite{4c, 4k, 6b, 7h, 8}. The DNA adsorption kinetics by AgNPs are slower than those by AuNPs and they cannot be accelerated by adding salt at neutral pH. This unique property of the specific molecular recognition of DNA-related colorimetric assays accounts for the difficulty of attaching DNA to AgNPs at neutral pH \cite{16}.

Similarly, AgNPs are good candidates as optical sensors because they display distance-dependent optical properties \cite{4f, 17}. Their stability can be improved by producing Ag/Au core-shell NPs that retains the Ag core optical properties. However, oligonucleotide-modified Ag/Au alloy particles are not as stable as oligonucleotide-modified core-shell particles and irreversibly...
aggregate in comparable conditions\textsuperscript{[17b]}. Furthermore, nanoparticles, such as nanorods, prisms, bipyramid of materials, have different SPR wavelengths\textsuperscript{[15]}. For this reason, sensing platforms based on AuNPs optical properties in combination with the molecular recognition of ligands, such as alkyl thiols, antibodies, nucleic acids, and proteins, are active areas of research\textsuperscript{[34]}. Mirkin’s group developed aptamer-based colorimetric assays for macromolecules using the more stable AuNPs, despite AgNPs having a greater extinction coefficient\textsuperscript{[6c, 18]}. NP colloidal stability can be adjusted by modifying the surface charges that affects electrostatic stabilization, and NP aggregation can be induced through loss (or screening) of surface charges. Basically, when AgNPs and AuNPs are exposed to light, they oscillate the electromagnetic field of light. This induces a collective coherent oscillation of conduction band electrons, giving rise to SPR. The SPR band intensity and wavelength depend on the factors that affect the electron charge density on the particle surface. According to Mie’s theory, these factors include the metal type, particle size, shape, structure, composition and dielectric constant of the surrounding medium\textsuperscript{[19]}. Thus, unmodified AuNPs are red while AgNPs are blue or maroon due to their specific and size-dependent SPR absorption. Addition of salt triggers electrostatic repulsion between negatively-charged NPs and antiparticle changes, resulting in NPs aggregation and, consequently, specific color and wavelength changes\textsuperscript{[3e, 4t, 6a, 13]}. Hence, by monitoring the changes in absorbance, it is possible to understand the characteristics of the enhanced scattering effect in aggregated NPs compared with non-aggregated NPs\textsuperscript{[10]}. Consequently, the band gap energies can also be used to improve the knowledge on NPs sensing and catalytic properties\textsuperscript{[20]}. The SPR profile is characteristic of the NP surface modification by small molecules, metal ions and bio-macromolecules\textsuperscript{[21]}.

3. **Detection of metals ions (aggregation induced by interparticle cross-linking)**

AgNPs and AuNPs colorimetric changes are due to the particle surface modification and aggregation. This can be achieved by NPs aggregation induced by interparticle bond formation (cross-linking aggregation) or by modifying colloidal stabilization (non-crosslinking aggregation)\textsuperscript{[7a, 22]}. In the non-crosslinking system, aggregation is driven by the London/van der Waals attractive forces between NPs\textsuperscript{[7a]}. Therefore, the presence of specific functional groups, such as hydroxyl (–OH), carboxyl (–COOH) and amine (–NH\textsubscript{2}), on the NPs surface plays a
critical role in aggregation. By modulating the strength of the NPs intermolecular ion and surface chemistry, this method can be improved [23]. For example, highly charged nucleotides or uncharged nucleosides can bind to citrate-capped AuNPs with the displacement of weakly bound citrate ions through metal–ligand interactions. This can increase AuNPs stabilization or trigger their aggregation, respectively, through gain or loss of surface charges [7a].

**Table 1: Detection of metal ions**

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Analyte</th>
<th>Sample/interferent</th>
<th>Functionalization</th>
<th>Analytical performance</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs</td>
<td>Mercury Hg^{2+}</td>
<td>River water and tap water</td>
<td>Citrate-AuNPs + 2, 2'-bipyridyl (Bipy)</td>
<td>LOD: 38 nM LDR: 0.2 to 2.0 μM</td>
<td>[24]</td>
</tr>
<tr>
<td>AuNPs (20 nm)</td>
<td>Mercury Hg^{2+}</td>
<td>Target-doped blood serum</td>
<td>AuNPs and conjugated polyelectrolyte</td>
<td>LOD: 50 μM</td>
<td>[23]</td>
</tr>
<tr>
<td>AuNPs (12 nm)</td>
<td>H_2O_2</td>
<td>Interferent solutions at different concentration</td>
<td>Citrate-AuNPs</td>
<td>LOD: 1.3 μM LDR: 1.3 to 41 μM</td>
<td>[4a]</td>
</tr>
<tr>
<td>AuNPs (17±2 nm)</td>
<td>Cr^{3+}</td>
<td>Tap water and underground water</td>
<td>Mercaptosuccinic acid-AuNPs</td>
<td>LOD: 0.6 μM LDR: 0.6 to 1.4 μM</td>
<td>[11a]</td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Ag^+</td>
<td>Bimetallic conjugate mixtures</td>
<td>Au–PolyT and Ag–PolyA</td>
<td>LODs: 86.8 nM</td>
<td>[14]</td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Pb^{2+}</td>
<td>Divalent metal ions</td>
<td>DNAzyme–AuNPs</td>
<td>LOD: 0.1 to 4 μM</td>
<td>[3a]</td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Pb^{2+}</td>
<td>RNA-cleaving DNA enzyme</td>
<td>Aptamer–AuNPs</td>
<td>LDR: 0.020 to 200 μM LODs: 10 μM</td>
<td>[7]</td>
</tr>
<tr>
<td>AuNPs (10 nm)</td>
<td>Pb^{2+}, Pd^{2+}, Hg^{2+}, Pt^{2+}</td>
<td>Standard solutions</td>
<td>Peptide-AuNPs</td>
<td>LODs: nM/ppb range</td>
<td>[3d]</td>
</tr>
<tr>
<td>AuNPs (3.5 nm)</td>
<td>K^+</td>
<td>Monovalent cations (Li^+, Na^+, Rb^+, NH_4^+) and bivalent (Ca^{2+} and Mg^{2+})</td>
<td>Aptamer–AuNPs</td>
<td>LOD: 1.0 nM</td>
<td>[3c]</td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>SCN⁻: thiocyanate</td>
<td>Tap water and common ions</td>
<td>Citrate-AuNPs</td>
<td>LOD: 140 nM</td>
<td>[4s]</td>
</tr>
<tr>
<td>AgNPs (9.5±2.0 nm)</td>
<td>Ca^{2+}</td>
<td>Serum and artificial cerebrospinal fluid</td>
<td>AgNPs and cysteine</td>
<td>LOD: 0.1 mM</td>
<td>[4d]</td>
</tr>
</tbody>
</table>

LDR, linear dynamic range; LOD, limit of detection.

Recently, colorimetric measurements based on metal-induced aggregation of small molecules (for instance, cysteine, dopamine), peptides and DNA-functionalized NPs have
received considerable attention \cite{3b, 3c, 4i, 11c}. Indeed, aggregation results in changes in the inter-particle distances, leading to a shift of the SPR absorption band of AgNPs to a longer wavelength \cite{4i}. These cross-linking aggregation-based approaches have been used for the detection of metal ions (Table 1). Farhadi et al. described the development of a sensitive and selective colorimetric assay based on the interaction between cysteine, sodium dodecyl sulfate (SDS)-capped AgNPs and calcium ions. In the presence of calcium ions and NaCl, AgNPs aggregation was induced, leading to a yellow-to-red color change \cite{3c} (Fig. 3). Mukherjee and co-workers used a similar approach for cysteine detection with AgNPs in the presence of Cr$^{3+}$ \cite{3b}. As in the case for colorimetric assays in which a ligand is used for complexation, these approaches ultimately are effective for detection of both ligands and metal ions. For instance, in the presence of Cu$^{2+}$, ascorbic acid rapidly induces AuNPs aggregation as a result of the Cu$^+$-catalyzed 1,3-dipolar cyclo-addition of azides and alkynes (click reaction). Thus, the AuNPs solution color changed and this was observed by naked eye for LOD of 3 nM \cite{4i}.
Fig. 3. Schematic illustration of the strategy for cysteine detection using (A) AgNPs and (B) AuNPs. The simultaneous presence of cysteine molecules and Ca$^{2+}$ ions that act as a “glue” to link two neighboring NP-cysteine complexes results in NP cross-linking. Reproduced with permission from [3c].

Enzymes, such as deoxyribozymes (DNAzymes) with high specificity for metal ions and can form blue aggregates with AuNPs, have also been used for the detection of metal ions. Specifically, Pb$^{2+}$ induces NPs aggregation, thus maintaining the color of the original solution [3a].

Peptide-based AuNPs also have been used as colorimetric sensors for metal ions. For example, in the Flg-A3 fusion peptide, the N-terminal Flg (-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-) includes charged and aromatic residues involved in metal ion complexation, whereas the
A3 peptide (Ala-Tyr-Ser-Ser-Gly-Pro-Ala-Pro-Pro-Met-Pro-Pro-Phe-) binds to gold surfaces. This led to an overall negative charge (pI=3.9) that prevents NPs aggregation by repulsive forces \[^{[3d]}\]. Interestingly, after addition into the solution of peptide-AuNPs, the colorimetric response of each metal ion (Co\(^{2+}\), Hg\(^{2+}\), Pb\(^{2+}\), Pd\(^{4+}\) and Pt\(^{2+}\)) was different and reproducible and occurs within 1 min \[^{[3d]}\] (Fig. 4).

![Colorimetric response of peptide-AuNPs to metal ions. A) Optical microscopy images of peptide-AuNP solutions (10 mM in Au atoms) after exposure to 10mM of different metal salts. B) Corresponding UV/Vis spectra of peptide-AuNPs in the presence of the different metal ions \[^{[3d]}\]. PFNs, peptide-AuNPs without metal ions.](image)

This indicates that peptides are versatile ligands and their complexation with metal ions is driven through interaction of the peptide backbone with their amino acid side chains. Modifications in the peptide sequences could affect the metal speciation and coordination geometry \[^{[3a, 3d]}\]. The limitation of the peptide-NP colorimetric sensor is that DNAzyme activity in the presence of NPs solution should be minimal.

4. Detection of small molecules: cysteine, dopamine

This section focuses on small molecules such as analytes and colorimetric substrates (Table 2), particularly cysteine and dopamine, because they have been extensively studied.
<table>
<thead>
<tr>
<th>Nanoparticle (size)</th>
<th>Analyte/s</th>
<th>Sample/s</th>
<th>Functionalization</th>
<th>Analytical performance</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs Monodisperse</td>
<td>Bisphenol-A</td>
<td>Urine and water samples</td>
<td>Aptamer–AuNPs</td>
<td>LOD: 0.01 pg mL(^{-1}) LDR: 10 000 to 0.1 pg mL(^{-1})</td>
<td>[4ab]</td>
</tr>
<tr>
<td>AuNPs (40–50 nm) Monodisperse</td>
<td>Concanavalin A</td>
<td>Lectin (jack beans)</td>
<td>Thioglucose-AuNPs</td>
<td>LOD: 9 nM LDR: 10–100 nM (R(^2) = 0.983)</td>
<td>[25]</td>
</tr>
<tr>
<td>AuNPs (12.33 nm) Monodisperse</td>
<td>Maltose, mannose, glucose, lactose and D-mannopentaose</td>
<td>Carbohydrate-AuNPs</td>
<td>Carbohydrate–protein interactions</td>
<td>NR</td>
<td>[26]</td>
</tr>
<tr>
<td>AuNPs (15, 20, and 2.5 nm) Monodisperse</td>
<td>Cysteine, glutathione and glutathione disulfide</td>
<td>Blood/serum</td>
<td>Citrate-AuNPs: 15 nm CTAB-AuNPs: 20 nm NaBH(_4)-coated AuNPs: 2.5 nm</td>
<td>LDR: cysteine, NR LDR: glutathione: 10–100 and 200–800 µM LDR: glutathione disulfide 10–300 and 400–800 µM LOD: cysteine &lt;0.5 LOD: glutathione 10 µM LOD: glutathione disulfide 10 µM</td>
<td>[11b]</td>
</tr>
<tr>
<td>AuNPs (~13 nm) Monodisperse</td>
<td>Dopamine</td>
<td>Interferent: ascorbic acid</td>
<td>AuNPs induced by copper ions</td>
<td>LOD: 30 nM LDR 1: 3.3 nM to 100 nM LDR 2: 0.1 µM to 4.5 µM</td>
<td>[4i]</td>
</tr>
<tr>
<td>AuNPs (15 nm) Monodisperse</td>
<td>Dopamine (DA)</td>
<td>Spiked serum</td>
<td>DA inhibits melamine-induced AuNP aggregation</td>
<td>LOD: 33 nM LDR: 33 nM to 3.33 nM</td>
<td>[12]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Monodisperse</td>
<td>Dopamine</td>
<td>Common interferents, such as 3,4-dihydroxyphenylalanine (DOPA), catechol, 3,4-dihydroxyphenylactic acid (DOPAC), homovanillic acid (HVA), epinephrine (EP) and ascorbic acid (AA).</td>
<td>The aptamer conformational change could facilitate salt-induced AuNP aggregation 58-mer dopamine-binding aptamer (DBA)</td>
<td>LOD: 360 nM LDR: 0.54 -5.4 µM</td>
<td>[27]</td>
</tr>
<tr>
<td>AuNPs ±15 nm Multi-dispersed</td>
<td>Dopamine (DA)</td>
<td>Human urine, human serum</td>
<td>DA-induced aggregation of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazol (AHMT) –AuNPs through hydrogen-bonding interactions</td>
<td>LOD: 70 nM LDR: 0.2–1.1 μM</td>
<td>[28]</td>
</tr>
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</tr>
<tr>
<td>AuNPs Monodisperse</td>
<td>Dopamine (DA)</td>
<td>Human serum</td>
<td>DA colorimetric sensing based on AuNP aggregation induced by copper ions</td>
<td>LOD: 200 nM LDR: 0.5–10 μM</td>
<td>[29]</td>
</tr>
<tr>
<td>AgNPs (NR) Monodisperse</td>
<td>Coraline</td>
<td>Selectivity test against intercalating ligands, ethidium bromide (EB) and daunomycin (DM)</td>
<td>Homoadenine-AgNPs, label-free colorimetric detection of small molecules using DNA oligonucleotides and AgNPs</td>
<td>LOD: 0.25 coralyne molecules/adenine base</td>
<td>[40]</td>
</tr>
<tr>
<td>AgNPs (5–15 nm) Multi-disperse</td>
<td>4-nitroaniline (4-NA)</td>
<td>self-assembled AgNPs on DNA</td>
<td>4-NA reduction to para-phenylenediamine</td>
<td></td>
<td>[20]</td>
</tr>
<tr>
<td>AgNPs (12 ± 2 nm) Multi-disperse</td>
<td>Adenine, guanine, cytosine, thymine</td>
<td>NR</td>
<td>Strength of interactions between the fundamental chemical components of DNA and AgNP surfaces</td>
<td>NR</td>
<td>[13]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Multi-disperse</td>
<td>Ampicillin ssDNA aptamer-AuNPs</td>
<td>Colorimetric assay of ampicillin using specific aptamers</td>
<td>LOD: 5 ng mL⁻¹</td>
<td></td>
<td>[41]</td>
</tr>
<tr>
<td>AuNPs Oxetetracycline</td>
<td>Tetracyclines (TCs) as counter targets</td>
<td>Ultrasensitive colorimetric detection of oxytetracycline using shortened aptamer</td>
<td>LOD: 0.1 nM</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Oxetetracycline</td>
<td>Aptamer-AuNPs</td>
<td>Aptamer-specific colorimetric assay</td>
<td>LOD: 25 nM LDR: 25 nM to 1 μM</td>
<td></td>
<td>[48]</td>
</tr>
<tr>
<td>AuNPs (18 nm) Monodisperse</td>
<td>Bisphenol a Water samples</td>
<td>AuNP aggregation by competitive binding of bisphenol A and aptamer</td>
<td>LOD: 0.1 ng mL⁻¹</td>
<td></td>
<td>[45]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Multi-disperse</td>
<td>Ochratoxin A Standards</td>
<td>Aggregation occurs as random coil structures to compact rigid antiparallel G-quadruplexes</td>
<td>LDR: 20 to 625 nM LOD: 20 nM</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Monodisperse</td>
<td>Cysteine Amino acids, glutathione, thioglycolic acid and mercaptoethyl alcohol</td>
<td>2:1 cysteine/Cu²⁺ complex</td>
<td>LOD: 10 nM</td>
<td></td>
<td>[13e]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Monodisperse</td>
<td>Ascorbic acid Fruit juices</td>
<td>Alkyne–azide click reaction</td>
<td>LOD: 3.0 nM</td>
<td></td>
<td>[44]</td>
</tr>
<tr>
<td>AuNPs (NR)</td>
<td>Cysteine</td>
<td>19 amino acids</td>
<td>ssDNA-AuNPs</td>
<td>LOD: 100 nM</td>
<td>LDR: 0.1 to 5 μM</td>
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</tr>
<tr>
<td>AuNPs (20.1±1.8 nm)</td>
<td>Influenza B/Victoria, influenza B/Yamagata</td>
<td>Virus dilution (hemagglutination assay titer, 512)</td>
<td>Sialic acid-AuNPs</td>
<td>LOD: 0.09 vol%. upper limit of linearity 2.5 vol%</td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Tryptophan enantiomers</td>
<td>D/L enantiomers</td>
<td>AuNPs</td>
<td>LOD: 0.1 μM</td>
<td></td>
</tr>
<tr>
<td>AuNPs (~11.89 nm)</td>
<td>Arginine, histidine, lysine</td>
<td>Urine samples</td>
<td>Quercetin-AuNPs</td>
<td>LOD: 0.04, 0.03, and 0.02 μM. LDR: 2.5–1,250 μM (Arg) and 1–1,000 μM (His and Lys)</td>
<td></td>
</tr>
<tr>
<td>AuNPs-I</td>
<td>Melamine</td>
<td>Pre-treated milk</td>
<td>Citrate-AuNPs</td>
<td>AuNPs-I (2.37 x10^-8 M) AuNPs-II (3.3 x 10^-8 M) AuNPs-III (8.9 x 10^-8 M)</td>
<td></td>
</tr>
<tr>
<td>AgNPs (NR)</td>
<td>Cysteine</td>
<td>Various metals</td>
<td>2:1 cysteine/Ca^{2+} complex</td>
<td>LOD: 83 nM</td>
<td>LDR: 0.25 – 10 μM</td>
</tr>
<tr>
<td>AgNPs (10–15 nm)</td>
<td>Cysteine</td>
<td>10 mM of nine amino acids</td>
<td>2:1 cysteine/Cr^{3+} complex</td>
<td>LOD: 1 nM</td>
<td></td>
</tr>
<tr>
<td>AgNPs (10-20 nm)</td>
<td>Dopamine, L-DOPA, noradrenaline adrenaline</td>
<td>Tyrosinase</td>
<td>AgNPs</td>
<td>LOD: dopamine, L-DOPA and noradrenaline 2.5 μM adrenaline 20 μM tyrosinase activity=10 units (~100 μg mL^{-1})</td>
<td></td>
</tr>
<tr>
<td>AuNPs (~ 13 nm)</td>
<td>Kanamycin</td>
<td>Other antibiotics: streptomycin, sulfadimethoxine and ampicillin</td>
<td>ssDNA aptamer-AuNPs</td>
<td>LOD: 10 nM</td>
<td></td>
</tr>
<tr>
<td>AuNPs (~ 13 nm)</td>
<td>Sulfadimethoxine (SDM)</td>
<td>NR</td>
<td>AuNPs</td>
<td>LOD: 50 ng mL^{-1} to 1.0 μg mL^{-1} LOD: 50 ng mL^{-1}</td>
<td></td>
</tr>
<tr>
<td>AuNPs (~15 nm)</td>
<td>Penicillin G</td>
<td>Different penicillins</td>
<td>CTAB-AuNPs</td>
<td>LOD: 0.007 mg mL^{-1}</td>
<td></td>
</tr>
<tr>
<td>Multi-disperse</td>
<td>Mono-disperse</td>
<td>Cysteine</td>
<td>19 essential amino acids</td>
<td>DNA-AuNPs</td>
<td>LOD: 100 nM</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
<td>----------</td>
<td>--------------------------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>AuNPs (20 nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Adenosine</td>
<td>inosine, guanosine, and cytosine</td>
<td>aptamer-OD-AuNPs</td>
<td>LOD: 10 µM</td>
<td></td>
</tr>
<tr>
<td>Multi-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Adenosine and caffeine</td>
<td>Other nucleosides</td>
<td>Aptamers</td>
<td>LOD: 0.3 mM</td>
<td></td>
</tr>
<tr>
<td>Mono-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (15 nm)</td>
<td>Digitoxin</td>
<td>Rat serum</td>
<td>AuNPs</td>
<td>LOD: 571 pM</td>
<td></td>
</tr>
<tr>
<td>Mono-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (10.8-13.1 nm)</td>
<td>Cysteine</td>
<td>Interferents: Na⁺, Cu²⁺, Cl⁻ and urea</td>
<td>AuNPs</td>
<td>LDR: 0.1 to 0.6 ppm</td>
<td>LOD: 0.01 ppm</td>
</tr>
<tr>
<td>Multi-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNPs (5 – 20 nm)</td>
<td>Cysteine/cystine</td>
<td>Other amino acids</td>
<td>AgNPs</td>
<td>LDR: 25–250 µM</td>
<td>LOD: 2.5 ppm</td>
</tr>
<tr>
<td>Multi-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNPs (8 nm)</td>
<td>Cysteine and homocysteine</td>
<td>Human urine and plasma samples</td>
<td>Non-ionic fluorosurfactant-AuNPs,</td>
<td>LOD: 0.4 µM.</td>
<td></td>
</tr>
<tr>
<td>Mono-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNPs (8±1.6nm)</td>
<td>Cysteine</td>
<td>Human urine and plasma samples</td>
<td>Fluorosurfactant-AgNPs</td>
<td>LOD: 0.05 µM.</td>
<td>LDR: 1.5–6.0 µM</td>
</tr>
<tr>
<td>Multi-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNPs (9.5±2.0 nm)</td>
<td>Cysteine</td>
<td>Serum and artificial cerebrospinal fluid</td>
<td>AgNPs and Ca²⁺</td>
<td>LDR: 0.1–1000 µM</td>
<td>LOD: 0.1 µM</td>
</tr>
<tr>
<td>Multi-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (20.0±1.4) nm</td>
<td>Cysteine</td>
<td>Human urine</td>
<td>Pectinase-protected AuNPs</td>
<td>LDR: 4.85x10⁻⁸ to 302 µM and 3.25 to 0.103 mM</td>
<td>LOD: 4.6x10⁻⁹ M.</td>
</tr>
<tr>
<td>Multi-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Cysteine</td>
<td>Brain microdialysate (sample), lactate, ascorbic acid and glucose (interferents)</td>
<td>Cysteine-AuNPs</td>
<td>LDR: 0.166 to 1.67 µM</td>
<td>LOD: 0.1 µM</td>
</tr>
<tr>
<td>Mono-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Pyruvic acid</td>
<td>Interferents: lactic acid (LA), ascorbic acid (AA) and glucose.</td>
<td>AuNPs</td>
<td>LDR: 5.6 µM to 168.0 µM</td>
<td>LOD: 3.0 µM</td>
</tr>
<tr>
<td>Mono-disperse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm)</td>
<td>Sulfadimethoxine (SDM), kanamycin (KAN) and adenosine</td>
<td>Mixture of KAN, SDM and ADE</td>
<td>Aptamer of KAN, SDM and ADE (1:1:1 mixture).</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>AuNPs (13 nm) Monodisperse</td>
<td>17β-estradiol</td>
<td>Interferents: methanol, diethylstilbestrol, bisphenol A, 19-nortestosterone, estrol, estrone</td>
<td>AuNPs</td>
<td>LOD: 0.1 ng mL⁻¹</td>
<td>[4k]</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
<td>-----------------------------------------------------------------</td>
<td>-------</td>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td>AuNPs (20 nm) Monodisperse</td>
<td>Caffeine</td>
<td>ATP and target-doped blood serum</td>
<td>AuNPs</td>
<td>LOD: 1.25 µM</td>
<td>[4k]</td>
</tr>
<tr>
<td>AuNPs (13 nm) Monodisperse</td>
<td>Parathion</td>
<td>Sea and tap water</td>
<td>Parathion inhibits AChE-induced aggregation of AuNPs</td>
<td>LDR: 15 to 65 ppb and 140 to 1000 ppb LOD: 0.7 ppb (2.4 nM)</td>
<td>[4a4l]</td>
</tr>
</tbody>
</table>

CTAB: cetyl trimethyl ammonium bromide; OD: oligonucleotides; SDM: sulfadimethoxine; KAN: kanamycin; ADE: adenosine; AChE: acetylcholinesterase, Cyt C: cytochrome c.; LDR: linear dynamic range; LOD: limit of detection.
Cysteine has been employed as a common strategy for the detection of metals, but it also can be quantified by colorimetric assays. Metal ions are used as cross-linking agents for cysteine-AuNP or –AgNP pairs to induce NP aggregation and the consequent red to blue color change of the NP solution is observed. The degree of aggregation depends on the cysteine concentration and the average AuNPs diameter in the presence of different cysteine concentrations. In such colorimetric assays, organic molecules bind to the Au/AgNPs surface via their amine (−NH₂) (blue) or thiol (−SH) (yellow) terminal groups (Fig 5).

(A)

(B) (C)
Fig. 5A) Chemisorption model for cysteine and dopamine on the AuNP surface. The solution color changes from red to blue in the presence of cysteine or dopamine. B and C shows linear relationship of nonionic fluorosurfactant-capped gold nanoparticles versus Cys and Hcy 650 nm. The linear range for Cys was 0.5–4.5 μM, and for Hey was 1.0–4.0 μM. Conditions: pH 6.5, 100 mM NaCl, 30 s incubation period at 50 °C

Ligands with the C₆H₃O₆ formula, without −NH₂ or −SH functional groups, preferably bind to the AuNPs surface, and are highly unlikely to induce AuNPs aggregation [4i, 4u, 32].

The thiol groups of cysteine interact with the surface of colloidal AuNPs or AgNPs via chemisorption-type interactions; however, cysteine can complex with metal ions, such as Pb²⁺, Zn²⁺, Cu²⁺ [4i, 11c], Ca²⁺ [3c, 4t], Cr³⁺ [3b], at a ratio of two cysteines for each metal ion [10, 11c]. In the presence of metal ions, cysteines can induce AgNP and AuNP aggregation with a color change from yellow to purple and blue to red, respectively [3b, 3c, 4u, 11c]. These assays are based on the NP distance-dependent optical properties after coordination, as confirmed by the change in the zeta potential from -30.7 mV for pure AgNPs to -19.63 mV after interaction with cysteine and Cr³⁺ [3b]. Specifically, Hajizadeh et al. reported that cysteine can rapidly induce AgNP aggregation (yellow-to-red color change) in the presence of Ca²⁺ and 10 mM NaCl, leading to a decrease in electrostatic repulsion and faster aggregation [3c]. Cysteine concentration can be determined also by using AuNPs and a UV-Vis spectrometer with a LOD of 10 nM (1.2 ng ml⁻¹) [11c]. The ratio between absorption at 524 and absorption at 396 nm (A₅₂₄/A₃₉₆) is linear with a cysteine concentration range from 0.25 to 10 mM (R² = 0.993) with a LOD of 83 nM [3c]. Jongjinakool and co-workers detected cysteine in a concentration range from 0.1 to 0.6 ppm with a LOD of 0.01 ppm [4u]. In Figure 5b-c, a multi-component mixtures demonstrated that cysteine and homocysteine were identified based on the different SPR wavelengths induced by aggregation of non-ionic fluoro-surfactant-functionalized AuNPs upon addition of a mixture of the amino acids. The absorbance changes due to AuNPs aggregation induced by cysteine and homocysteine increase the individual absorbance values (LDR from 0.5 to 4.5 μM for cysteine and LOD (S/N=3) of 0.4 μM for homocysteine) [4o].

When dopamine (DA) and Cu²⁺ solutions are mixed, the amine group directly coordinates with Cu²⁺ without nitrogen atoms bonded to the gold surface [4i]. The LOD for DA is 30 nM with, differently from cysteine-based methods, a linear calibration curve for two concentration ranges (3.3×10⁻⁸ to 1.0×10⁻⁷ M and 3.0×10⁻⁷ to 4.5×10⁻⁶ M) with correlation coefficients of 0.9981 and 0.9979, respectively [4i]. Likewise, addition of 5 mM Cu²⁺ improves
the colorimetric probe to a LOD of 200 nM \[^{29}\]. AuNPs also can be used for the quantitative colorimetric detection of neurotransmitters that mediate the generation and growth of AuNPs, with a LOD of 2.5 µM for dopamine, L-DOPA and noradrenaline, and of 20 µM for adrenaline \[^{4b}\]. The metal ion-Au/AgNPs interaction is mainly a coulombic interaction and its strength is directly related to the molecular structure and charged groups. Therefore, selectivity can be improved by working on these two parameters \[^{4r}\]. For instance, researchers highlighted the excellent selectivity of AuNP-based colorimetric assays for cysteine compared with other biomolecules, such as thioglycolic acid and mercaptoethyl alcohol \[^{11c}\], glutathione \[^{11b, 11c}\] glutathione disulfide \[^{11b}\], aspartic acid and glutamic acid \[^{23}\]. Moreover, selectivity changes also when using cysteine derivatives, namely glycine, dipeptide Cys-Gly, cysteamine, mercaptopropionic acid, S-protected (S-methyl-L-cysteine), N-protected (N-acetylcysteine) and O-protected cysteine (L-cysteine methylester hydrochloride), as indicated by the color change from yellow to pink and peak broadening \[^{4m}\]. Therefore, it is imperative to investigate the detection of sulfur-containing amino acids compared with other standard amino acids \[^{4m}\]. Chen et al. used 19 naturally occurring amino acids, but they could not improve the colorimetric response of cysteine \[^{4e}\].

Another strategy for cysteine detection is based on the observation that when cysteine is added to AuNPs/ssDNA, the ssDNA molecules that stabilize AuNPs against salt-induced aggregation are displayed spontaneously by cysteine encapsulation on the AuNPs surfaces, via an Au–S bond \[^{4e}\]. The salt-induced aggregations result in a characteristic AuNPs color change from red to blue \[^{4c, 4m}\]. According to Chen et al. \[^{4e}\] this approach is not feasible with other amino acids (Fig 6).
In the presence of cysteine, the ssDNAs is displayed by cysteine on AuNPs surface resulting in AuNP aggregation and in a color change from red to blue upon addition of NaCl. Other amino acids do not lead to a color change due to the absence of thiol groups \[4e\]. (B) Cysteine colorimetric detection using AuNPs probes that contain T-T mismatches complexed with Hg\(^{2+}\): competitive approach in which cysteine can displace Hg\(^{2+}\)\[4d\].

In this system, the A\(_{640}/A_{525}\) ratio is linearly dependent on the cysteine concentration (from 0.1 to 5.0 \(\mu\)M with LOD of 100 nM \[4e\]). Differently from methods that require AuNPs modification, this approach is simple and fast, but it requires specific links between the biomolecules and AuNPs to allow ssDNA displacement from the NPs surface. On the other hand, Mirkin’s group developed a cysteine detection assay where two sets of AuNPs probes are functionalized with different oligonucleotide sequences (probe A: 5’ HS-C10-A10-T-A10 3’; probe B: 5’ HS-C10-T10-T-T10 3’) and rapidly form aggregates upon combination through the thymidine-thymidine (T-T) mismatches complexed with Hg\(^{2+}\) with LODs as low as a 100 nM \[4d\]. Comparison of the two methods (ssDNA-AuNPs and mismatch assay) highlights that they rely on the distance-dependent optical properties of AuNPs, the sharp melting transition of oligonucleotide-AuNPs
aggregates and the very selective coordination of \( \text{Hg}^{2+} \) with cysteine during which the purple-to-red color change occurs \(^{[4d, 4e]}\). However proteins with one free cysteine residue, such as human serum albumin, can spontaneously attach to AuNPs surfaces through Au-S bond formation \(^{[2]}\). These studies demonstrate that the presence of negatively-charged carboxyl groups in the cross-linkers remains essential to induce AuNPs aggregation through ion pair interactions between amino groups present in cysteines and carboxyl groups in the cross-linkers \(^{[4m, 23]}\). On comparison of the dopamine- and cysteine-based methods it was evident that functional groups present on the surface also plays a key role in metal detection.
4.1. Recognition of chiral molecules

Stereochemistry plays a central role in molecular recognition and interactions. Indeed, the molecule’s chemical and biological properties depend not only on the nature of their constituent atoms, but also on their position in space \[^{32b}\]. Currently, chiral molecules are mostly separated with techniques like capillary electrophoresis, high-performance liquid and gas chromatography. Nanoparticles allow for the easy detection of chiral molecules by the naked eye. For instance, the color of the AuNPs solution changes from red to blue in the presence of D-tryptophan (LOD of 0.1 µM and LDR of 0.2–10 µM), but not of L-tryptophan \[^{4y}\]. Interestingly, AuNPs can selectively adsorb D-tryptophan, and therefore, L-tryptophan molecules can easily be separated by simple centrifugation of the tryptophan/AuNP solution \[^{4y}\]. The infrared spectra confirms the D-tryptophan absorption to AuNPs by disappearance of the NH (NH\(^{3+}\)) stretching absorption peaks (3078 and 3038 cm\(^{-1}\)), leaving the carboxylic group (-COOH) and nitrogen atom of the indole ring free for further coordination. This distinctive feature allows for binding of one Cu\(^{2+}\) ion to two tryptophan molecules by coordination with the COOH and nitrogen atom of the indole ring, hence allowing for recognition \[^{4y}\]. Another reported visual differentiation is between the D- and L-forms of mandelic acid (MA) was based on their chirality towards 13 nm l-tartaric acid-capped AuNPs. The L-MA solution changes the red colour of l-TA-capped AuNPs to a bare-eye observable blue, while d-MA does not trigger any color changes \[^{33}\]. The AgNPs capped with a novel chiral R-mandelic acid-derived calix[4]arene (R-MAC4), for it good optical and structural properties. These self-assembled NPs were used to recognize the N-Fmoc-d/l-aspartic acid (d/l-FAA)\[^{34}\].

4.2. Detection of macromolecules

Nanoparticles can be easily modified by replacing surface-adsorbed weak ligands (e.g., negatively charged citrate ions) with thiolated macromolecules that are difficult to displace due to their strong binding to the surface.

Xue and co-workers described citrate-AuNP-based assays for trypsin and arginine residues screening (Fig. 8). The aggregation of negatively charged citrate-capped AuNPs in the presence of a peptide composed of six arginine residues (Arg\(_6\)) occurred mainly through electrostatic interactions, and led to a red-shift of the usual SPR profile \[^{35}\]. However, when the Arg\(_6\) peptide was hydrolyzed into fragments upon trypsin addition in the solution, the
electrostatic interactions between AuNPs and arginine residues were weakened and, therefore, neither AuNPs aggregation nor SPR shift was observed.

Fig. 7. Colorimetric assay for trypsin by using AuNP crosslinking/aggregation based on trypsin-catalyzed hydrolysis of Arg6 for random dispersion of citrate capped AuNPs. Reproduced with permission from [35].

Moreover, Arg6 hydrolysis catalyzed by trypsin is retarded if trypsin inhibitors are present in the solution. This feature was used to develop a label-free assay for trypsin (LOD: 1.6 ng ml⁻¹) and Arg6 residues screening with AuNPs [35]. Similarly, interaction of citrate-AuNPs with fibrinogen to form fibrinogen–AuNPs through electrostatic and hydrophobic interactions was used for the detection of thrombin (LOD: 0.04 pM and LDR: 0.1–10 pM; R² = 0.96) [71]. Although the mechanism of detection was the same, the molecular interactions was different. Indeed, fibrinogen was adsorbed on NPs before the addition of thrombin [71]. Conversely, trypsin interacts with Arg6 before the NPs addition into the system [35]. Detection of thrombin has been improved by the use of catalytic enlargement as shown in Fig. 9. [5a].
Fig. 8. Amplified thrombin detection on surfaces by catalytic enlargement of thrombin-aptamer-functionalized Au-NPs\textsuperscript{[5a]}. As the concentration of thrombin increases, the surface loading of bound thrombin is higher, resulting in a number of Au NP seeds for enlargement.

Specifically, upon aptamer-AuNPs reaction with thrombin, AuNPs aggregate. But in the presence of other proteins (200 nM BSA or human IgG antibodies), the aptamer-AuNPs does not precipitate implying that the precipitation originates from the specific interaction between aptamer and thrombin (LOD: 20 nM)\textsuperscript{[5a]}.

Similarly, Chen’s group evaluated the possibility of using mannopyranoside-encapsulated AuNPs/concanavalin (Man-AuNPs/Con A) complexes for a competitive colorimetric assay for ten proteins. However, only thyroglobulin, \textit{bandeiraea simplicifolia} lectin I (BS-I), soybean agglutinin (SBA) and \textit{maackia amurensis} (MAL) significantly modified the absorption spectrum of Man-AuNPs/Con A complexes\textsuperscript{[5b]}. In contrast to the method proposed by Xue \textit{et al.}\textsuperscript{[35]}, where upon the introduction of thrombin in the fibrin–AuNP solutions, catalyzes the polymerization of the free and conjugated fibrinogen species to form insoluble fibrillar fibrin–AuNP agglutinates\textsuperscript{[7l]}. Finally, Guarise \textit{et al.} exploited the fact that, compared with the native peptide substrates, protease-cleaved peptides do not induce NPs aggregation (and thus the color of the solution does not change) to detect two proteases (thrombin and lethal factor)\textsuperscript{[36]}.

Quercetin-AuNPs have been used as a colorimetric probe for the detection of amino acids, such as arginine (Arg), histidine (His) and lysine (Lys). Indeed, quercetin-AuNPs aggregation caused by amino acids leads to a color change from red to blue\textsuperscript{[30]}. In optimal
conditions, a linear relationship exists between the absorption ratios at different wavelengths (A\textsubscript{702}/A\textsubscript{525} for Arg, A\textsubscript{693}/A\textsubscript{525} for His, and A\textsubscript{745}/A\textsubscript{525} nm for Lys) and the concentration ranges (from 1.25 to 2.50 μM for Arg; from 1 to 1,00 μM for His and Lys), with LOD values of 0.04, 0.03, and 0.02 μM, respectively, at pH 5.0 \cite{30}. Siddhartha and Debabrata reported that protein estimation is within a LOD of 10-80 μg mL$^{-1}$ using unmodified AgNPs \cite{5b}.

Lately, the sequence-length-dependent adsorption of ssDNA on AuNPs has been investigated for colorimetric nuclease assays and measurement of oxidative DNA damage \cite{6g}. Based on ssDNA adsorption rate on citrate-AuNPs, it can be hypothesized that incubation with AuNPs for a specific period of time can lead to differential adsorption of short and long ssDNA. Consequently, the stability of the ssDNA-AuNPs complex in the presence of salt could be influenced by the ssDNA length \cite{6g}. The confirmation of this hypothesis led to the development of colorimetric assays taking advantage of ssDNA length to improve adsorption. For example, when ssDNA is cleaved by the S1 nuclease or −OH radicals in small fragments, these shorter ssDNA can be rapidly adsorbed on AuNPs and significantly enhance the negative charge density on each AuNP surface for the same time of incubation (Fig. 9).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig9.png}
\caption{Schematic representation of AuNP-based colorimetric strategies for ssDNA cleavage assays. (a) Salt-induced NP aggregation before and after ssDNA cleavage by the S1 nuclease or OH radicals. Reproduced with permission from \cite{6g}. (b) Nuclease activity assay using positively charged AuNPs and polyanionic ssDNA ((+)AuNPs). Reproduced with permission from \cite{7n}.}
\end{figure}

Basically, charging the NPs surface increases the electrostatic repulsion between the ssDNA-AuNPs complexes enough to inhibit NP aggregation at the same salt concentration, and the solution color does not change. Thus, DNA cleavage can be directly visualized by naked eye \cite{6g}. 

\textbf{A}
Charge interaction between positively charged AuNPs and polyanionic ssDNA leads to AuNPs aggregation that can be monitored by the color change from red to blue (Fig. 9) \cite{7n}. However, in the presence of the S1 nuclease, the ssDNA substrate is cleaved into small fragments, and the AuNPs solution remains red. Thus, the nuclease activity can be easily monitored by naked eye or with a simple colorimetric reader \cite{7n}. In this case, the exocyclic amino group of nucleotides is the main cause of nucleotide-dependent aggregation because cysteamine-capped AuNPs are positively-charged at pH 3.6 (with a pKa of 10.75), leading to efficient electrostatic interactions with the negatively charged DNA. These results complement each other because during S1 enzymatic activity, ssDNA is degraded into smaller fragments that cannot induce aggregation \cite{6g,7n}.

Additionally, the non-crosslinking AuNPs aggregation induced by the loss of surface charges also is exploited for enzymatic activity testing and screening for potential inhibitors \cite{7a}. This approach has been extended to the serotype I-specific detection of dengue virus DNA. In the absence of the DNA target solution, peptide nucleic acids (PNA) induce AuNPs aggregation with a red-to-purple color change and the appearance of a second absorbance peak at 650 nm due to the AuNPs surface coating by PNA \cite{37}. Likewise, sialic acid-reduced and -stabilized AuNPs (d=20.17 ± 1.8 nm) can be used for the colorimetric detection of influenza viruses \cite{10}. The discrimination of such molecules can also be standardized using chemometric techniques including hierarchical cluster analysis and principal component analysis. This approach was used to accurately classified and measure the array response of cysteine, glutathione, glutathione disulfide and interferences without any misclassification \cite{38}. 
5. DNA-functionalized nanoparticles (aptasensors)

This is the most fascinating area of NP-based colorimetric assays. Much attention has been focused on aptamers (i.e., ssDNAs and oligopeptides with high binding affinity and selectivity for target molecules) as powerful biological macromolecules (Table 3). Particularly, the aptamer advantages compared with antibodies, such as possibility of chemical synthesis and modification and lower immunogenic response, contribute to their potential. They are generally selected in vitro by using the systematic evolution of ligands by exponential enrichment (SELEX) technique and random-sequence nucleic acid libraries. This allows selecting the highest binding aptamer(s). Then, aptamers can be capped with thiol groups that bind to two AuNPs. The principle of colorimetric sensing based on aptamers to detect specific DNA sequences was introduced about a decade ago and is now a key tool in biodiagnostics. Since then, other aptamer-based sensors have been developed for the detection of metal ions, small molecules and proteins.

**Table 3: DNA/protein sensing based on AuNP aggregation**

<table>
<thead>
<tr>
<th>Nanoparticle (size)</th>
<th>Analyte/Target</th>
<th>Functionalization</th>
<th>Analytical application</th>
<th>Analytical performance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs (13 nm) and AgNPs (10-20 nm) Monodisperse</td>
<td>DNA3 complementary DNA</td>
<td>DNA1-AuNPs DNA2-AgNPs</td>
<td>Complementary DNA recognition</td>
<td>NR</td>
<td>[16]</td>
</tr>
<tr>
<td>AuNPs (12.33 nm) Monodisperse</td>
<td>Maltose &gt; mannose &gt; glucose &gt; lactose &gt; MAN5.</td>
<td>Carbohydrates-AuNPs</td>
<td>carbohydrate–lectin interactions</td>
<td>NR</td>
<td>[26]</td>
</tr>
<tr>
<td>AuNPs (13-nm) Monodisperse</td>
<td>Lysozyme</td>
<td>HSA-AuNPs</td>
<td>Colorimetric detection</td>
<td>LOD: 50 nM.</td>
<td>[7]</td>
</tr>
<tr>
<td>AgNPs (10-15 nm) Monodisperse</td>
<td>Globular proteins (BSA and IgG)</td>
<td>AgNPs</td>
<td>Concentration-dependent particle agglutination</td>
<td>LOD: 10 to 80 μg mL⁻¹</td>
<td>[18]</td>
</tr>
<tr>
<td>AuNPs and AgNPs Monodisperse</td>
<td>Specific DNA sequence</td>
<td>AuNPs and AgNPs</td>
<td>Detection of DNA through nucleic acids (PNA) hybridization</td>
<td>LOD: DNA/PNA ratio of 0.05</td>
<td>[6]</td>
</tr>
<tr>
<td>AuNPs (50 nm) Multidisperse</td>
<td>Con A</td>
<td>p-Aminophenyl-D-mannose–AuNPs</td>
<td>Carbohydrate–lectin system</td>
<td>LOD: 9.0 nM ($R^2 = 0.983$) LDR: 10-100 nM</td>
<td>[25]</td>
</tr>
<tr>
<td>AuNPs Multidisperse</td>
<td>PDGFs and PDGFR</td>
<td>Apt-AuNPs</td>
<td>Protein analysis and cancer diagnosis</td>
<td>-2.5-10 and 10-20 nM, respectively, for 0.42 nM Apt-</td>
<td>[5c]</td>
</tr>
<tr>
<td>AuNPs (32 nm) Monodisperse</td>
<td>AuNPs (13 nm) Monodisperse</td>
<td>AuNPs (20 nm) Monodisperse</td>
<td>AuNPs (15 nm) Monodisperse</td>
<td>AgNPs (31 nm) Multidisperse</td>
<td>AuNPs (13 nm) Monodisperse</td>
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<tr>
<td>Con A binding partners</td>
<td>AuNPs capped with 3ε- and 5ε- (alkanethiol) oligonucleotides</td>
<td>AuNPs</td>
<td>Bla molecules</td>
<td>DNA-AgNPs</td>
<td>Single-stranded DNA cleavage</td>
</tr>
<tr>
<td>Man-AuNPs</td>
<td>Hybridization of the target with the probes</td>
<td>Sensing phosphatase activity of alkaline phosphatase</td>
<td></td>
<td></td>
<td>Enzymatic cleavage and oxidative damage of single-stranded DNA</td>
</tr>
<tr>
<td>Competitive colorimetric assay for ConA binding partners through protein-protein interactions</td>
<td>54: 3.4 nM of peptide : alkaline phosphate</td>
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<tr>
<td>NR</td>
<td>60 pM</td>
<td>NR</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>NR</td>
<td>10 nM</td>
<td>LOD: 10 nM</td>
<td></td>
<td>LDR: 0 to 167 nM</td>
<td>LOD: 0.83 nM</td>
</tr>
<tr>
<td>NR</td>
<td>0.83 nM</td>
<td></td>
<td></td>
<td></td>
<td>LOD: 10 nM</td>
</tr>
<tr>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
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NR: Not Reported
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<tr>
<th>AuNPs (13.3±1.2 nm)</th>
<th>Thrombin</th>
<th>Fibrinogen-AuNPs (56 nm)</th>
<th>Colorimetric assay for blood plasma</th>
<th>LOD: 0.04 pM</th>
<th>LDR: 0.1 to 10 pM</th>
<th>[33]</th>
</tr>
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<tr>
<td>AgNPs (NR)</td>
<td>CIAP and PKA</td>
<td>AgNPs</td>
<td>Adenosine phosphorylation and dephosphorylation</td>
<td>LOD: CIAP: 1.0 unit mL(^{-1}) PKA: 0.022 unit mL(^{-1})</td>
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<td>AuNPs (~ 12.74 nm)</td>
<td>staphylococcal enterotoxin B</td>
<td>AuNPs</td>
<td>Colorimetric assay based on aggregation in the absence of the aptamer</td>
<td>LDR: 10 to 50 ng mL(^{-1}) LOD : 10 ng mL(^{-1})</td>
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<td>AuNPs (NR)</td>
<td>Aminopeptidase N</td>
<td>Gold nano-composites conjugated with a thermo-responsive copolymer</td>
<td>Activity based on inhibition of the disassembly of Gold nano-composites</td>
<td>AR: 20 to 50 U L(^{-1})</td>
<td>[43]</td>
<td></td>
</tr>
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<td>AuNPs (15.1 nm)</td>
<td>β-galactosidase and β-glucosidase</td>
<td>Gal-Lip-AuNPs and Glc-Lip-AuNPs</td>
<td>Glycosidase inhibitor screening</td>
<td>LODs: β-galactosidase 9.2 nM and β-glucosidase 22.3 nM at 20°C</td>
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</tr>
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<td>AuNPs (26 nm)</td>
<td>ADA</td>
<td>AuNPs</td>
<td>Nucleotide-dependent aggregation</td>
<td>LOD: 0.8227 U L(^{-1})</td>
<td>[31]</td>
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<td>AuNPs (13 nm)</td>
<td>Endonuclease</td>
<td>DNA-AuNPs with duplex interconnection</td>
<td>Endonuclease activity and inhibition</td>
<td>NR</td>
<td>[72]</td>
<td></td>
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<td>AuNPs (13 nm)</td>
<td>Biotin (biotinylation)</td>
<td>Peptide-AuNPs and avidin-AuNPs</td>
<td>Kinase-catalyzed substrate biotinylation</td>
<td>NR</td>
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<td>AuNPs (20 nm)</td>
<td>Protein kinase A,</td>
<td>AuNPs</td>
<td>Kinase activity based on the coagulating ability of a cationic substrate peptide and its phosphorylation</td>
<td>NR</td>
<td>[76]</td>
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<td>AuNPs (NR)</td>
<td>Acetylcholinesterase</td>
<td>Citrate-AuNPs</td>
<td>AChE-catalyzed hydrolysis of acetylthiocholine</td>
<td>0.6 mU mL(^{-1})</td>
<td>[45]</td>
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<td>AuNPs (16 nm)</td>
<td>Con A</td>
<td>Mannose-AuNPs Mannose-AgNPs</td>
<td>Con A induced aggregation</td>
<td>Mannose-AuNPs LOD: 0.04 µM LDR: 0.04-0.10 µM Mannose-AgNPs: LDR: 0.08 – 0.26 µM LOD: 0.1µM</td>
<td>[2]</td>
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</tbody>
</table>
| AuNPs (~15 nm) | Kaposi’s sarcoma associated herpesvirus and Bartonella DNA | Oligonucleotides-AuNPs | Aggregation reaction with multi-color changes | LOD: 1 nM | [15]
| AuNPs (20 nm) | Monodisperse | AgNPs (20 nm) | Oligonucleotides-AgNPs | | |
| AgNPs (10-15 nm) | Multidisperse | BSA and immunoglobulins | AgNPs | Nanoparticle agglutination | LOD: 10 -80 μg mL⁻¹ | [50]
| AuNPs (21 nm) | Monodisperse | polyA, polyC, polyU, polyI, BSA, lysozyme, dsDNA, ssDNA | AuNPs | Aggregation due to self-assembly (discrimination and detection) | LOD: Protein ~100 pM, Homopolynucleotide ~10 pM | [24]
| AuNPs (13.2 nm) | Monodisperse | Dengue virus | AuNPs | PNA/DNA hybridization | LOD: 0.12 μM | [37]
| AuNPs (~13 nm) | Monodisperse | ssDNA and dsDNA | AuNPs | DNA sequences based on electrostatic interactions | AR: 100 fmol | [64]
| AuNPs (13 nm) | Monodisperse | Abrin | Catalytic AuNPs | Peroxidase-like activity | LDR: 0.2 to 17.5 nM, LOD: 0.05 nM | [48]
| AuNPs (13 nm) | Monodisperse | Lipase | Tween 20-GNPs | Enzyme-regulated AuNP aggregation | LOD: 0.028 mg mL⁻¹, LDR: 0.15 to 1.80 mg mL⁻¹ | [74]
| AuNPs (50 nm) | Multidisperse | Native proteins | DNA-AuNPs | 50% human urine | Cluster analysis | [93]

NR: Not Reported; CIAP: Calf Intestine Alkaline Phosphatase; HSA-AuNPs: Human serum albumin-modified gold nanoparticles, PKA: Protein Kinase A; SEB: Staphylococcal enterotoxin B; LOD: Limit of detection; AR: Activity Range; PDGFs: Platelet-derived growth factors; PDGFR: platelet-derived growth factor receptors, Con A: Concanavalin A; Man-AuNPs: Mannopyranoside-encapsulated gold nanoparticles; Gal: β-galactosidase and Glc: β-glucosidase; ADA: Adenosine Deaminase; AChE: Acetylcholinesterase, KSHV: Kaposi’s sarcoma associated herpesvirus; BePI: benzo[e]pyridoindole; CORA: coralyne, BSA: Bovine serum albumin; PNA: peptide nucleic acid; LDR: linear dynamic range; LOD: limit of detection.

The development of DNA-aptamer-based colorimetric assays by Mirkin’s group was inspired by the fact that the steps necessary for NPs+ modification with ligands can be tedious or time-consuming and relatively expensive⁶⁶,¹⁸,⁴⁷. NPs stabilized by ssDNA aptamers do not aggregate with the addition of salt only³³,⁴⁴. Conversely, in the presence of the target/analyte, the aptamer is folded because it binds to the target while desorbing from the NPs surface, which leads to NPs aggregation and colorimetric changes. Ideally, folded aptamers or dsDNAs should hardly adsorb onto the NPs. This is related to the higher structure rigidity and high proton density inside
dsDNA \(^{3e, 4g, 4x, 6e}\). Indeed, ssDNAs cannot hybridize with each other to form dsDNAs. Thus, their strategy is based on the observation that unmodified AuNPs and AgNPs can differentiate between ssDNA and dsDNA, mainly due to the higher structure rigidity of the latter \(^{3e, 4n, 6e}\). However, not only the strands but also their lengths contribute to NP stabilization. For instance, Chen and co-workers tested ssDNAs of different lengths (18nt: 5′ TAG AAT ACT CCC CCAGGT 3′; 24nt: 5′ GGT TGG TCA GAT TCA GTG GGT TAG 3′, and 30nt: 5′ AAA CCC CCC TGC TAAAAC CCC AAA CCC 3′) for AuNP stabilization and consequently for detection and sensitivity. They found that the longer ssDNAs have a better stabilization effect because at the same molar concentration, longer ssDNAs have more monomeric deoxynucleotide units. Moreover, it is difficult for cysteine to replace highly stable DNA-protected AuNPs \(^{4e}\). DNA bases possess higher affinity towards gold than silver via coordination between Au and nitrogen atoms (thus favoring DNA adsorption). However, the negatively charged surfaces of AuNPs electrostatically repel DNA phosphate backbones (reducing DNA adsorption) \(^{3e}\). The key challenge to their successful application is in transforming the aptamer-binding events into physically detectable signals \(^{4c}\).

The development of the nanotechnology for NPs functionalization with DNA and the biotechnology for the \textit{in vitro} selection of target-specific nucleic acids offer a unique opportunity for designing colorimetric biosensors \(^{7c, 17a}\). The four types of DNA aptamer-based colorimetric approaches are highlighted below:

\textbf{5.1. TYPE I: Aptamers adsorbed on nanoparticles}

Type I aptasensors includes two common steps: i) adsorption of the DNA unit onto the NPs surface and ii) recognition of the target molecule by the DNA strands while serving as an optical sensing element. DNA adsorption on NPs surface is favored by the high charge density and stability provided by the aptamer \(^{4f}\) (Fig. 10). This is a crucial step because the selectivity of the targeting molecule must be retained during adsorption onto NPs, especially when the aptamer is designed for qualitative assays \(^{4f}\). The aptamer conjugation constant is stronger than that of antibodies and that of non-specific adsorption between aptamer and NPs \(^{4a}\). With these conditions in mind, Xu and co-workers used unmodified DNA and AgNPs to detect ligands binding to homoadenine, by monitoring the color change from yellow to brown due to AgNP aggregation after salt addition. When coralyne binds to the homoadenine sequence in the aptamer, the aptamer is removed from the AgNP surface and AgNP can aggregate \(^{4f}\).
A$_{550}$/A$_{397}$ ratio shows a good linear correlation with coralyne concentrations between 0.0 and 10 mM with a LOD of 0.3 mM $^{[4f]}$. Using a similar strategy, DNA-AuNPs/AgNPs were used to detect several targets, such as bisphenol A (LOD: 0.1 ng mL$^{-1}$ $^{[4q]}$ and LOD: 0.01 pg mL$^{-1}$ $^{[4ab]}$), digoxin (LOD: 571 pM $^{[4aa]}$), oxytetracycline (OTC) (LOD: 25 nM) $^{[4g]}$, thrombin, (LOD: 0.83 nM) $^{[5g]}$, kanamycin (LOD: 25 nM) $^{[4j]}$, OTC (LOD: 0.1 nM), ampicillin (LOD: 5 ng mL$^{-1}$) $^{[4l]}$, staphylococcal enterotoxin B (LOD: 10 ng mL$^{-1}$) $^{[4p]}$, and pyruvic acid (LOD: 3.0 μM) $^{[4w]}$.

**Fig. 10.** Schematic representation of Type I colorimetric aptasensors for detection (A) small $^{[4q, 4aa]}$ and (b) large molecular targets $^{[5g]}$.

It is acknowledged that for many type I aptamers, addition of the target/ligand induces the aptamer release from the NP surface and consequently the color change as the salt tolerance decreased by NPs $^{[4f, 4q, 4aa, 4l]}$. Two groups reported an intriguing observation using a similar approach $^{[4c, 49]}$. When aptamers are added to NPs, their interaction via hybridization leads to NP aggregation and consequently to the color change from red to blue. Addition of the target molecule (e.g., adenosine) to the aptamer-NP solution induces a dramatic conformational change.
of the aptamer structure that leads to the dissociation of the NP network and to a new color change (blue to red) \[4c, 49\].

AuNPs have also been used by Dong’s group \[5g\] to understand the conformational changes of thrombin-binding aptamers (TBA) when they are removed from the AuNP colloidal solution in the presence or not of thrombin. Addition of 100 mL of 0.5 M NaCl causes a quick color change (red to purple) in the solution with thrombin, but not in that with only TBA. Due to the color change, the TBA conformation modification from unfolded to G-quadruplex/duplex formation could be directly monitored by naked eye, thus allowing the easy detection of thrombin \[5g\]. Likewise, the addition of enough salt could be used to inhibit the repulsion between unmodified negatively charged AuNPs and result in their aggregation and in the corresponding red-to-blue color change. As previously reported, there is stronger coordination interaction between the nitrogen atoms of unfolded ssDNA and AuNPs than electrostatic repulsion between the negatively charged phosphate backbone and the negatively charged AuNPs \[5g\]. Conversely, the relatively rigid structure of dsDNA or folded ssDNA (e.g., G-quadruplexes) prevents the exposure of the DNA bases to AuNPs and the high density of negative charges increases the repulsion between DNA and AuNPs. However, aggregation of DNA-functionalized AuNPs can be induced also by hybridization of target DNA that does not cross-link the NPs. A conceivable disadvantage of this non-crosslinking system, compared with the crosslinking system, is the consumption of target DNA \[48\].

Recently, a viable approach to overcome the limitations of type I aptamers due to the DNA length was reported. Briefly, the design of shortened aptamers is mainly based on selecting nucleotide bases characterized by high homogeneity in accordance with their conserved regions \[4l, 4v\]. Shortened aptamers that contain common regions have approximately the same binding affinity as the original. For instance, based on the conserved sequences with high homogeneity of the original five 76-mer aptamers, A1 and A2 (8-mer sequences) were successfully obtained and still exhibited high affinity and specificity for tetracycline (TC) \[4v\] (Fig. 11).
Fig. 11: Truncation process after analysis of the sequences of the original five 76-mer aptamers that bind to oxytetracycline, 20 to 8 mer [4v].

Although only the original stacking pocket and six additional specific bases are present in A1 and A2, they display higher binding affinity (K$_d$ 1.067 nM for TC). The LOD of A2 for oxytetracycline (OTC) was 0.1 nM, which is about 500-fold better than that of the original 76-mer aptamer, and the color change can be detected in the presence of 10 nM OTC [4v]. Similarly, based on their common sequence and predicted structure, Changill Ban’s group [4l] shortened three 90-mer ssDNA aptamers that specifically bind to ampicillin to obtain AMP4 (21-mer 5’-CACGGCATGGGCGTCGTG-3’), AMP17 (19-mer 5’-GCGGCGGTTGTATAGCGG-3’), and AMP18 (21-mer 5’ TTAGTTGGGTTCACTGTTGG-3’) [4l]. Comparison of AMP17, AMP4 and AMP18 (at concentrations of 100 mM, 150 mM, and 200 mM, respectively) showed that ampicillin can be detected at concentrations as low as 5 ng mL$^{-1}$ using the AuNP-based dual fluorescence–colorimetric method and in a milk sample at 10 ng mL$^{-1}$ [4l].

Thus, the results by Song et al. [4v] and Kwon et al. [4l] illustrate and confirm that using aptamers harboring only the binding site/active site sequence can further improve their selective features.

Importantly, the target must not react or crosslink with NPs. Moreover, the ratio between NPs and aptamer could affect the final sensitivity. Too many aptamers in the sensing system reduce the sensitivity, while too few decrease the stability of the sensing systems [4q]. One of the primary challenges of Type I approaches relies on the different binding affinities of ssDNA and dsDNA towards unmodified NPs. However, the important feature is that negatively charged
ssDNA sequences can effectively stabilize NPs against salt-induced aggregation, providing a convenient route for colorimetric assays without NP surface biomodification.

5.2. **TYPE II: Aptamer-target adsorbed on nanoparticles**

Type II aptamers also include two common steps: (i) aptamer linkage to the target molecule to form a complex, and (ii) aptamer-target complex adsorption to the NP surface. With Type II approaches, it is always wise to check the interaction of the pure aptamer with NPs (Type I) because the system may follow a similar mechanism \[^{4c, 50}\]. Ideally, aptamer adsorption onto the NP surface should not lead to NP aggregation (and thus color change) after addition of high salt concentration. In the presence of the target, the aptamers should bind in competition with AuNPs, resulting in a color change in the presence of salts. A typical Type II system has been used by Chen *et al.* for sulfadimethoxine (SDM) detection using unmodified AuNPs. In optimal conditions (pH 8, 0.2 mM of aptamer and 2 M of salt), the LDR was 50 ng mL\(^{-1}\) to 1 mg mL\(^{-1}\) and the LOD was 50 ng mL\(^{-1}\) \[^{4n}\].

Upon addition of SDM, the conformation of the SDM-binding aptamer changes from a random coil structure to a more folded rigid structure that promotes the detachment of the adsorbed aptamers from AuNPs and results in the subsequent AuNP aggregation after salt addition (Fig. 15). This leads to a color change from red to purple-blue that can be easily observed by naked eye \[^{4n}\]. Recently, Liu *et al.* assessed whether aptamer truncation could improve the sensitivity also in Type II aptamers \[^{4x}\] (Fig. 1).
Briefly, the long (76-mer) aptamer specific for 17β-estradiol was split in two shorter sequences (P1 and P2) that still retain the original aptamer affinity and specificity, but with 10-fold higher LODs. Indeed, 17 β-estradiol could be detected with a LDR from 0.1 ng mL$^{-1}$ to 105 ng mL$^{-1}$ [4x]. The authors hypothesized that this increased sensitivity is caused by the lower aptamer adsorption concentration and lower affinity for AuNPs of the shorter ssDNA sequences [4v, 4x]. Likewise, Xie et al. developed an assay in which incubation of a RNA-DNA duplex with the HIV-1 reverse transcriptase (RT) leads to the production of ssDNAs and ssRNAs that can form a charged protecting layer on the AuNPs surface and consequently, to NP stabilization at a precisely defined salt concentration (Fig. 17). In the absence of RT, the selected RNA–DNA duplex remains intact, and the unprotected AuNPs aggregate in the presence of salt with a concomitant change in color [7o].
Fig. 13. Schematic of the approach by Xie et al. A synthetic RNA–DNA duplex substrate is first incubated or not with HIV-1 RT. HIV-1 RT should cleave the RNA into fragments, resulting in the dissociation of ssDNA and ssRNA probes at room temperature (≈ 28 °C). Reproduced with permission from [7o].

Wang et al. [3e] used K⁺ as a target because it stabilizes ssDNA, thus facilitating the formation of G-tetrads within 4 min. AuNPs incubated with G-tetrads changes color (red-to-purple) like unmodified AuNPs, suggesting that the G-tetrad structure is not significantly adsorbed onto AuNPs [3e]. The presented assay, which uses C-rich (5'-CCTCCCTCCTTTTCC ACCCACC-3') oligonucleotide aptamers, cationic polymers and AuNPs, provides a platform for the detection of other ions and molecules [3g] (Fig. 18). For instance, in the presence of Ag⁺, the two oligonucleotide form a tightly bound complex with a C-Ag⁺-C notation and change conformation, from a random coil to a hairpin structure with a stronger π-π* transition of the bases with deoxyribose. The resulting C-Ag⁺-C complex poorly interacts with a cationic polymer known as Poly (diallyldimethylammonium chloride) (PDDA) and subsequently the polymer aggregates AuNPs through electrostatic interactions, with a color change from wine red to blue [3g]. In the absence of Ag⁺, the positively charged polymer can electrostatically interact with ssDNA and destroy the charge balance, leading to induction of AuNP aggregation (LOD of 48.6 nM and LDR from 100 to 1000 nM for Ag⁺). Together, the results by Wang et al. [3e, 3g] are in
agreement with the hypothesis that unstructured DNA oligonucleotides strongly adsorb onto the NP surface and prevent salt-induced NP aggregation.

Fig. 14. Schematic description of the colorimetric lead biosensor for Ag⁺ detection based on AuNP aggregation induced by PDDA and Ag⁺ aptamers [3p].

Recently, Zhang et al. [7p] demonstrated that AuNPs possess peroxidase-like activity that can catalyze 3, 3, 5, 5-tetramethylbenzidine (TMB) in the presence of H₂O₂. AuNP peroxidase-like activity can be improved by surface activation with target-specific aptamers. However, by increasing the concentration of abrin (i.e., the target), AuNP peroxidase-like activity decreases and the aptamer is desorbed from the AuNP surface, resulting in a decrease of AuNP catalytic activity. The LDR for the current analytical system ranges from 0.2 nM to 17.5 nM with LOD of 0.05 nM [7p].

Comparison of Type I and Type II systems shows that aptamer-NP complexes are preferentially formed in Type I and aptamer-target complexes in type II systems. It is reasonable to expect less sensitivity from Type I systems because the colorimetric changes are related to the aptamer detachment from the NP surface. The amount of aptamer removed will depend on the amount of target. On the other hand, Type II systems are limited by the fact that the aptamer is expected to retain its adsorption properties after complexation with the target. This suggests that if it is
folded during target binding, it should be flexible enough to facilitate colorimetric changes.

Overall it all depends on the aptamer capability because we would prefer induced aggregation in the absence of the aptamer.

5.3. TYPE III: Competition in “One-pot detection systems”

Type III approaches are an intermediate between Type I and Type II systems because the aptamer and the target (or targets) interacts in the presence of NPs, and therefore, this approach is often referred to as “one-pot detection systems”. Sometimes, more than one class of aptamers are used to stabilize NPs \(^{[4k]}\). Yang and co-workers \(^{[4k]}\) described a “one-pot detection system” for ochratoxin A (OTA) where phosphate buffered saline (PBS), Mg\(^ {2+}\), OTA and the aptamer are mixed with AuNPs that can undergo salt-induced aggregation within 5 min \(^{[4k]}\) (Fig. 15).

![Fig. 15. One-pot detection system of ochratoxin A (OTA). The target is bound to the aptamer and upon salt addition, AuNP aggregation can be detected by the solution color change.](image)

Although the method is different, the authors hypothesized that “the duly formed G-quadruplex structure could not protect AuNPs against salt-induced aggregation, and thus the color change from red to blue could be observed by the naked eye”, as previously proposed by Wei et al. \(^{[5g]}\) and Wang et al. \(^{[3c]}\) for Type I and Type II detection systems. Interestingly the LOD is 20 nM, while the LDR from 20 to 625 nM \(^{[4k]}\). The major limitation of the Type III approach is that there is more than one source of electrostatic interactions that could change the solution color. For instance, if the metal is in excess, it will also contribute to the electrostatic interactions, and this
can only be prevented by having ssDNA in excess. Therefore selectivity is a major limiting factor for this approach.

5.4. **TYPE IV: Multiplex Aptasensors**

In this section, the systems that use more than one aptamer based on the previously described structure-switching strategies are described. In homogeneous multiplex aptasensors, more than one class of aptamer is used to stabilize NPs for detection of single or several targets \[^{[4x]}\]. Several approaches are based on the likelihood that the target DNA molecules with one nucleotide mismatch have different melting temperatures, and therefore they can be distinguished by NPs disassociation based on temperature \[^{[6b, 6c, 6f, 7e, 17a, 50]}\]. A typical example of such a DNA sensor was reported by Mirkin and co-workers, in which the target DNA molecules triggered AuNPs aggregation by hybridizing two complementary DNA strands on the AuNPs \[^{[18]}\].

![Fig. 16. Representation of the structure and color change of nano-assemblies in the presence of a triplex binding agent at room temperature \[^{[42]}\].](image)

Another assay includes AuNPs of different sizes (AuNP1 and AuNP2) that are functionalized with non-complementary DNAs (3’ or 5’ pyrimidine-rich thiol-modified oligonucleotides) (Fig. 16). Functionalized AuNP1 and AuNP2 are then cross-linked with another complementary DNA to form non-aggregating duplexes \[^{[42]}\]. Introduction of a triple binding agent induces triplex formation through base hydrogen bonds and consequently, reversible NP aggregation that result in a red-to-blue color change \[^{[42]}\]. Analysis of the aggregate melting properties in terms of cooperative binding theories suggests a lower DNA surface coverage on AgNPs functionalized with 12 mer-thiolated homo-oligonucleotides containing only adenine (AgNPs/ST) than that on
AuNPs functionalized with 12mer-thiolated homo-oligonucleotides containing only thymine (AuNPs/ST), while exhibiting changes that are significantly different from those of AuNPs upon hybridization \cite{51}.

Interestingly, Sato et al. \cite{48} demonstrated that ssDNA-AuNPs have different stability against salt-induced aggregation in the presence of complementary DNA, although there is no triplex binder \cite{48}. Using a similar assay format, Zhao et al. configured oligonucleotide-modified AuNPs duplexes with a short complementary oligonucleotide. Upon addition of adenosine (the target), the aptamer switches its structure from a DNA duplex to an aptamer/target complex, because the aptamer preferentially binds to the target molecule \cite{7b}. Importantly, the aptamer on NP surfaces must retain its switching capability \cite{7b, 18, 42, 48}. Erickson’s group \cite{15} developed a multiplexed one-pot detection system for Kaposi’s sarcoma-associated herpesvirus (KSHV) and Bartonella using both AuNPs and AgNPs. Specifically, when the Bartonella-targeted DNA (BA-DNA) is introduced in the solution, AgNPs aggregate and the solution turns pink, more dependent on the SRP characteristics of non-aggregated AuNPs. When KSHV-DNA was introduced, AuNP aggregate and the solution changes to a murky yellow-orange color, more dependent on AgNP aggregation \cite{15}. The multi-color change tuning of AuNPs and AgNPs gave LODs down to 1 nM and 2 nM, respectively \cite{15}.

Niu et al. \cite{4x} used more than one class of aptamers to stabilize AuNPs (Type I). Specifically, a kanamycin-specific aptamer (750 nM), a sulfadimethoxine-specific aptamer (250 nM) and an adenosine-specific aptamer (500 nM) are mixed (1:1:1 volume ratio) and adsorbed directly onto the surface of unmodified AuNPs by electrostatic interaction. Upon addition of any of the three targets, the conformation of the corresponding aptamer changes from a random coil structure to a rigid folded structure that cannot adsorb and stabilize AuNPs \cite{4x}. Although this looks more like a type III system, more than one aptamer is present and the reaction does not proceed sequentially.

Ultimately, multiplex systems are not straightforward because multiplex detection largely depends on the concentration of each aptamer and the buffer used for the aptamer reaction with its target \cite{4x}. Moreover, all the aptamers in solution can be adsorbed onto the NP surface; however, the level of adsorption also depends on the neighboring aptamers. For this reason, the use of aptamers with short sequences gives better adsorption yields. It has been shown that changing the length of the ssDNA sequences yields different particle dispersion profiles on unmodified AuNPs, and that short DNA sequences might improve the colloidal
stability against salt-induced aggregation \[41, 4v, 6e\]. Most importantly, the aptamer on the NP surface must retain its switching capability and for this reason the switching capability of the structures with aptamers is a key factor that determine the LOD of the assay \[7b\].

The multiplex type of aptamers has proven to be useful for enzyme activity and inhibitor assays.

### 5.5. Nanoparticle-based enzyme assays

NPs can be used also to improve colorimetric assays of enzymes \[52\]. In these systems, the substrate for the target enzyme should also be a suitable NP stabilizer. For instance, it should be stable at high salt concentration (approximately 100 mM). Moreover, a charged molecule that can provide electrostatic and steric stabilization is likely to yield better selectivity. The strong interaction between amino groups and AuNPs surfaces has been well confirmed and the bond energy is comparable to that of a thiol–Au bond. The rapid aggregation induced by the non-crosslinking process is a useful approach for enzyme inhibition-based colorimetric screening, as shown by several studies using assays that rely on polymeric aggregates of DNA-functionalized AuNPs (DNA-AuNPs) with DNA-duplex interconnections \[7e, 7j, 7k\]. Mirkin’s group functionalized two separate batches of 13-nm AuNPs with two different thiol-modified oligonucleotide strands (DNA-1: 5’-CTCCCTAATAACATTTAATAATTCCTA-A10-SH-3’, and DNA-2: 5’-TAGGAATTAGTTATATAATTGTATAGGGAG-A10- SH-3’) (blue and red ribbons, respectively, in Fig. 17) \[7e\].

**Fig. 17.** Aggregation and dissociation of the DNA-AuNPs probe used in the colorimetric screening of endonuclease inhibitors. The probe consists of spherical AuNPs functionalized with two complimentary oligonucleotides (blue and red ribbons). Individual NPs (red) aggregate into a cross-
linked network of NPs (blue) through hybridization of their oligonucleotide chains. Upon addition of DNase I, the aggregates remain intact longer in the presence of a strong endonuclease inhibitor [7e].

The endonuclease (DNase I) degrades the DNA-duplex interconnections and NPs are released, thus generating a red color [7e] (Fig 17). In the presence of inhibitors, the DNase I activity is decreased and the aggregates are strongly hydrolyzed ($T_H$). Consequently, the time required for the color change is much longer. Most importantly, strong inhibitors (in contrast to weak inhibitors) hinder DNase I activity to such an extent that the color change is no longer possible [7e]. In their method, endonucleases cleaves dsDNA in the absence of inhibitors and cross-linked AuNPs can separate into single AuNP molecules, as indicated by the instant color change, from blue to red. With this approach, the inhibitor performance can be directly evaluated. Similar observations were made using a system that includes a single type of DNA-AuNPs probe and an appropriate oligonucleotide linker that can hybridize with the DNA probe. The linker was designed to contain a self-complementary region that can form a duplex structure with a base-pair overlap that contains the recognition sites and overhanging 3’-ends [7k] (Fig. 18). Significant color change is observed when the endonuclease (DNA methyltransferase, DNA MTase) degrades the DNA duplex [7k].
Fig. 18. (A) Schematic representation of the assay to assess endonuclease and methyltransferase activity and inhibition. (B) Sequences of the DNA probe, DNA-1 (recognition site for the DpnII/Dam MTase) and DNA-2 (recognition site for the EcoRI/EcoRI MTase). The arrows show the cleavage sites, and the red letters indicate the methylation sites. 

Although highly selective and more sensitive than conventional methods, this visual inspection assay is limited for the preparation of probes by functionalizing two separate AuNP batches with two different thiol-modified oligonucleotide strands. On the other hand, this approach can be used for most endonucleases by simply changing the recognition sequence in the linker DNA.

For instance, similar assays were used for assessing adenosine triphosphate (ATP) dephosphorylation by calf intestine alkaline phosphatase (CIAP) and peptide phosphorylation by protein kinase A (PKA). ATP can protect AgNPs from salt-induced aggregation only in the absence of enzymes. Phosphorylation and dephosphorylation can be readily detected by the color change of AgNPs (CIAP LOD: 1 unit mL\(^{-1}\), and PKA LOD: 0.022 unit mL\(^{-1}\)). Zhao and coworkers took advantage of the non-crosslinking AuNPs aggregation phenomenon to develop a simple colorimetric assay for monitoring an enzymatic dephosphorylation reaction, where ATP is converted into adenosine by CIAP. AuNPs capped by adenosine 5’-monophosphate (AMP), adenosine 5’-diphosphate (ADP), or adenosine 5’-triphosphate (ATP) are progressively...
more stable than bare AuNPs, but their stability gradually decreases (and thus the color of the 
solution) with the dephosphorylation process\cite{7a}. Likewise, Choi et al.\cite{40} described an alkaline 
phosphatase assay based on AuNPs aggregation\cite{40}. To develop an adenosine deaminase assay, 
Zhang and co-workers hypothesized that the interaction between adenosine amino group and 
AuNPs surface will displace the weakly bound citrate ions from the AuNPs surface and diminish 
the stability of citrate-capped AuNPs, resulting in the aggregation of AuNPs in the presence of 
NaCl and a corresponding red to blue color change. Adenosine, guanosine and cytidine 
(molecules that contain amino groups) strongly interact with AuNPs, causing aggregation. 
Conversely, inosine, thymidine and uridine have negligible effects on AuNPs stability, therefore 
the solution remains red because of the stronger electrostatic repulsion between negatively-
charged AuNPs\cite{8}. Xinhui et al. described a suitable method for nucleases, such as the S1 
nuclease\cite{7j}. In the presence of nucleases and their substrates, unmodified AuNPs are stabilized 
by dNMPs at high salt concentration and the solution remains red. Conversely, in the absence of 
nucleases or substrates, the unmodified AuNP solution turns blue at high salt concentration due 
to aggregate formation\cite{7j}.

Xu and co-workers developed a colorimetric assay to screen for inhibitors of several 
kinases with the same type of NPs\cite{7d} (Fig. 19). The method takes advantage of peptide-capped 
NPs, in which 10% of peptide ligands carry an extension that is the substrate for a specific kinase 
(PKA or calmodulin-dependent kinase II, CaM KII). Using γ-biotin-ATP as a co-substrate, the 
kine reaction results in substrate-AuNP biotinylation.
Fig. 19. Schematic representation of phosphorylation/ biotinylation of substrate-NPs followed by addition of avidin-modified NPs, in the presence and absence of a kinase inhibitor [74]

When biotinylated substrate-AuNPs are mixed with avidin-AuNPs, they immediately aggregate due to the specific binding between avidin and biotin [74]. Similarly, Wei et al. [7h] reported that in the absence of the kinase, or in the presence of an efficient inhibitor, no observable color change occurs after addition of avidin-modified NPs, and the solutions are indefinitely stable without showing signs of aggregation [74]. Furthermore, a hydrolysis-based colorimetric assay for acetylcholinesterase (AChE) was developed based on the finding that AChE can catalyze acetylthiocholine hydrolysis into thiocholine [45]. AChE and acetylthiocholine are added in the AuNP solution, the generated thiocholine can take the place of citrate on the AuNP surface, promoting NP aggregation and a change of color from red to gray. Addition also of the AChE inhibitor tacrine (1,2,3,4-tetrahydroacridin-9-amine) leads to less AuNP aggregation and a slower color change [45]. Uehara et al. [43] reported that gold nanocomposites conjugated with a thermo-responsive copolymer can be used in a colorimetric assay to quantify the activity of aminopeptidase N (APN). By heating the solution, the assembled gold nanocomposites disassemble and the solution color change from blue, purple to red. This process is inhibited by cysteine, therefore the enzymatic decomposition of cysteinylglycine into cysteine and glycine by APN can be monitored [43].
Tiwari *et al.* hypothesized that particles could be used for the detection of the hydrolytic activity of penicillin G acylase (PGA) on penicillin G. This hydrolysis reaction leads to a shift in the surface plasmon band of AuNPs from 527 to 545 nm accompanied by a solution color change from red to blue. The presence of 0.007 mg ml\(^{-1}\) PGA can be detected \[^{7m}\]. The enzyme is known to hinder the salt-induced NP aggregation. Xie and co-workers found that DNA-RNA duplexes cannot stabilize unmodified NPs at a certain salt concentration, a typical type II approach. However, addition of the active HIV-1 RNase H enzyme leads to the specific cleavage of RNA strands into RNA fragments and ssDNAs that can stabilize NPs against salt-induced aggregation \[^{7o}\].

In an assay for glycosidases based on self-immolative elimination to release amines, functionalized trigger-AuNPs aggregate by electrostatic attraction upon cleavage of the trigger. The assay gives LODs for β-galactosidase (Gal) and β-glucosidase (Glc) of 9.2 and 22.3 nM, respectively, at 20 min, and they improve slightly over time \[^{44}\]. The functionalized AuNPs (2.0 nM), which were capped with the enzyme substrate ligand Gal-Lip (or Glc-Lip) and lipoic acid at a ratio of 1:1, showed a typical SPR peak at 521 nm (i.e., a red shift of 2 nm compared with citrate-AuNPs) and good stability in PBS. The respective addition of Gal or Glc caused a time-dependent decrease of the peak absorbance induced by NP aggregation that could be observed by naked eye.

Two different systems (the DNA endonuclease DNase I and the Pb\(^{2+}\)-dependent RNA-cleaving DNA enzyme 8-17) were chosen to demonstrate the utility of an assay for the detection of metal ions and enzyme activities based on rapid NP aggregation driven by van der Waals attraction \[^{7i}\] (Fig. 20).
Fig. 2. Schematic illustration of AuNP aggregation and color change triggered by the enzymatic cleavage of DNA on AuNPs. Before enzymatic cleavage, DNA-modified AuNPs are stable at a relatively high salt concentration, due to their electrostatic and steric stabilization. DNA removal from the AuNP surface by enzymatic cleavage destabilizes AuNPs and results in their rapid aggregation. A) Cleavage of a DNA duplex by DNase I. B) Pb$^{2+}$-mediated cleavage of an RNA-containing DNA substrate by the 8-17 DNA enzyme.$^{[7i]}$

The authors reasoned that the removal from the NP surface of DNA strands, which serve as electrostatic and steric stabilizers at relatively high salt concentrations (e.g., 40 mM MgCl$_2$), should result in AuNPs destabilization and aggregation, a process driven by van der Waals attraction.$^{[7i]}$ Indeed, addition of 100 mM Pb$^{2+}$ generated a rapid red-to-purple color change at room temperature.$^{[7i]}$

AuNPs generation induced by neurotransmitters can be used to analyze the activity of tyrosinase, an enzyme that catalyzes the O$_2$-induced hydroxylation of tyrosine to L-DOPA.$^{[4b]}$ As tyrosinase concentration increases, the SPR bands of NPs are intensified and slightly blue-shifted, suggesting that larger particles are formed, and small Au nanoclusters enlarged.$^{[4b]}$ This system can be used for the sensitive detection of dopamine, L-DOPA and noradrenaline (LOD of
2.5 µM) and adrenaline (0.2 µM) that act as active reducing agents for Au-NP generation of Au-NPs \[4b\].

Colorimetric aptasensors for the quantitative analysis of abrin using catalytic AuNPs were reported for the first time by Zhang et al. and Wu et al. The AuNP peroxidase-like activity can catalyze 3, 3, 5, 5-tetramethylbenzidine (TMB) formation in the presence of \( \text{H}_2\text{O}_2 \), leading to a color change \[4a, 7p\]. Particularly, Song et al. \[7k\] quote the work by Mirkin’s group \[7e\] and say that the rest of the methods eliminate the binder use of different DNAs to obtain functionalized-NP1 and functionalized-NP2. However, such method requires cumbersome preparation of modified NPs and the formation of crosslinked NPs. Thus, it is not suitable for high-throughput screening.

Crosslinking also offers a unique selectivity in reversibility of NP aggregation, where analytes dissociate the cross-linker and re-disperse the NP aggregates to their original color. For instance, Lu and co-workers described detection assays for \( \text{Pb}^{2+} \), adenosine and cocaine where DNA molecules with a single RNA linkage serve as cross-linkers that bring complementary DNA-AuNPs into aggregates. The DNA enzyme catalyzes the specific hydrolytic cleavage of the substrate strand that disrupts the NP assembly, changing the color from purple to red and thus indicating the presence of \( \text{Pb}^{2+} \) \[3a, 7c\].

Zhao and co-workers speculated that if the substrate and product of an enzymatic reaction affect differently AuNP stability by changing their electrophoretic properties, such a reaction can be monitored colorimetrically and the enzymatic activity can therefore be determined \[7a\].

6. Core-Shell Nanoparticles and Ratios

Core–shell nanoparticles (CSNs) are a class of nanostructured materials that have recently received increased attention owing to their interesting properties and broad range of applications in catalysis, biology, materials chemistry and sensors. By rationally tuning the cores as well as the shells of such materials (ratios), a range of core–shell nanoparticles can be produced. In this review, the combination of gold and silver enhancement, an electron-dense deposits that can be read by a simple colorimetric array workstation \[6d\]. However, Cao et al. stated that AgNPs cannot be effectively passivated by alkylthiol-modified-oligonucleotides using the protocols for modifying AuNPs, because they irreversibly aggregate when heated in a solution with 0.05MNaCl, the concentration needed for DNA hybridization \[17b\]. For this reason,
an Au shell can be grown on AgNP, forming a particle with an Au outer surface (3.1 ± 0.6 Å). This surface can be easily modified with alkylthiol-oligonucleotides and indefinitely suspended in high salt solutions. Ag/Au core-shell NPs retain the optical properties of the silver core, but have optical properties different from pure gold NPs, thus providing another “color” option for 30-mer DNA target-directed colorimetric detection [6f, 17b].

By taking advantage of their reversible aggregation and melting nature, oligonucleotide-modified Ag@SiO\(_2\) nano-probes can be prepared by using 5’ TCT-CAA-CTC-GTA-(CH\(_2\))\(_7\)-NH\(_2\)3’ and non-complementary oligonucleotides with a 5’ TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT3’ sequence in which no hybridization product was reported [50]. The high stability of oligo-modified Ag@SiO\(_2\) nano-probes at elevated temperatures (30-70°C) was confirmed by the absence of peak shift and of broadening of well-dispersed nano-probes in their UV-Vis spectra after long heating. The fast hybridization kinetics of the resulting Ag@SiO\(_2\) nano-probes with complementary target oligonucleotides render them very useful for fast colorimetric detection based on the sequence-specific hybridization properties of DNA [50].

CSNs can also be used to introduce a second colorimetric change, distinct from the gold system, for monitoring two different oligonucleotide targets in one sample [17b]. For instance, when lactose-stabilized AuNPs are mixed with mannose (2-mercaptoethyl R-D-mannopyranoside)-stabilized AgNPs [2], addition of concanavalin A (Con A) leads to the aggregation only of mannose-stabilized AgNPs, thereby demonstrating the system selectivity. Limited non-specific interaction occurs with lectin Con A, thereby enabling the subsequent specific interaction with the lectin from *Ricinus Communis* Agglutinin (RCA120). Although mannose-stabilized AgNPs show a longer LDR and faster reaction kinetics for the target lectin, mannose-stabilized AuNPs provide the most sensitive bioassay [2].

Nevertheless, the synthesis of the CSNs can be a daunting task for quick assays [4f].

### 7. Summary and outlook

The detection of metals and molecules based on bare-eye observable colorimetric changes depends on various parameters that are even more complex for macromolecules. The functional groups on the NP surface are very influential in various areas of research, such as sensor arrays and biosensor detection. Overall in any given condition, both thiols and nucleotides compete for adsorption sites, and the equilibrium shifts towards thiols adsorption as the salt concentration...
Therefore, nucleotide adsorption could be completely eliminated after being ‘crowded out’ (steric hindrance) as more thiols are adsorbed onto the metal. In one-pot detection approach (where nanoparticles, analyte, DNA or enzyme are mixed together) qualitative analyses is deemed imprecise because the color changes are not only due to the analyte, as they could be triggered also by DNA or enzyme adsorption to the NP surface. Also, the multiplex aptasensors have some limitations. For instance, it is not possible to determine which target is detected when the sample gives a positive result (change of color). While enzyme-assisted assays have a huge potential for many applications in biomedicine and bio-imaging, the application of the described in vivo techniques faces formidable challenges. Indeed, the interactions of NPs with enzyme molecules are not yet fully understood.

Through the review, there are viable approaches that have been introduced, Firstly, aptamer truncation to overcome the limitations due to the DNA length, while maintaining the original binding affinity. Secondly, overcoming the limitation imposed by the stability of AgNPs by coating them with a thin layer of gold to produce a core–shell structure that retains the spectrophotometric signature of the silver core. Thirdly, use of chemometric approaches provides synergy in colorimetric discrimination or classification of small molecules and macromolecules. Overall, the AuNP-based colorimetric aptasensors are currently used for many analytes, largely because of the ease of detection, high sensitivity and potential for high-throughput analysis. Moreover, the synergy between chemometrics and biotechnology selectivity narrows the gap for the development of smart apta-based colorimetric sensing devices. In any case, careful interpretation of the findings is critical because the colorimetric changes are not general or universal, suggesting that they are highly dependent on the analytes and matrices.

REFERENCES


