


# A Review on the Synthesis and Functionalization of Gold Nanoparticles as a Drug Delivery Vehicle

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**Abstract:** Metal nanoparticles are being extensively used in biomedical fields due to their small size-to-volume ratio and extensive thermal stability. Gold nanoparticles (AuNPs) are an obvious choice for biomedical applications due to their amenability of synthesis, stabilization, and functionalization, low toxicity, and ease of detection. In the past few decades, various chemical methods have been used for the synthesis of AuNPs, but recently, newer environment friendly green approaches for the synthesis of AuNPs have gained attention. AuNPs can be conjugated with a number of functionalizing moieties including ligands, therapeutic agents, DNA, amino acids, proteins, peptides, and oligonucleotides. Recently, studies have shown that gold nanoparticles not only infiltrate the blood vessels to reach the site of tumor but also enter inside the organelles, suggesting that they can be employed as effective drug carriers. Moreover, after reaching their target site, gold nanoparticles can release their payload upon an external or internal stimulus. This review focuses on recent advances in various methods of synthesis of AuNPs. In addition, strategies of functionalization and mechanisms of application of AuNPs in drug and bio-macromolecule delivery and release of payloads at target site are comprehensively discussed.

**Keywords:** gold nanoparticles, drug delivery, biological synthesis, functionalization, drug release

## Introduction

Nanotechnology is referred to the designing and application of components which occur at the nano-scale: up to 10–1,000 nm in size.<sup>1</sup> Nanotechnology encompasses the study of structural properties of nano-structures at the molecular and sub-molecular level along with their electrical, optical, and magnetic attributes. At present, nanotechnology is an interdisciplinary field which takes engineering, biomedicine, chemistry, and physics under one umbrella.<sup>2</sup> The application of nanomaterials in different fields ranging from oil and gas and cosmetics to nanomedicine has taken this world to the new era, which is the era of nanotechnology.<sup>3,4</sup> The best investigated nanostructures include carbon nanotubes, gold nanoparticles, liposomes, and paramagnetic nanostructures.<sup>5–8</sup> Gold colloids are now increasingly utilized in different fields like chemistry, biology, engineering, and medicine. In the biomedical field they have vast applications in diagnostics, therapy, and immunology.<sup>9</sup>

Gold nanoparticles provide an outstanding material for study due to the fact that they are one of the most stable, non-toxic, and easy to synthesize nanoparticles and exhibit various fascinating properties like assembly of various types and quantum size

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effect.<sup>6</sup> The optical behavior of gold nanoparticles is dependent on their surface plasmon resonance (SPR), located in a wide region ranging from visible to the infrared region of the spectrum, which is determined by collective oscillation of conducting electrons. The range of the spectrum depends on various features of gold nanoparticles, including size and shape.<sup>9</sup> Methods have been developed to synthesize these materials reproducibly, which can further be modified using countless chemical functional groups. Many new sensitive and specific assays are based on the gold nanoconjugates.

Gold nanoparticles have emerged as an excellent candidate for the application in delivery of various payloads to the target site.<sup>10,11</sup> These payloads range from small drug molecules including drugs to large biomolecules like DNA, RNA, and proteins. Some drugs molecules do not require modification of a monolayer of gold nanoparticles for their delivery and can be directly conjugated with gold nanoparticles through physical absorption and ionic or covalent bonding.<sup>12</sup> Whereas for the delivery of other payloads, gold nanoparticles require functionalization like PEGylation,<sup>13</sup> peptide and amino acid conjugation,<sup>14,15</sup> or functionalization with oligonucleotides.<sup>16</sup> Apart from that, another prerequisite for the efficient delivery of therapeutic agents is their release. Various internal stimuli (glutathione, pH and enzymes)<sup>17–19</sup> and external stimuli (light, etc.)<sup>20</sup> have been investigated for the efficient release of these payloads from gold nanoparticles.

Due to the vast amount of information available and the level at which it is being renewed we have chosen the generalized data from the past few years to present this review encompassing the most promising application of gold nanoparticles in drug delivery.

## Synthesis of AuNPs

For the synthesis of AuNPs, there are two basic strategies that are used, which are “Top-Down” and “Bottom-Up” approaches. The top-down approach involves the synthesis of AuNPs starting from bulk material and cracking it into nanoparticles using different methods. In contrast, the bottom-up approach synthesizes nanoparticles starting from atomic level. Figure 1 shows the basic steps that are involved in the top-down and bottom-up approaches. Synthesis methods that involve the top-down approach include laser ablation,<sup>21</sup> ion sputtering,<sup>22</sup> UV and IR irradiation,<sup>23,24</sup> and aerosol technology,<sup>25</sup> whereas the reduction of Au<sup>3+</sup> to Au<sup>0</sup> is the bottom-up approach.

The formulation of AuNPs involves two main stages:

In the first stage the gold precursor, which is usually an aqueous gold salt solution, is reduced to gold nanoparticles using a specific reducing agent like citrate.

In the second stage the stabilization of gold nanoparticles is done by a specific capping agent. The capping agents hinder the agglomeration of metallic nanoparticles.

## Chemical Synthesis Turkevich Method

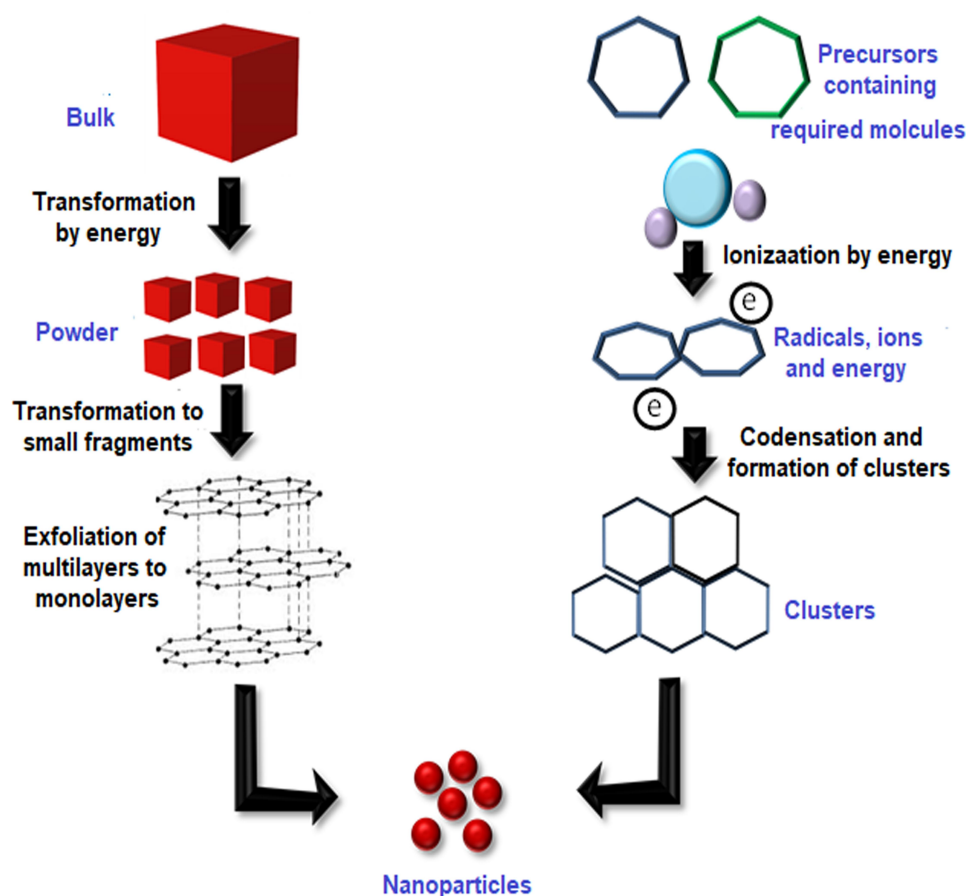
This method for the synthesis of AuNPs was first reported in 1951. It is one of the most commonly used techniques for formulation of spherical AuNPs. AuNPs prepared using this method have the size in the range of 1–2 nm.<sup>26</sup> The basic principle of this technique involves the reduction of gold ions (Au<sup>3+</sup>) to produce gold atoms (Au<sup>0</sup>) by using some reducing agents like amino acids,<sup>27</sup> ascorbic acid,<sup>28</sup> UV light, or citrate.<sup>29,30</sup> Stabilization of AuNPs is carried out by using different capping/stabilizing agents. At the beginning, the applications of Turkevich method were finite because of the limited range of AuNPs that could be synthesized by this technique. With the passage of time several advancements in the basic method have enabled researchers to extend the size range of particles synthesized using this method. In 1973, it was established that by varying the ratio of reducing as well as stabilizing agents, AuNPs of particular size with the range from 16–147 nm can be produced.<sup>31–33</sup> Figure 2A shows the basic method involved in the Turkevich method.

## The Brust Method

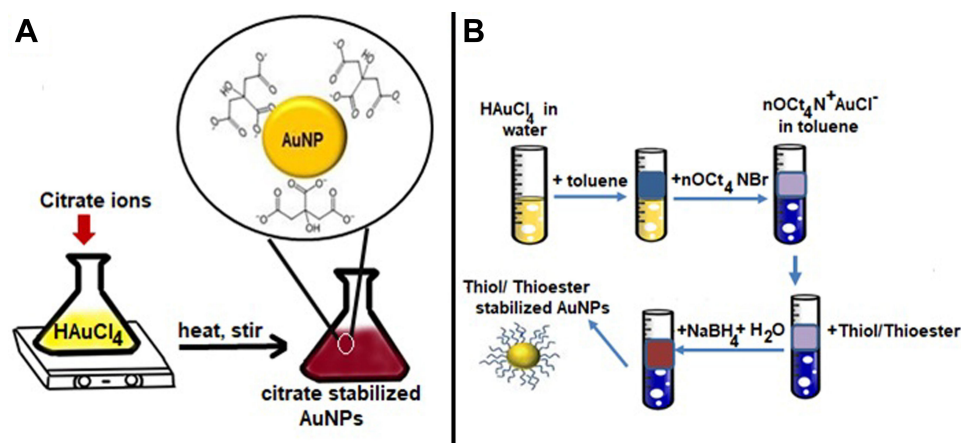
This method was first reported in 1994 and involves a two-phase reaction to synthesize AuNPs with the size range of 1.5–5.2 nm by using organic solvents.<sup>34</sup> The method encompasses the use of a phase transfer such as tetraoctylammonium bromide to carry out transferring of gold salt to organic solvent from its aqueous solution (eg, toluene). The gold is then reduced by the use of a reducing agent such as sodium borohydride along with an alkanethiol. The alkanethiol carries out the stabilization of AuNPs.<sup>35</sup> As a result of this reaction the color changes from orange to brown.<sup>34,36</sup> Figure 2B shows the schematic illustration of main steps involved in Brust method.

## Seed-Mediated Growth

The previous two methods can synthesize only spherical AuNPs; however, they can also be formulated in a number of geometries and shape such as rods.<sup>37,38</sup> The most commonly used technique to synthesize rod shaped AuNPs is



**Figure 1** Top-down and bottom-up approaches for the synthesis of NPs. The top-down approach involves the transformation of bulk material by using energy to produce the powder form which is then transformed into smaller fragments with multiple layers and then to the monolayers leading to the formation of nanoparticles. On the other hand, the bottom-up approach uses the precursor molecules which are then ionized by using energy. Radicals, ions, and electrons thus produced are condensed to form clusters which are then transformed to nanoparticles.



**Figure 2** (A) Turkevich method for the synthesis of AuNPs. (B) Series of steps involved in the Brust method for the synthesis of AuNPs.

seed-mediated growth. This method is based on the fundamental principle which involves first synthesizing seed particles by reducing gold salts. This reaction is done in the presence of reducing agents like  $\text{NaBH}_4$ . The next step

involves the transferring of the seed particles to a metal salt and a weak reducing agent like ascorbic acid which prevents further nucleation and speeds up the synthesis of AuNPs of rod shape. Shape and geometry of gold

nanoparticles depends on the concentration of reducing agents and seeds. Figure 3A shows schematic illustration of seed-mediated growth of short and long gold nanorods as reported by a study.<sup>39</sup>

### Digestive Ripening

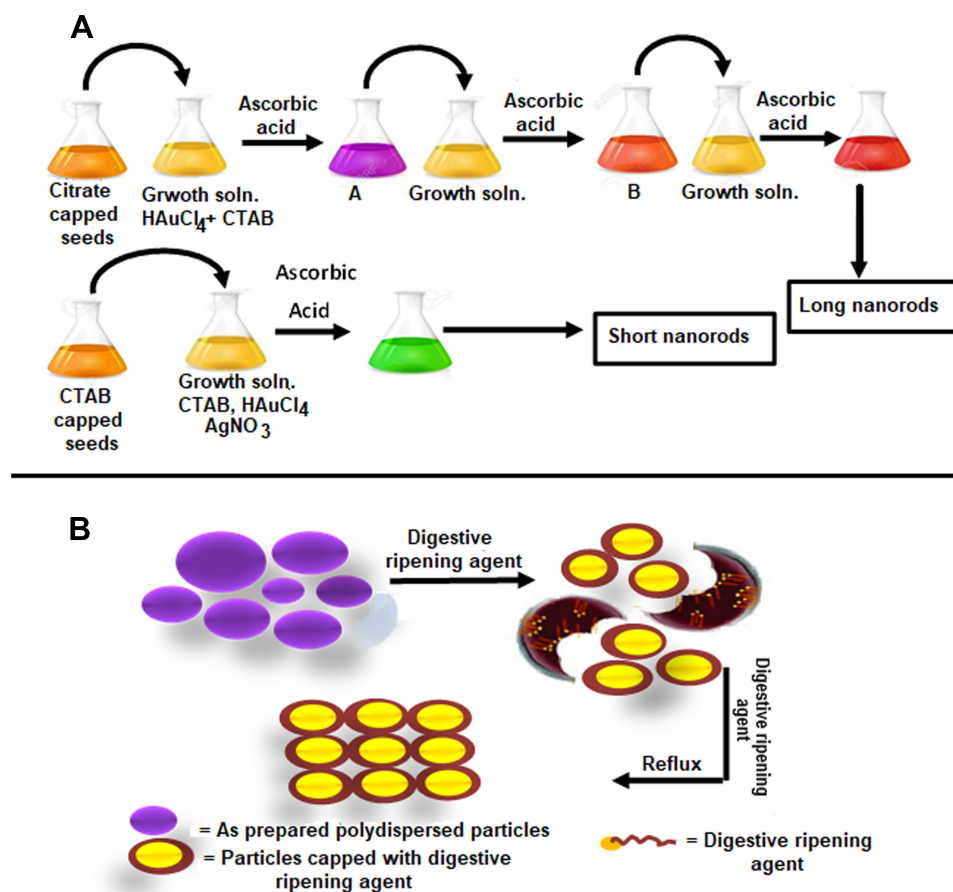
Digestive ripening is considered to be a convenient method to prepare monodispersed gold nanoparticles in the presence of excessive ligands (digestive ripening agents). The basic process comprises heating a colloidal suspension at high temperatures ( $\sim 138^\circ\text{C}$ ) for 2 minutes and then heating at  $110^\circ\text{C}$  for 5 hours by using alkanethiol, as shown in Figure 3B. Temperature is the major factor for determining the size distribution of the gold colloids.<sup>40</sup>

In addition to these methods, other methods involve the use of ultrasonic waves for the synthesis of AuNPs.<sup>41,42</sup>

### Advantages and Limitations of the Methods

Turkevich method is a fairly uncomplicated and reproducible procedure for the formulation of spherical particles with the size range 10–30 nm. But, as the size of particles

increases above 30 nm, they become less spherical in shape with broader size distribution. Moreover, this reaction gives low yield and involves the use of only water as a solvent.<sup>43</sup> Brust method, on the other hand, involves an easy strategy for the formulation of thermal and air-stable AuNPs having controlled size and less dispersity. One possible limitation of Brust method is synthesis of AuNPs which are less dispersed and used of organic solvents immiscible with water, therefore, limiting their biological applications.<sup>44</sup> Seed-mediated growth is a reliable method for the synthesis of rod-shaped AuNPs, but various factors affect the size of rod and so must be carefully controlled. In a study when higher concentrations of  $\text{HAuCl}_4$  were used it produced bigger seed rods with smaller aspect ratios. Temperature also plays a significant role in the synthesis of rods and at higher temperatures rods with lower aspect ratio were produced. Also, the number of seeds added to the reaction mixture must be critically considered to stimulate the growth of rods.<sup>45</sup> The digestive ripening method is also an easy and valuable



**Figure 3** Series of steps involved in the synthesis of AuNPs. (A) Seed-mediated method. (B) Digestive ripening method.



chemical technique to produce monodispersed nanoparticles. Another benefit of this strategy is the high yield of nanoparticles.<sup>46</sup> A possible disadvantage of the digestive ripening method is that controlling the shape of nanoparticles via the digestive ripening process is difficult as it involves very high temperatures.<sup>47</sup>

In addition, chemical methods are inherited with their own limitations which include environmental and biocompatibility concerns. Some of the chemicals that are used in the synthesis of gold nanoparticles during chemical synthesis can affect our environment and are the cause of risks for administering them into the living organisms, thus limiting the biological applications of such NPs.<sup>48</sup> Therefore, various biological methods have been devised for the synthesis of AuNPs to limit these concerns.

## Biological Synthesis

Recently, efforts have been made for biological synthesis of AuNPs, which is a clean, dependable, and bio-friendly alternative to harsh chemicals used in chemical synthesis reactions. The biological resources used in synthesis of nanoparticle range from simple bacterial cells to complex eukaryotes. Interestingly, the capability of organisms in synthesis of metal nanoparticles has given rise to a new thrilling approach toward the development of these biological nano-factories.<sup>49</sup> A plethora of organisms have been reported to carry out successful synthesis of AuNPs, ranging from bacteria to plants, algae, and fungi.

### Bacteria

Microorganisms have been reported to be an excellent candidate for the synthesis of both intracellular and extracellular AuNPs.<sup>50–52</sup> The negatively charged cell wall of bacteria can electrostatically interact with positively charged Au(III) ions. During the intracellular synthesis, gold ions are transported inside the cell where enzymes and biomolecules carry out the synthesis of AuNPs. On the other hand, during extracellular synthesis the gold ions are trapped on the cell membrane by membrane enzymes. These enzymes on the membrane or reductase enzymes secreted out by the microbial cell can carry out the synthesis process outside the bacterial cell.<sup>53</sup> Extracellular synthesis, however, is more fascinating as it does not require extra downstream processing steps which are required for the separation of nanoparticles from the intracellular matrix. A study has shown that, during the extracellular synthesis reaction NADPH-dependent enzymes are secreted by bacteria which can reduce Au(III) ions to

Au<sup>0</sup> such as nitrate reductase secreted by *Pseudomonas denitrificans*. The results showed that the action reductase enzyme diminished once AuNPs had been synthesized.<sup>54</sup> Shah et al<sup>55</sup> reported that both NADH and NADH-dependent enzymes function as a scaffold or nucleating agent for the synthesis reaction. Singh et al reported that *Rhodopseudomonas capsulate* secreted NADH and NADH-dependent enzymes during extracellular synthesis of AuNPs. The transfer of electrons from NADH carried by NADH-dependent enzyme causes the reduction of Au(III) to Au<sup>0</sup>, resulting in the synthesis of AuNPs.<sup>51</sup> *Thermomonospora* sp. (Order: Actinomycetes) was reported to carry out intracellular enzymes mediated synthesis of AuNPs by achieving the reduction of Au(III) ions at the surface of membrane and mycelia.<sup>56</sup> Similarly, *Shewanella algae* efficiently carried out enzymes mediated bioreduction of AuCl<sup>4-</sup> ions to AuNPs which were found to be dispersed in periplasmic membrane of bacterium.<sup>57</sup> Certain materials produced by microbial cells like proteins, enzymes, and organic substances can act as capping agents to stabilize nanoparticles and, hence, prevent their agglomeration.<sup>58</sup> Micro-organisms possess certain reductase enzymes which can reduce metal salts to metal nanoparticles with narrow size distributions and monodispersity. By altering the essential growth parameters, the shape and size of AuNPs can be controlled. Synthesis of AuNPs using bacteria is a tedious reaction and requires additional precautionary measures while handling bacteria, and also takes hours and days as bacterial cultural is a time consuming process. These drawbacks have limited the use of bacteria for the synthesis of AuNPs.<sup>59</sup>

### Fungi

Fungi have also been used as a biological source for the synthesis of AuNPs. Fungi secrete a number of biomolecules, including metabolites and extracellular enzymes, such as hemicellulose, acetyl xylem esterase, 3-glucanase, cell wall lytic enzyme  $\beta$ -1, etc., which have been reported to play a role during the synthesis of metallic nanoparticles.<sup>60</sup> Numerous studies have reported the synthesis of gold nanoparticles using unicellular and multicellular fungi.<sup>61,62</sup> A fungal species *Fusarium oxysporum* was used in a study for the extracellular synthesis of Au-Ag alloy NPs by the reduction action of nitrate-dependent enzyme and shuttle quinone.<sup>63</sup> A fungal species *Verticillium* has also been reported to carry out intracellular synthesis of AuNPs. AuNPs were found to be trapped

in the cell membrane and the cell wall of fungi, indicating that Au<sup>3+</sup> ions were bio-reduced by the reduction action of reductase enzymes in fungi.<sup>64</sup> A study on the biosynthesis of AuNPs from *Phanerochaete chrysosporium* proved that laccase was the enzyme secreted by the fungi for extra-cellular synthesis of AuNPs and, for intracellular synthesis, ligninase was found to be responsible.<sup>65</sup>

## Plants

Phytonanotechnology has gained attention with time as it comprises an eco-friendly, cheap, and rapid process for the synthesis of nanoparticles. A number of studies have reported biosynthesis of AuNPs using different plants or plant extracts involving the use of harmless bio-components from plants to carry out the reduction and capping of AuNPs, reducing the waste generation and limiting the requirement for additional purification steps. Numerous bio-components present in plants such as flavonoids, phytosterols, quinones, etc., play a role in the synthesis of AuNPs because of the functional groups which speed up the reduction and stabilization of AuNPs.<sup>66</sup> Although nearly every part of plants has been reported to successfully carry out the synthesis of AuNPs, leaves are most commonly used. The difference in the level of various compounds present in different plants and even in different parts of a plant affects the synthesis of AuNPs. For example, a study has reported the effect of difference in level of phenolic contents present in leaves and fruit of *Garcinia mangostana* plant on the synthesis of AuNPs. As the leaves are rich in phenolic content so the rate of synthesis of AuNPs was faster in the presence of leaves than fruit.<sup>67,68</sup> Moreover, recently the synthesis of gold nanoparticles using medicinal plant *Acorus calamus* and *Cassia auriculata* has been reported.<sup>69,70</sup>

Reactive compounds; Lignans [(+)-pinoresinol, (+)-medioresinol], alkaloids, flavonoids, steroids (sitosterol-3-0-glucoside), and terpenoids present in the leaves of *Justicia glauca* have been reported to complete the synthesis reaction of AuNPs in 1 hour. AuNPs had spherical and hexagonal morphology and were 32 nm in size.<sup>71</sup> Leaves of the *Terminalia arjuna* plant also carried out the synthesis of AuNPs within 15 minutes. AuNPs synthesized in this study were 20–50 nm in size and had spherical morphology. The author claimed that the reactive compounds Arjunetin, leucoanthocyanidins and hydrolysable tannins present in leaves of *Terminalia arjuna* contributed to the synthesis of AuNPs.<sup>72</sup> Similarly, the leaves of olive plant and *Cassia auriculata* were shown to complete the

synthesis reaction of AuNPs in 20 minutes and 10 minutes, respectively. The active metabolites and biomolecules in the leaves of the olive plant are proteins, oleuropein, apigenin-7-glucoside, and luteolin-7-glucoside, which resulted in the formation of spherical and anisotropic AuNPs with the size range of 50–100 nm.<sup>73</sup> Polysaccharides and flavonoids are the major active substances in the leaves of *Cassia auriculata* and AuNPs synthesized from leaves of this plant were 15–25 nm in size and had spherical and anisotropic morphology.<sup>70</sup> *Mangifera indica* leaves used by Philip<sup>74</sup> synthesized spherical AuNPs within 2 minutes of reaction time. The size of AuNPs was found to be in the range of 17–20 nm. Terpenoids, flavonoids, and thiamine are the active compounds present in mango fruit, which might have contributed to the synthesis of AuNPs.

Apart from leaves, various other parts of plants, including fruits, roots, stems, etc., have been used for the synthesis of AuNPs. The fruit of *Citrus maxima* was used in one study and synthesized spherical AuNPs with the size range of 15–35 nm within 5 minutes of reaction time. Proteins, terpenes, and ascorbic acid were the major compounds that were claimed to act as reducing agents during reaction.<sup>75</sup> The high phenolic content of *Sambucus nigra* (elderberry) was the major factor in the synthesis of AuNPs.<sup>76</sup> Apart from that, flowers of *Lonicera Japonica* contain amino acids as active compounds and successfully synthesized AuNPs of triangular and tetrahedral morphology with the size range of 8 nm in the reaction time of 1 hour.<sup>77</sup> Similarly flowers of the *Moringa oleifera* plant synthesized AuNPs of size 3–5 nm. This plant was reported to contain a high content of flavonoids, carotenoids, phenols, sterols, and amino acids, which were claimed to be responsible for carrying out the reduction reaction during the synthesis process.<sup>78</sup> Various types of roses have been demonstrated to possess the reducing ability for the synthesis of AuNPs.<sup>79,80</sup> Similarly, banana and mango peels can synthesize AuNPs with the sizes 50 nm and 6.03±2.77 to 18.01±3.67 nm, respectively. Banana peels synthesized spherical shaped AuNPs and mango peel synthesized quasi-spherical shaped AuNPs. The reaction time for both processes was 20 and 25 minutes, respectively.<sup>81,82</sup> Apart from the above-mentioned parts of plants, rhizomes of turmeric,<sup>83</sup> yam beans,<sup>84</sup> ginger,<sup>85</sup> and seeds of cocoa,<sup>86</sup> pulp of green pepper,<sup>87</sup> bark of bay cedar,<sup>88</sup> galls of zebra wood,<sup>89</sup> latex of *Hevea brasiliensis*,<sup>90</sup> nuts of *Areca catechu*,<sup>91</sup> and effluent from palm oil mill<sup>92</sup> were found to carry out the synthesis of AuNPs.

## Algae

There are a few studies which have demonstrated the synthesis of gold NPs using algae. A few species of both marine and fresh algae were used in these studies. Among the marine red algae, *Gracilaria corticata*,<sup>93</sup> *Acanthophora spicifera*,<sup>94</sup> and *Galaxaura elongata*,<sup>95</sup> and marine brown algae, *Stoechospermum marginatum*,<sup>96</sup> *Ecklonia cava*,<sup>97</sup> *Sargassum wightii*,<sup>98</sup> *Cystoseira baccata*,<sup>99</sup> *Laminaria japonica*,<sup>100</sup> and *Turbinaria conoides*<sup>101</sup> have been previously reported to carry out the synthesis of AuNPs. On the other hand, biomass from freshwater algae including *Prasiola crista*,<sup>102</sup> *Lemanea fluviatilis*,<sup>103</sup> and *Chlorella pyrenoidosa*<sup>104</sup> can also synthesize AuNPs. Previous studies have shown that hydroxyl and carbonyl groups present in algal biomass can act as reducing agents for carrying out the synthesis of AuNPs. It has also been shown that these group can also act as the capping agent for gold nanoparticles.<sup>105–107</sup> Table 1 shows the list of various organisms that have been reported to carry out successful synthesis of AuNPs.

## Biomolecules

Molecules synthesized by living organisms to speed up their biological processes of the body are known as biomolecules and these include macromolecules such as amino acids, nucleic acids, carbohydrates, and lipids. Previous studies have reported the synthesis of gold nanoparticles using chitosan which does not only act as a reducing agent but also as a stabilizing agent during synthesis reaction.<sup>108</sup> Apart from that, starch is another polysaccharide used for the synthesis of AuNPs. In an alkaline environment starch can be degraded into short chains having carboxyl groups and the –OH group of carboxylic acid can reduce Au<sup>3+</sup> ions to gold nanoparticles.<sup>109</sup> Among proteins, consensus sequence tetratricopeptide repeat proteins and corn protein,  $\alpha$ -zein can be used to carry out the synthesis reaction of AuNPs.<sup>110,111</sup> The biological method of synthesis of AuNPs can conveniently overcome the complications of biosafety of the chemicals used for the generation of AuNPs.

## Advantages and Limitations of Biological Synthesis

Synthesis of AuNPs using biomass from bacteria is an advantageous process as some species of bacteria are not affected by the presence of heavy metals. Also, the extracellular synthesis approach produces pure nanoparticles as compared to the intracellular synthesis process which requires additional purification steps. Conversely, culturing of bacteria is a slow and tedious process so the

**Table 1** Various Types of Living Organisms That Can Carry Either Intracellular or Extracellular Synthesis of AuNPs

Name of Organism	Intra/ Extracellular	Reaction Type	References
Bacteria			
<i>Deinococcus radiodurans</i>	Extra/intra	Reduction	[311]
<i>Bacillus cereus</i>	Extra	Reduction	[50]
<i>Pseudomonas aeruginosa</i>	Extra	Reduction	[312]
<i>Rhodospseudomonas capsulate</i>	Extra	Reduction	[51]
<i>Rhodococcus</i>	Intra	Reduction	[313]
<i>Marinobacter pelagius</i>	Extra	Reduction	[52]
<i>Bacillus megaterium</i>	Extra	Reduction	[112]
Fungi			
<i>Neurospora crassa</i>	Intra	Reduction	[61]
<i>Trichothecium sp.</i>	Extra/Intra	Reduction	[314]
<i>Candida albicans</i>	Extra	Reduction	[315]
<i>Penicillium brevicompactum</i>	Extra/Intra	Reduction	[316]
Algae			
<i>Rhizoclonium fontinale</i>	Intra	Reduction	[317]
<i>Sargassum wightii</i>	Extra	Reduction	[98]
<i>Tetraselmis kochinensis</i>	Intra	Reduction	[318]
<i>Prasiola crista</i>	Extra	Reduction	[102]
<i>Shewanella</i>	Extra	Reduction	[319]
Plants			
<i>Magnolia kobus</i>	Extra	Reduction	[320]
<i>Sesbania drummondii</i>	Intra	Reduction	[321]
<i>Coriandrum sativum</i>	Extra	Reduction	[322]
<i>Tanacetum vulgare</i>	Extra	Reduction	[323]
<i>Abelmoschus esculentus</i>	Extra	Reduction	[324]

synthesis reaction of AuNPs can take a long time comprising hours and even days. On the other hand, fungi produce a large quantity of proteins and reactive compounds. Therefore, the reaction process can be easily scaled up.<sup>112–114</sup> Moreover, as compared to bacteria it is easier to culture and grow fungi. But preparing biomass from fungi for the synthesis reaction requires careful steps as it

is complicated to separate mycelia from culture filtrates. Manipulation of the genetic makeup of eukaryotes to produce desired proteins is also challenging. Also, some species of fungi are pathogenic.<sup>115–117</sup> Synthesis of AuNPs using plants based material is a facile and uncomplicated process. Various attributes of AuNPs such as shape and size can be regulated by controlling the reaction parameters. Additionally, the reaction process is fast and economical. The disadvantage of using plants for the synthesis of AuNPs is that the identification of reactive components is difficult as plant biomass comprises a large number of organic components.<sup>118–120</sup> Synthesis of AuNPs from algal biomass is also easy and simple, but algae take a lot of time to grow so the overall process can become tedious and time consuming. Biomolecules on the other hand contains various functional groups which can aid in the synthesis of AuNPs. Contrarily, as different biomaterials show different reducing ability it is imperative to first determine their reducing ability before using them in the synthesis reaction.<sup>110,111,121,122</sup>

## Stabilization of AuNPs

Nanoparticles can be stabilized using a stabilizing agent which basically assists in maintaining repulsive forces to overcome Van der Waals forces in the solution of nanoparticles to avoid agglomeration.<sup>123</sup> During the chemical synthesis of AuNPs sodium borohydride or sodium hydride, sodium citrate or ascorbic acid may act as capping and stabilizing agents for AuNPs. However, during the biological synthesis of AuNPs, stabilization of nanoparticles can be successfully achieved by using the bio-material rich in antioxidant properties. The large variety of reactive compounds present in the biomass can take part in the synthesis and stabilizing process. Various studies have reported the synthesis of highly stable AuNPs via green approach. AuNPs synthesized from *Actinidia deliciosa* showed a zeta potential value of  $-22.3$  mV,<sup>124</sup> whereas two different types of AuNPs synthesized from *Cannabis sativa* showed zeta potential values of  $-12.3$  mV and  $-20.6$  mV.<sup>125</sup> The high values of zeta potential mean that AuNPs are highly stable due to the presence of high surface charge which prevents agglomeration. Various studies have reported that phenolic compounds,<sup>126</sup> terpenoids,<sup>127,128</sup> proteins,<sup>129</sup> and nicotinamide adenine dinucleotide<sup>54</sup> might act as stabilizing and capping agents during the biological synthesis of AuNPs.

Moreover, changing the concentration of gold salt used for the synthesis reaction, pH, and temperature can also

provide control over the size and geometry of AuNPs. Derjaguin Landau Verwey Overbeek theory (DLVO) explains the whole process for stabilization of metallic nanoparticles.<sup>130,131</sup> The stabilization of NPs done by using various capping agents can be divided into three different categories, including steric, electrostatic, and unification of steric and electrostatic stabilization.<sup>132</sup>

## Electrostatic Stabilization

Ionic groups present in the liquid dispersion media can attach to the surface of a colloidal nanoparticle giving rise to a charged layer. As a result, an equal number of oppositely charged ions will border the colloidal nanoparticles giving rise to overall electro-neutral double layers.

This stabilization which involves an electric double layer originating from the presence of both repulsive as well as attractive forces between the nanoparticles as a result of the action of some ionic composites is shown in Figure 4A. These ions include polyoxyanions, carboxylates, as well as fluorides. This type of stabilization involving electrostatic repulsions inhibits the agglomeration of nanoparticles in the solution phase. Electrostatic stabilization is regulated by controlling certain significant variables including pH, concentration, and temperature.<sup>133</sup>

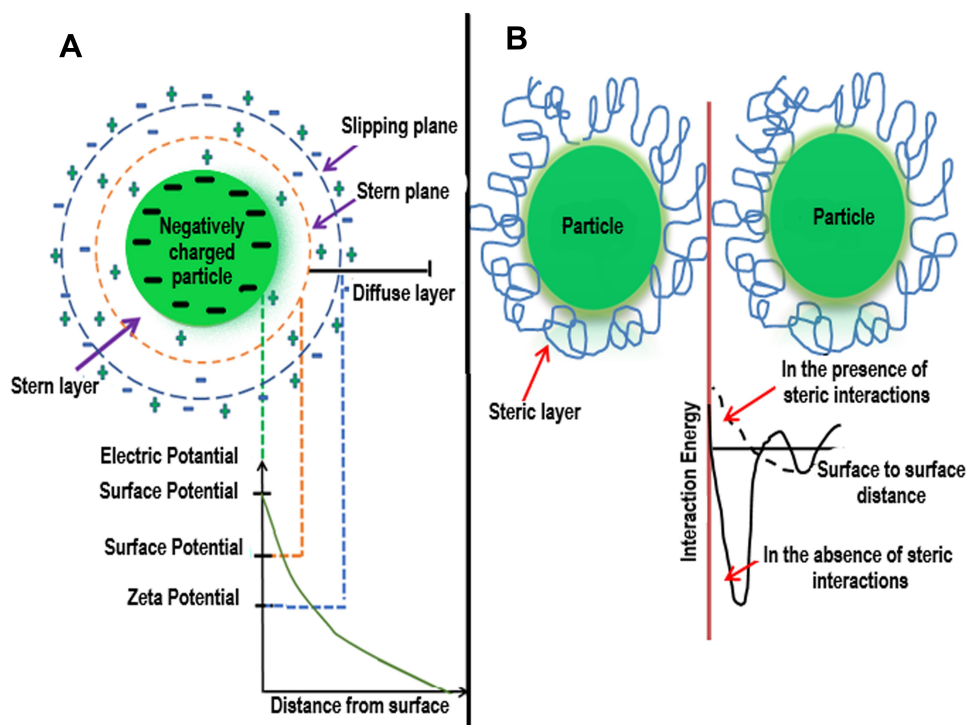
## Steric Stabilization

Steric stabilization hinders the free movement of metal nanoparticles during synthesis reactions. Stabilizing agents used in this type of stabilization include various functional groups such as hydroxyl groups, surfactants, and different oligomers/polymers. This results in the generation of a protective layer by the assimilation of the stabilizing agent at the outer surface of nanoparticles which plays an important role in the stability of metallic nanoparticles.<sup>134</sup> The mechanism of steric stabilization is shown in Figure 4B.

## Electrosteric Stabilization of AuNPs

The stability of metallic nanoparticles in solution phase can also be maintained by another type of stabilization which involves unification of electrostatic and steric stabilization. A polyelectrolyte employed as a polymeric surfactant gives combined effects of electrostatic and steric stabilization in one molecule. A double electric layer around the nanoparticle is generated by an ionic surfactant possessing extended end chains and polar head group which offers steric repulsion within the nanoparticles, thus preventing the agglomeration and giving rise to





**Figure 4** (A) Electrostatic stabilization of gold nanoparticles. (B) Steric stabilization of gold nanoparticles.

a mutual stabilization system.<sup>135</sup> Figure 5 shows the stabilization of AuNPs by the unification of steric and electrostatic interactions.

## Advantages and Disadvantages of Stabilization Methods

Although electrostatic stabilization is easier to maintain in colloidal media, there are certain limitations to it. Firstly, electrostatic stabilization cannot be achieved in electrolyte sensitive media. Additionally, due to strong forces of interactions between oppositely charged ions it is impossible to separate agglomerated particles. Moreover, it cannot be applied to multiple phase systems as different solids establish distinct surface charge and surface potential. As compared to the electrostatic stabilization, which is a kinetic stabilization method, steric stabilization is a thermodynamic stabilization method; therefore, particles can be redispersed. It is also not sensitive to electrolytes and can be applied to multiple phase systems.

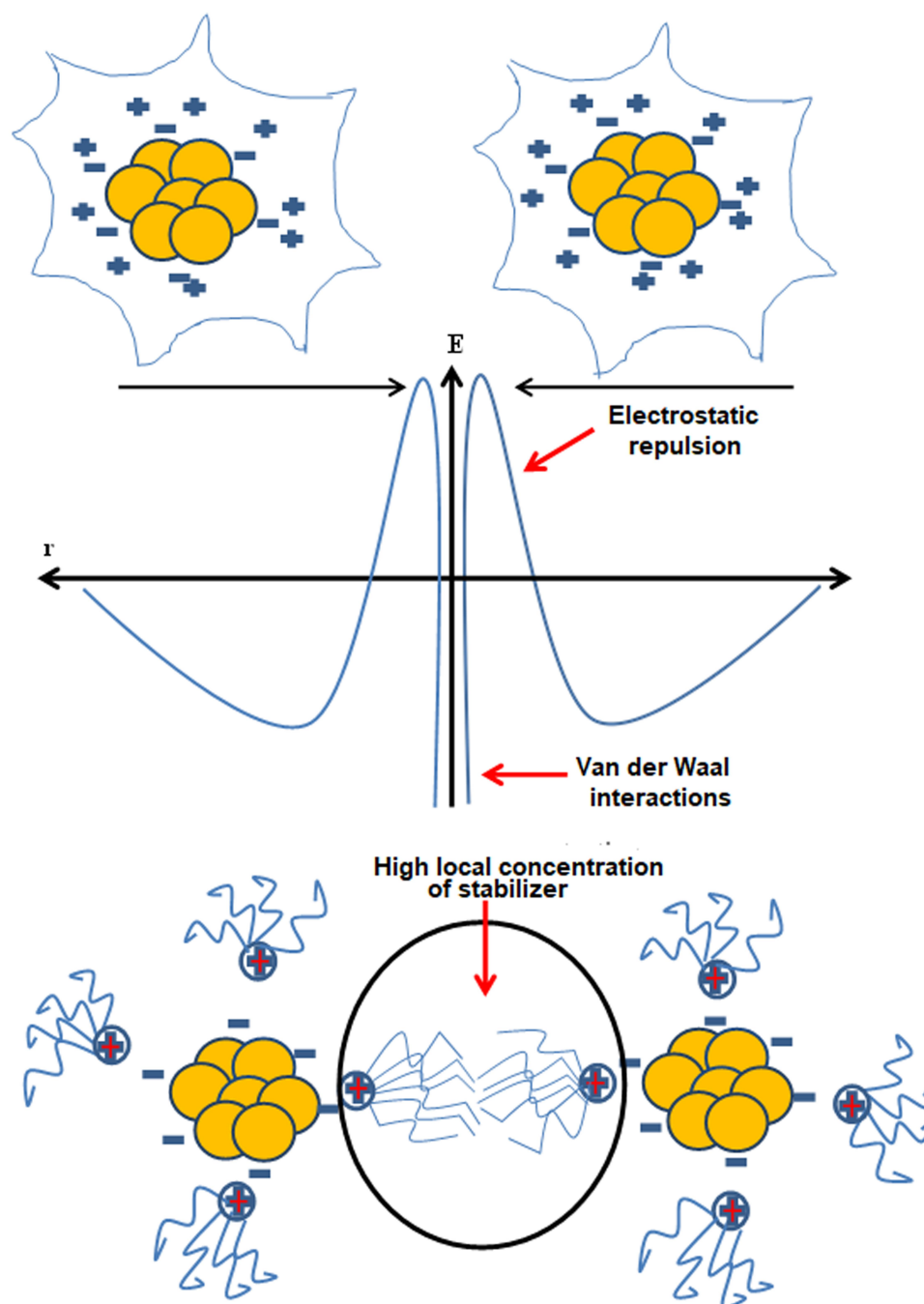
## Properties of AuNPs

AuNPs exhibit properties which are different from those shown by bulk material. These properties of AuNPs depend on their size and shape. Gold nanoparticles exhibit a wide variety of colors which include brown, purple, blue,

orange, and red in the solution form, and the color also depends on the size of the particles. Gold nanoparticles exhibit the SPR band in the range of 500–550 nm, which also depends on the size of nanoparticles.<sup>136–138</sup> The SPR band arises due to the collective oscillations of conduction electrons caused by the incident photon. Such a band is absent in AuNPs of very small size, particularly those which have a diameter <2 nm as well as in the bulk materials.<sup>139,140</sup> Apart from the size of the particles, the shape of nanoparticles, ligands, temperature and charge also influence SPR of AuNPs.<sup>141,142</sup> If the gold nanoparticles exist in the form of aggregates there is a red shift in SPR which results in the broadening of SPB resulting in the change of color of particles from red to blue.<sup>143</sup>

AuNPs also exhibit the property of quenching fluorescence of proximate fluorophore by inducing the deactivation pathway. This is done by generation of an excellent overlap between the SPB of AuNPs and emission spectra of fluorophores.<sup>144,145</sup> This phenomenon is called fluorescent resonance energy transfer (FRET) and has been observed in AuNPs as small as 1 nm in size.<sup>146</sup> AuNPs can also cause quenching by another process called photo-induced electron transfer (PET) during which AuNPs accept the photon and cause the quenching of fluorescence.<sup>147</sup>





**Figure 5** Unification of electrostatic and steric stabilization. Gold nanoparticles surround the ionic surfactants having polar ends and extended side chains. The area with high local concentration of stabilizer hinders the agglomeration of AuNPs.

AuNPs have been widely applied as surface enhanced Raman scattering (SERS) substrate for the detection of various elements in living cells. The basic mechanism of SERS is caused by two major amplifications that result in the increase in the cross-section of Raman scattering; first being the electromagnetic enhancement. The resonance of applied light field along with the collective oscillations of electrons of nanostructures cause amplification in the local

electric field at nanoparticle surface. Second is the short range chemical enhancement caused by the alteration in polarizability of molecules due to its charge-transfer interaction with the surface of nanoparticles.<sup>148</sup>

The oxidation reduction reactions of AuNPs make them efficient tools for applications in electrochemical sensing<sup>149,150</sup> and electronic devices.<sup>151,152</sup> On the other hand, the applications of AuNPs in the field of

imaging<sup>153,154</sup> and sensing<sup>155,156</sup> can be attributed to their surface enhanced raman scattering (SERS). Studies show that AuNPs can be used for calorimetric sensing<sup>157,158</sup> and laser ablation<sup>159,160</sup> because of their SPR, whereas fluorescence quenching properties of AuNPs make them well suited for the applications in the field of material engineering<sup>161,162</sup> and sensors development.<sup>163,164</sup>

Melting point of gold nanoparticles changes with the change in the size of particles. Gold nanoparticles are shown to have a lower melting point as compared to the bulk materials. This decrease in the melting point is because of the fact that the attractive forces of interaction of core get weaker due to a decrease in the number of neighboring atoms. As a result of this, the interaction between inner and surface atoms is reduced and surface atoms get higher surface energy. This leads to the decrease in the melting points.<sup>165,166</sup> Electrical properties of AuNPs are also reported to be different from that of bulk material. As the particle size decreases the surface area is increased which causes a decrease in electrical conductivity.<sup>167</sup> However, different materials can be used in combination to enhance their electrical and optical properties. For example, AuNPs can be used to enhance electrical and optical properties of zinc oxide nanoparticles.<sup>168</sup>

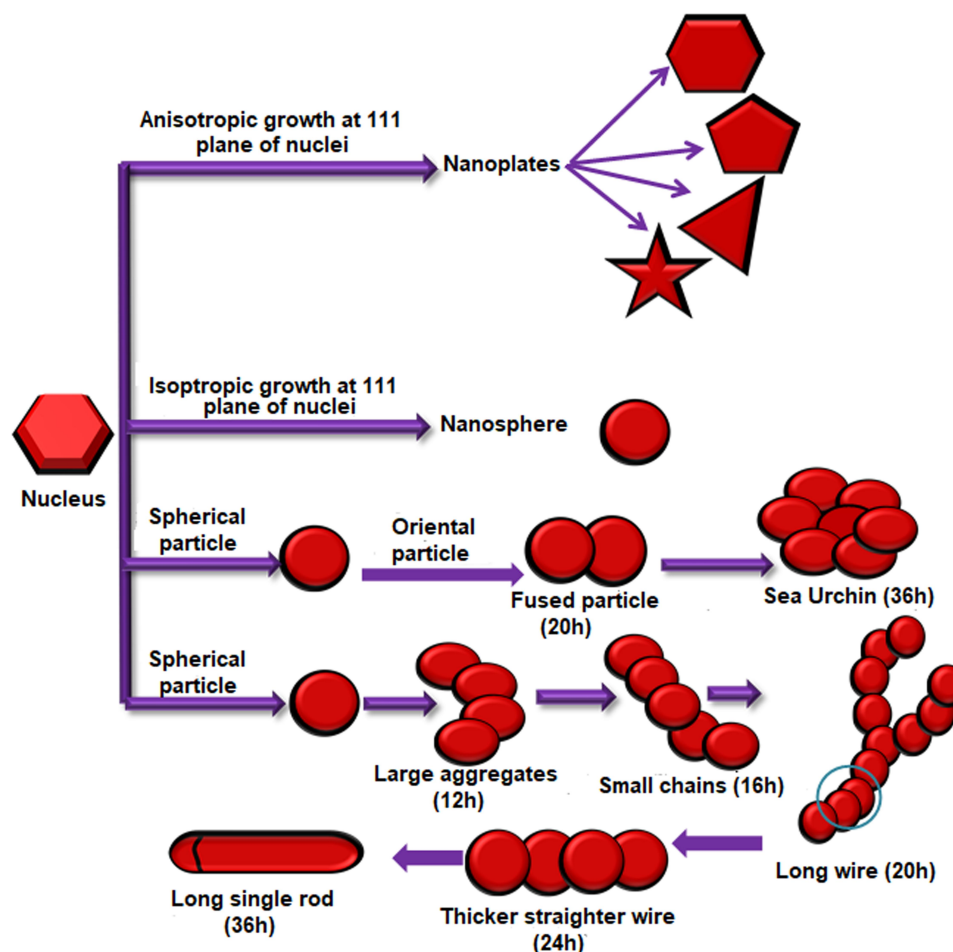
## Anisotropic AuNPs

The physical and chemical properties of AuNPs greatly depend on the shape and size of AuNPs. AuNPs of spherical shape can be synthesized by isotropic growth on gold nuclei. On the other hand, gold nanoparticles of different shapes can be synthesized by anisotropic growth on gold nuclei. As described above in the synthesis methods for AuNPs, the synthesis of anisotropic gold nanoparticles can be carried out by a two-step process, ie, seed-mediated growth. In the first step, spherical gold seeds are synthesized which have uniform size. In the second step, the reaction conditions are changed including the addition of more gold ions, along with a capping agent and reductant. The gold seeds produced in the first step act as a template on which newly reduced AuNPs deposit and form AuNPs of larger size with varied shapes. The reducing agent used in the second step is a weak reducing agent and  $\text{Au}^{3+}$  can be converted to  $\text{Au}^0$  only in the presence of seeds which act as a catalyst for the reaction. Because of the use of weak reducing agent, the second step is a slower process than the first step.<sup>169</sup> Various studies have reported synthesis of AuNPs of different shapes including gold nanorods,<sup>170,171</sup> nanocages,<sup>172</sup> nanowires,<sup>173,174</sup> nanoplates,<sup>175,176</sup> polyhedral,<sup>177,178</sup> flower shaped,<sup>179,180</sup> and star shaped.<sup>181,182</sup>

A study which involved the use of cell free extract from a fungus *Rhizopus oryzae* showed that different factors such as protein concentration, pH of solution, and time of the reaction contribute to the synthesis of AuNPs of varied shapes. When proteins got deposited at 111 plane of nuclei it resulted in the formation of nanoplates which were either triangular, pentagonal, hexagonal, or star shaped. Similarly, isotropic deposition of proteins on all planes resulted in the formation of nanospheres, and orientated attachment under the limited supply of cell free extract caused the spherical gold nanoparticles to give rise to urchin shaped nanoparticles. For the formation of 2D nanowires, first the spherical agglomerates formed followed by the deposition of protein molecules on the concave surfaces of two particles which joined together finally resulting in the formation of long wires. When these wires underwent the process of Oswald ripening it resulted in the formation of nanorods.<sup>183</sup> The schematic illustration of the study is shown in Figure 6.

## Properties of Anisotropic AuNPs

The shape of nanoparticles has an effect on their optical<sup>184,185</sup> and catalytic properties.<sup>186,187</sup> Nanoparticles having different shapes have the atoms of different faces which have different electronic distribution and are thus used to catalyze different types of chemical reactions. Anisotropic nanoparticles can show various plasmon resonances other than dipole resonance which can be attributed to their higher order modes. These additional resonances include quadruple and octopole resonances.<sup>188,189</sup> AuNPs show their typical LSPR with the maximum absorption observed at around 520 nm, but as the symmetry of AuNPs changes to gold nanorods (AuNRs) two different LSPRs can be observed, with one being at the short axis, which is transverse LSPR, and the other one being along the long axis, which is longitudinal LSPR. There is a red shift in longitudinal LSPR of the peak with the increase in the aspect ratio.<sup>190</sup> The studies have demonstrated that a peak shift and aspect ratio of anisotropic AuNRs have a linear relationship.<sup>136</sup> AuNRs have diverse applications in the field of biology. When AuNRs are exposed to laser light, they absorb the portion of that resonant light with their surface plasmon oscillations. The light that they absorb is quickly converted to heat and if the rate of heat absorption is higher than the rate of heat loss it results in the accumulation of heat inside the lattice and sometimes this heat is enough to cause the conformational change in these AuNRs, changing them to spherical AuNPs.<sup>191,192</sup>



**Figure 6** Synthesis of AuNPs of various shapes under different conditions due to different type of growths on the gold nuclei.

The aspect ratio of AuNRs is an important factor in determining the applications of AuNRs. For example, AuNRs that have higher aspect ratio respond effectively to NIR light and therefore have application in the field of Photothermal therapy. However, larger sized AuNRs having fixed and larger aspect ratios can scatter the light effectively and thus can be used for optical imaging. Higher absorption efficiency can be attributed to smaller AuNRs which make them an efficient tool for photothermal therapy.<sup>193</sup> The surface plasmon resonance of gold nanostructures having other shapes has also been studied. Gold nano-stars exhibit various surface plasmon resonances which result from hybridization of resonances related to their core and tips.<sup>194</sup>

## Functionalization of AuNPs

AuNPs can be functionalized in a number of ways which generate the possibilities for a variety of approaches for their use in designing various drug delivery systems

(DDS). When non-covalent interactions are used for the loading of drugs on nanoparticles, no specific bond cleavage is required to carry out the efficient drug release and only alterations in native physical forces are needed.<sup>17</sup> For example, the release of hydrophobic drugs can be carried out by inducing changes in local hydrophobicity. Similarly, AuNPs can be covalently bonded to the drug through cleavable bonds forming a prodrug which can be delivered to the cell liberating the drug from AuNPs using either external or internal stimuli.<sup>195,196</sup> Irrespective of the method used for the drug delivery, the modification of the monolayer of AuNPs is very important for the extracellular or intracellular discharge mechanisms.

Various mechanisms for the synthesis of AuNPs containing functional moieties are being developed in order to increase their bonding with biological molecules and to make them better drug-carriers with improved specificity. Current methods for the functionalization of AuNPs involve the use of either one or a combination of the

functional groups including oligo or polyethylene glycol (PEG), bovine serum albumin (BSA), amino acids and polypeptides, oligonucleotides, antibodies, receptors, and diverse similar particles. Table 2 shows various functional groups, their ligand moieties, and key features which make them suitable for their biological application.

## PEGylation of AuNPs

During the PEGylation, AuNPs are conjugated with PEG alone or in the presence of some other molecule in order to make the cellular uptake of AuNPs efficient. These molecules include biotin, peptides, and oligonucleotides. These functionalized AuNPs can be used for targeted drug delivery owing to their binding ability with cell membranes. Studies have been reported for the synthesis of AuNPs functionalized with lectin, lactose, and biotin along-with PEG.<sup>197–199</sup> Such functionalized AuNPs cannot only be used for cellular internalization but for intracellular internalization as well. PEGylated gold nanoparticles can also be conjugated with thiol having fluorescent dye coumarin attached on one side. These fAuNPs work as hetero-bifunctional moieties and can

make their way into the cells and be tracked simultaneously because of dye molecule attached at one end.<sup>200</sup> The study which involved the internalization of such fAuNPs for application in tumor ablation in mice has been reported. The result of this study showed that the internalization of AuNPs into cells was also affected by factors such as size of AuNP,  $M_r$  of PEG, the ligand conjugated with PEG, and physiochemical properties.<sup>201</sup>

## Peptides and Amino Acid Conjugation

Nanoparticles have also been conjugated with peptide/ amino acid in order to produce effective and targeted delivery systems. Synthesis of AuNPs conjugated with aspartic acid,<sup>14</sup> glutamic acid, phenylalanine and tryptophan,<sup>202</sup> L-cysteine,<sup>203</sup> lysine,<sup>204</sup> and peptides<sup>15,205</sup> has been reported. Amino acids and peptides bind with negatively charged AuNPs through amine groups, whereas the negatively charged carboxylic groups extend outwards to stabilize AuNPs. Some amino acids including lysine, poly-lysine, and glycine exhibit higher efficacy to conjugate with DNA and can be used for DNA or gene delivery

**Table 2** List of Various Functional Groups, Their Ligands, and Key Features Which Make Them Suitable for Biological Applications

S. No.	Functional Groups	Ligands	Key Feature	Application	Ref.
1	Polyethylene Glycol (PEG)	Thiol (-SH) group	Adhesion with plasma membrane	Cell targeting, intracellular targeting, biodistribution analysis	[197,199,325–327]
2	Peptides	Cell surface receptors, amyloid inhibitory peptide + sweet arrow peptide, antibody, octreotide peptide	Cytoplasmic and nuclear translocation, adjuvant, targeting carcinoma cells analog of somatostatin	Cellular and intracellular targeting, macrophage and proinflammatory cytokine elicitation bioimaging imaging of cancer cells	[209,328–331]
3	Carboxyl group	Amino acids	Delivery of various agents	Various applications which depend on the amino acid used	[27]
4	Amine Group	PEG	siRNA delivery	RNA technology	[207]
5	DNA	Aptamer, PEGylated gold poly ( $\beta$ -amino ester), Thiolated ssDNA of RNA I gene, antisense DNA oligonucleotides	Targeting Prostate cancer cells, siRNA carrier, binds to antisense RNA of p53	Bioimaging, gene delivery RNAi-regulation of transgene expression, detection of specific genes, eg, for microbial detection	[220,332–335]
6	RNA	Polyvalent RNA-gold nanoconjugates	RNA delivery	RNA technology	[336]
7	Folate	Folate	Targets cancer cells that are folate receptor positive	Cancer targeting	[337]

without inducing any cytotoxicity. Anionic DNA shows higher binding capacity with the positively charged ammonium ions present on amino acids. One possible method could be the use of AuNPs having thiol end which can then be conjugated with amino acids to give out positive amino groups. These amino groups will bind with DNA through ion pairing. A study which reported the similar mechanism for the synthesis of lysine and poly-lysine conjugated AuNPs expression for gene delivery found that lysine dendrons were better at expressing reporter  $\beta$ -galactosidase gene as compared to polylysine.<sup>206</sup> Similarly another study has reported that AuNPs functionalized with positively charged amine groups were able to effectively conjugate with negatively charged PEGylated siRNA. When these fAuNPs were tested against prostate cancer cell lines they efficiently inhibited cancer genes. Also, specific cancer genes were efficiently inhibited using these amine functionalized AuNPs carrying siRNA-PEG conjugates when used against human prostate cancer cells.<sup>207</sup> Later a study reported triethylenetetramine (TETA) functionalized gold cores of size 2 nm which featured biodegradable glutamic acid scaffolds and showed that positively charged amino groups of TETA moieties interact electrostatically with anionic siRNA. These dendronized AuNPs were very effective at suppressing expression of  $\beta$ -gal (-50%) with the least toxicity.<sup>208</sup> Proteins can also be conjugated with AuNPs via glutamic acid which conjugates with AuNPs through their amino groups and their carboxyl groups extending outwards bind with amino groups of proteins. On the other hand, the attachment of protein with glutamic acid can induce certain conformational changes in proteins.<sup>27</sup> Lysine conjugated AuNPs were first synthesized and then conjugated with doxorubicin and the results showed that most of the drug was released within 12 hours.<sup>204</sup>

Gold nanoparticles functionalized with peptides have also been reported for the application in targeted drug delivery. Peptide-drug conjugates (PDC) are an efficient and effective tool for delivering drugs to cancer cells. Phage Peptide P4 conjugated with 2-chlorotriyl resin was tested against A20 leukemic-like cell line. The results showed that chlorambucil which previously was less effective as compared to bendamustine was equally as effective as bendamustine when used in conjugation with peptides fAuNPs.<sup>15</sup> Phospho-peptides have also been investigated for application in drug delivery. The results have shown that phospho-tyrosine modified AuNPs when used as a delivery vehicle for doxorubicin were more effective at killing SGC-7901 cells.<sup>205</sup>

Another study reports the conjugation of peptide CALNN and its derivative CALNNR8 with AuNPs for targeting components present inside the cell. AuNPs with the size of 30 nm could cross the cell membrane more easily through the processes of endocytosis and micropinocytosis. Both of these functionalized nanostructure types demonstrated higher binding capacity for DNA, RNA as well as for the organelles like endoplasmic reticulum. Upon testing CALNN and CALNNR8 together for internalization in cells it was found that both of them could enter in the nucleus but the most of the CALNNR8 was still bound with endoplasmic reticulum as ER has a high binding affinity for signal peptides rich in arginine.<sup>209</sup>

## Oligonucleotide Functionalized Nanoparticles

Synthesis of DNA functionalized inorganic nanoparticles is an area of fascination for researchers because of their definite structures and functions,<sup>210–212</sup> and possesses a programmable assembly process as far as the sequence, length, and structure of DNA is concerned.<sup>213,214</sup> Similarly, the synthesis process can be controlled either to form oligomers or large agglomerations. Moreover, it is even possible to control the separation distance between the assemblies of nanoparticle to produce crystals of nanoparticles.<sup>215–217</sup> DNA functionalized nanoparticles can be synthesized under specific conditions. DNA can be used as in the form of a single strand capped with thiol as well as by saturation of the AuNPs' surface with single stranded DNA molecules.<sup>218</sup> Studies carried out on kinetics and thermodynamics of DNA conjugated with AuNPs have demonstrated that ssDNA first attach to AuNPs and then gradually spread on their surface.<sup>219</sup> Another study has shown that conjugation of aptamers through hybridization reactions on oligonucleotide functionalized gold nanoparticles is a better approach than carrying out the direct conjugation of aptamers with AuNPs. The first advantage is that the integrity of aptamers remains intact and a smaller amount of aptamers is required to carry out the conjugation process. This technique was employed for the detection of prostate cancer cells.<sup>220</sup>

## Other Common Functionalization Methods

Several molecules other than proteins, amino acids, and nucleic acids have also been used for the functionalization of AuNPs for diverse applications. One study has reported



the functionalization of AuNPs with anti-human IgG to develop a technique for the detection of human IgG in blood serum samples and the results were found to be consistent with enzyme-linked immunosorbent assay (ELISA).<sup>221</sup> Apart from that studies have been carried out for functionalization of AuNPs with antibodies for application in the detection of *E. coli*. Antibody conjugated AuNPs interacted with *E. coli* O157:H7 through EDC coupling chemistry.<sup>222</sup> In addition to that, in a study the enzyme glucose oxidase was attached on chitosan conjugated AuNPs for the detection of glucose. The results showed that the method was effective for the enzyme to retain its enzymatic activity, even at extreme conditions, including higher temperature.<sup>223</sup> Another study has reported the application of AuNPs in the detection of 5-fluorouracil; an anti-cancer drug, as AuNPs show a quenching effect against the fluorescence produced by 5-fluorouracil. Along with the detection of 5-fluorouracil, this conjugate was also shown to have antifungal and antibacterial properties.<sup>224</sup> Depending on the application of AuNPs, there are many studies which have used the combination of proteins, oligonucleotides, and antibodies for the functionalization of AuNPs. This type of functionalization of AuNPs is increasing now and has diverse applications in the field of biomedical sciences.

## Advantages and Limitations of Functionalization Methods

Functionalization allows us to impart AuNPs with multimodal features.<sup>225</sup> Physical aspects of AuNPs can be modified through functionalization, making them efficient for clearance. AuNPs when layered with small molecule or polymers such as poly(ethylene glycol) (PEG), the resultant nanostructure displays improved blood circulation with better biodistribution and active cellular uptake. PEG also reduces the degree of attractive forces between AuNPs by expanding the steric gap between the particles and developing hydrophilicity through hydrogen bonds with solvent.<sup>226</sup> PEGylation can also result in the alteration of the size of the particle. The rate of renal filtration of particles with the size >10 nm reduces  $t_{1/2}$ ; though a bigger size (>100 nm) enhances their uptake by liver and reduces EPR extravasation.<sup>227</sup> PEG also alters the flexibility of NP which can become “softer” due to PEGylation, thus effecting extravasation. Though the toxicity caused by PEG is little but is inversely proportional to the molecular weight, mainly after oral consumption. NPs and not the PEG corona are the cause of toxicity caused after

intravenous injection.<sup>228</sup> Though the hindrance of PEG to the degradation by serum is advantageous from a stability perspective, functionalized AuNPs which are harmlessly biodegraded in-vivo after a certain time period are desired. An ultimate challenge of PEG coating is its fragmentation by light, heat, or stress, resulting in the diminishing of its coating ability.<sup>229</sup>

Similarly, functionalization of gold nanoparticles with some amino acids can cause the aggregation of nanoparticles. Thiol chemistry can be used to our advantage, but the thiol groups can be replaced by other thiol groups present in the high concentration in living organisms.<sup>230</sup> The other challenge is formulation-function challenge which is to determine the exact sequence of peptides to be used to obtain the desired function. The results of another study which involved the synthesis of L-Arginine (Arg) functionalized AuNPs showed that Arg and nanoparticles interact through covalent or coordination-like bond, and the resultant steric limitation on binding of gold nanoparticles to Arg results in reducing the coverage of Arg.<sup>231</sup> On the other hand, the potential of oligonucleotides functionalized AuNPs has been generally shown using in-vitro analysis; but, there are questions to be addressed before conjugates of AuNPs and oligonucleotides can be moved to clinical applications. First of all, diminishing short- and long-term cytotoxic effects of AuNPs is indispensable. Various investigations have reported the biocompatibility of such therapeutic AuNPs through uncomplicated cytotoxicity studies, though comprehensive toxicological assessment needs to be appropriately carried out. Secondly, delivery of these carriers to targeted organs and tissues is essential to lessen side-effects. Coating AuNPs surface with precise antibodies destined to the damaged cells and decorating them with functional groups like polyethylene glycol and zwitterionic moieties to avoid adsorption of plasma protein, bettering the pharmacokinetics and escaping immune system can be done to achieve the desired drug targeting. Lastly, immunological problems are required to be entirely researched prior to the clinical application of any novel material.<sup>232</sup>

## Applications of AuNPs in Drug Delivery

Gold nanoparticles have lately been exploited as an excellent applicant for delivering numerous drugs to their target sites.<sup>10,11</sup> These payloads range from small drug molecules to bigger biomolecules such as RNA, DNA, and proteins.

Effective discharge of these payloads is an essential factor to be considered for efficient therapy. The release of therapeutic agent from gold nanoparticles can be achieved by using internal stimuli such as glutathione,<sup>233</sup> pH etc.,<sup>234</sup> and as well as external stimuli such as light.<sup>235</sup> Drug targeting can be generally categorized into passive or active targeting. In the “passive targeting,” drug or nanoparticle is built-up at the targeted site by using their physiochemical properties like weight and size, extravasation, and pharmacological aspects.<sup>236</sup> However, during the “active target”, the drug molecule or nanoparticle are modified by attaching them with a definite active molecule for targeting particular cells. For instance, studies have reported the targeting of nanoparticles to specific phagocytic cells<sup>237</sup> and to tumor cells.<sup>238</sup> But the major factor that plays a significant role in such targeting is surface modification and functionalization.

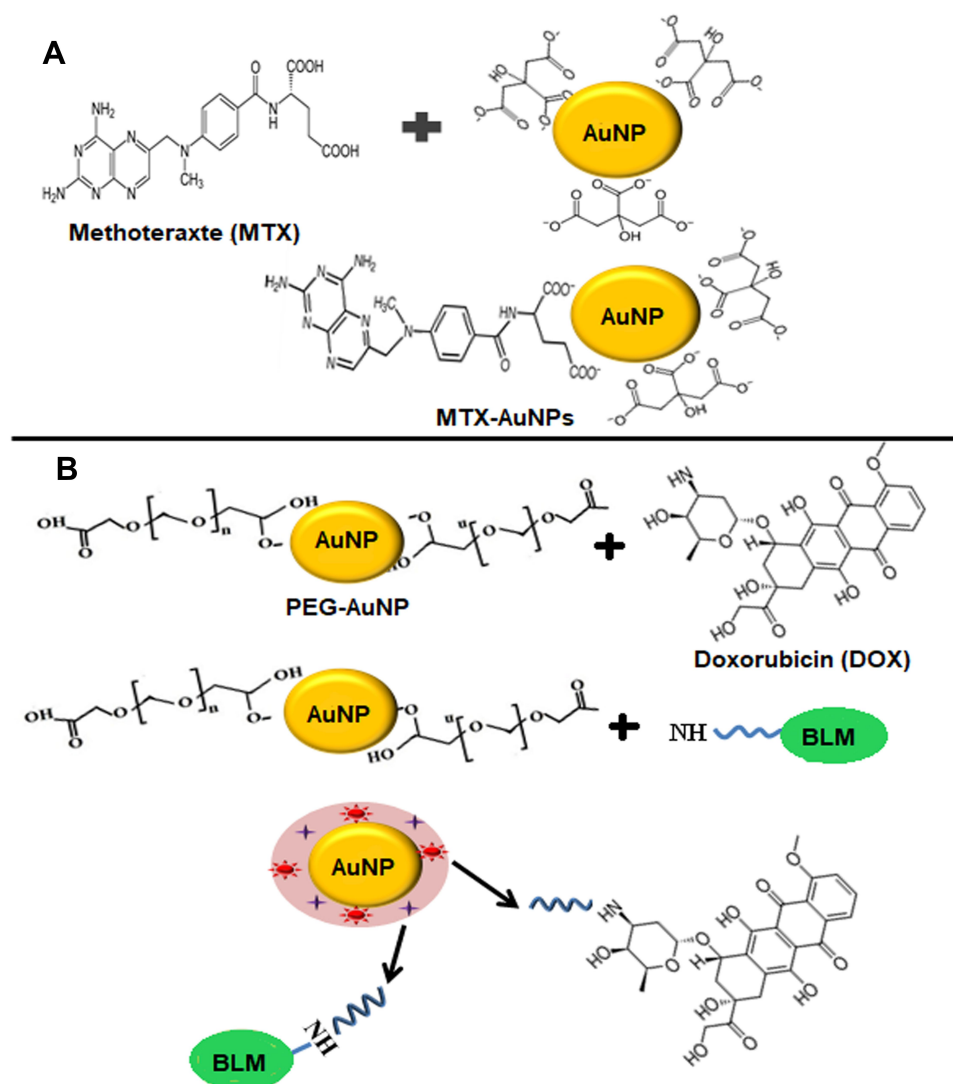
## Direct Conjugation of AuNPs with Drug

AuNPs can be conjugated directly with drug molecules or antibiotics with the help of physical absorption, covalent, or ionic bonding. One example is methotrexate (MTX); a folic acid analog that was conjugated to 13 nm colloidal gold nanoparticles to disrupt the folate metabolism in the cancer cells and has mainly been used as a cytotoxic anticancer drug. After the overnight incubation, carboxylic groups on the molecule of methotrexate had the ability to bind with the surface of gold nanoparticles and as reported the tumor cells had higher concentration of AuNP conjugated methotrexate as compared to that of free methotrexate. Also, the conjugated form exhibited a 7-times higher cytotoxic effect in Lewis lung carcinoma mouse models as compared to free methotrexate.<sup>12</sup> The schematic illustration of mechanism used for the synthesis of MTX conjugated AuNPs is shown in Figure 7A. Saha et al,<sup>239</sup> in another example, directly conjugated non-functionalized spherical gold nanoparticles of about 14 nm diameter to several different antibiotics such as streptomycin, ampicillin, and kanamycin by physical means. As a result, conjugated antibiotics showed more stability and higher inhibition of bacterial growth than their free forms.

## Alterations in AuNPs' Surface for Drug Conjugation

During the conjugation process of nanoparticles and biomolecules, the surface chemistry of a nanomaterial plays the vital role. For the drug delivery system there are four

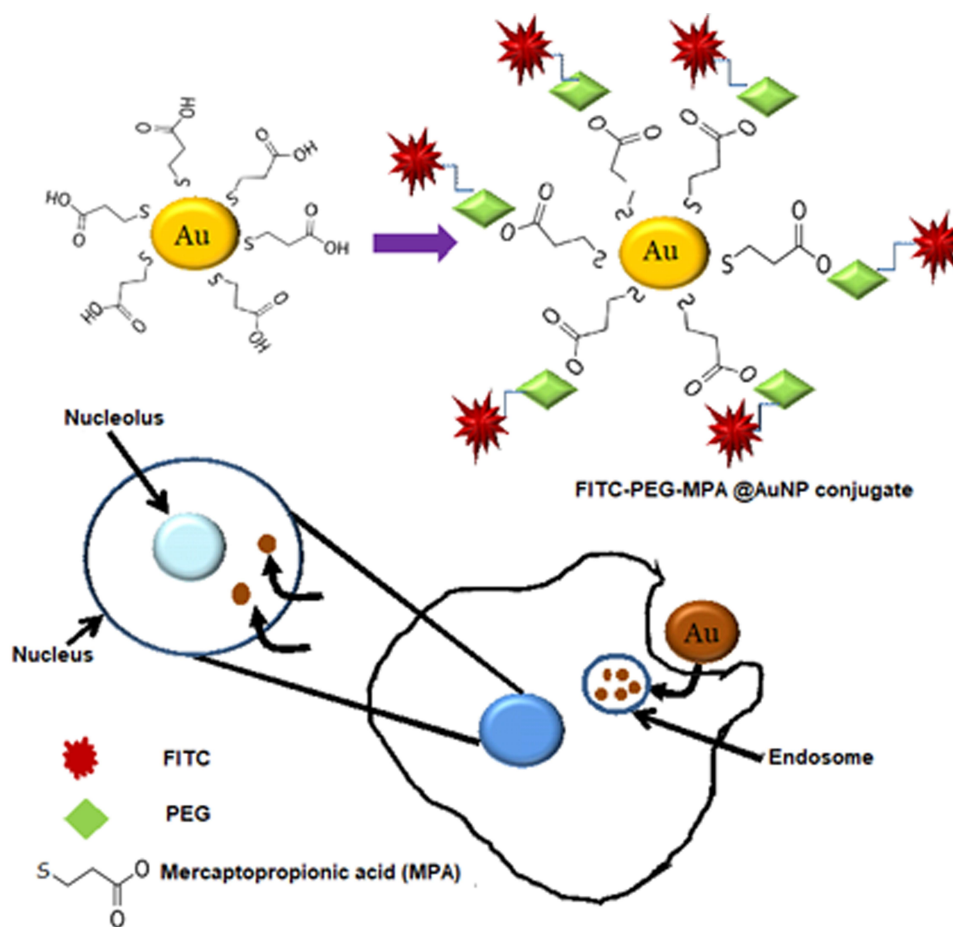
main reasons to define why the modifications in the surface of AuNP could be worthy. The primary reason is slowing or preventing the removal of conjugate by reticulo-endothelial system (RES) and to increase the conjugate's lifetime of circulation. The second reason is the proper attachment of therapeutic molecules and desired targeting. Another reason is preventing the aggregation of nanoparticles and improving their stability. Lastly, surface modification can solve the problem of cytotoxicity in gold nanoparticles due to original capping ligands. There are many studies on the methodology to improve the bio-stability, biocompatibility, and water-solubility of gold-bioconjugates. A number of studies have highlighted the properties of polymer-modified gold nanoparticles. Farooq et al<sup>240</sup> showed the synthesis of doxorubicin (DOX) conjugated PEGlyated AuNPs, bleomycin (BLM) conjugated PEGlyated AuNPs, as well as both DOX and BLM conjugated PEGlyated AuNPs. The results provided evidence that nanohybrid drug carriers significantly resulted in the decrease of half-maximal drug concentration to be effective. The mechanism for the synthesis of nanohybrid drug carrier is shown in Figure 7B. Studies have shown that PEG inhibits the agglomeration of gold nanoparticles in an environment with high concentration of ions and favors a longer particle circulation in the vivo systems.<sup>200,241</sup> In a different study carried out in mice, the biological distribution of PEG modified AuNRs was compared with non-modified AuNRs and results showed that when PEG-modified gold nanorods were injected in mice 54% of the gold nanorods were found in blood at 0.5 hours after injection while, at 72 hours, 35% of AuNRs were found accumulated in the liver. On the other hand, at 0.5 hours the gold nanorods without modification were already 30% accumulated in the liver.<sup>242</sup> In a different study, coumarin dye was conjugated with one end of PEG spacer and then AuNPs were conjugated with coumarin-PEG-thiol and the results showed that these particles were not toxic and can enter into the cells after 1 hour of incubation and were found localized in the peri-nuclear region. These particles internalized into cells by non-specific endocytosis.<sup>200</sup> The PEG-modified gold nanorods can be used as a contrast agent for in vivo screening of organs using the light in the near-infrared region.<sup>242,243</sup> Bhattacharya et al<sup>244</sup> reported that gold nanoparticles functionalized with PEG-amines and folic acid via non-covalent interactions target the cancerous cells which have folate receptors. Takahashi et al<sup>245</sup> used the technique “layer-by-layer” surface modification to modify phosphatidylcholine-gold nanorods (PC-NR) with polyethylenimine (PEI) and bovine serum albumin (BSA) which showed



**Figure 7 (A)** Direct conjugation of AuNPs with methotrexate drug (MTX). Methotrexate molecule possesses two amine and carboxylic groups and exchanges citrate ions present on the surface of citrate capped AuNPs to form MXT-AuNPs. **(B)** Surface modification of AuNPs with PEG for conjugation of Doxorubicin (DOX) and Bleomycin (BLM). During the conjugation reaction, carboxylic groups on PEG forms amide bonds with amino groups present on BLM and DOX.

greater stability in electrolyte buffer solution and as a result prevented their aggregation under physiological conditions and caused increased uptake of the nanoparticles and cellular binding. Recently, Gu et al<sup>246</sup> exhibited another type of surface-functionalized gold nanoparticle that had the ability to focus a payload to the core of the cell. The surface of the circular gold nanoparticles with a diameter of 3.7 nm was altered with 3-mercaptopropionic acid (MPA) to frame a self-assembled monolayer. The carboxylic end groups on the particles were then conjugated with amine end-groups on the NH<sub>2</sub>-PEG-NH<sub>2</sub>. This conjugation brought great stability and high proficiency of intracellular vehicles for the targeted delivery to the nucleus. The schematic representation of this study is shown in Figure 8. Gold

nanoparticles functionalized with paclitaxel are another example of a nano-sized drug delivery framework. The C-7 position of paclitaxel was bonded with hexa-ethylene glycol (carboxyl-terminated linker) which was then directly conjugated to 4-mercaptophenol-coated gold nanoparticles of diameter 2 nm. The results showed that approximately 70 molecules of paclitaxel were conjugated per nanoparticle.<sup>247</sup> As illustrated above, there are diverse ways of surface modification of gold nanoparticles. Among covalent and non-covalent reaction based modifications, the covalent interactions are stronger but the interesting assemblies are provided by non-covalent interactions of NPs with the biomolecules.<sup>248</sup> Due to the stable covalent interaction the problem of effective release of the drug



**Figure 8** Surface modification of AuNPs with mercaptopropionic acid. These fAuNPs were conjugated with PEG- FITC (fluorescein isothiocyanate). These AuNPs entered the cell through endocytosis and were found localized in the endosomes and nucleus.

payload on the target site needs to be addressed. To solve this problem, surface modification with functional ligands having acid sensitive or amine groups can act as a site for non-covalent binding of drug molecules.<sup>249</sup> Surface coating with polyelectrolytes such as poly(diallyldimethyl ammonium chloride; PDADMAC), poly(sodium-4-styrenesulfonate; PSS), and poly(allylamine hydrochloride; PAH) has already been used.<sup>250,251</sup> So, to avoid any agglomeration, a careful surface modification strategy is needed to deliver the payload.

### Limitations of AuNPs in Drug Delivery

Although gold nanoparticles show a promising future in the field of drug delivery, the other side of the coin is imperative to be considered, ie, their potential side-effects. Although most of the concerns related to biological applications of gold nanoparticles have been addressed in various studies, their results seem to contradict each other, and final answers to all the questions related to

biocompatibility, biodistribution, cytotoxicity, retention, and clearance time are still required. One major limitation of gold nanoparticles in drug delivery is their non-specific targeting and the capability of stimulating the host's immune system. These problems have been addressed by modifying the surface of AuNPs with PEG, thereby masking their surface and leaving them inactive regarding protein adhesion on the surface, thus minimizing the likelihood of immune system stimulation. Unexpectedly, such perfect coating not only causes nanoparticles to become "invisible" to the immune system but it also makes them lose their capability to adhere to definite receptors. To overcome such in vivo obstacles, gold nanoconjugates are additionally altered using targeting ligands. But this detailed alteration of the surface may give rise to undesirable toxic effects. Considering the mechanism by which conjugated ligands may result in the alteration of the pharmacokinetics, biodistribution, and ultimate potential side-effects is also very crucial. Some toxicity can be

attributed to specific type of ligands. For instance, in-vitro toxicity has been reported only for cationic ligands.<sup>252</sup> Moreover, as a result of chemoresistance and the diversity of genetic makeup of cancer cells, not all the therapeutics may be effective for every patient. The efficacy of nano cancer drug carriers can be increased by decorating nanoparticles with stromal antagonists. However, to facilitate the specific targeting of nanotherapeutics, additional studies are imperative to discover new molecular targets expressed only in the cancer microenvironment. One possible way to solve the problem of cancer heterogeneity is to possibly target stromal cells. Other important candidates for active drug targeting are cancer stem cells (CSC). Potential solution for eradicating chemoresistance of cancer cells is by eliminating chemoresistance of CSCs.<sup>253</sup> Consequently, there several serious concerns that require serious attention, for instance, dependable formulation assays, deep rooted side effects, and cellular and immune reactions. This calls for continued research in the development of the techniques described above, particularly with respect to active targeting.<sup>254</sup>

## Delivery of Large Biomolecules Using AuNPs

The capability of gold nanoparticles in delivering large biomolecules, such as peptides, nucleic acids, and proteins, has also gained success. Various biomolecules including genes, oligonucleotides, proteins, and peptides are various types of biomolecules which have been delivered to target cells using AuNPs as delivery vehicles.

## Nanoparticle-Based Genetic Therapy

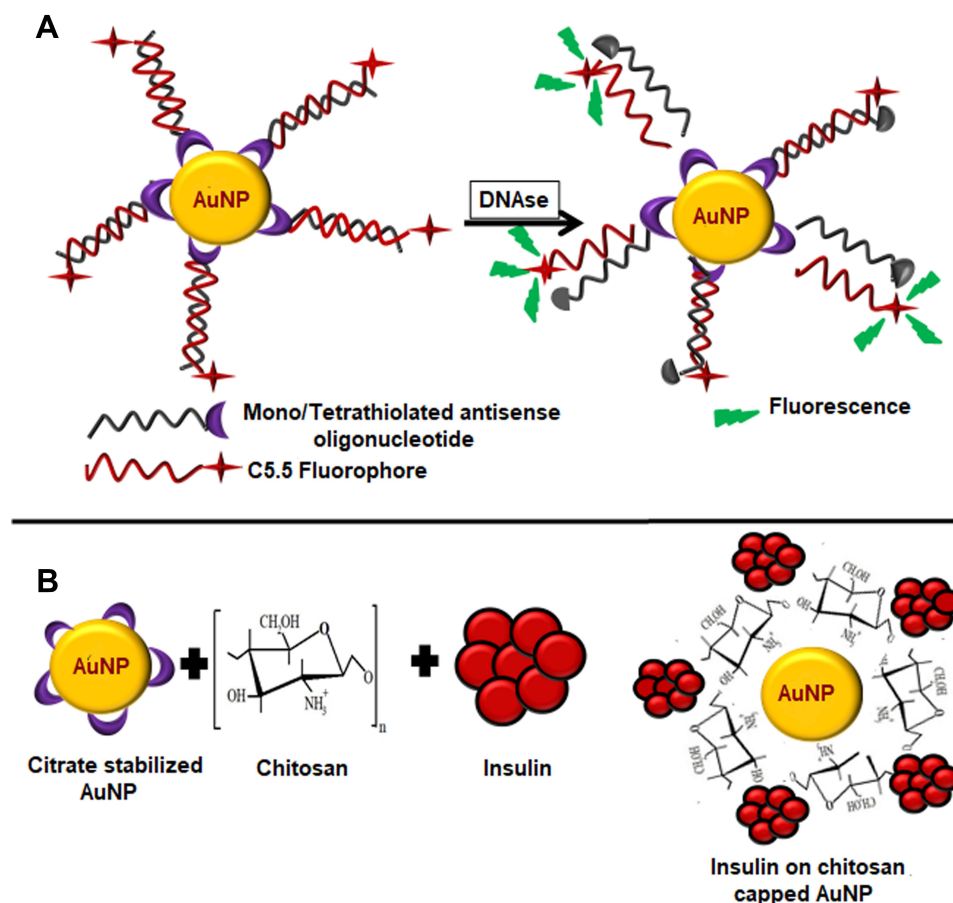
The ideal approach to treat genetically acquired disease is via Gene therapy.<sup>255</sup> Viruses also provide a vehicle for highly efficient gene therapy,<sup>256</sup> but they have raised safety concerns which arise due to immune response and random cytotoxicity.<sup>257</sup> Conversely, at present less efficiency has been reported via non-viral gene delivery systems.<sup>258</sup> An effective delivery vehicle should provide efficient entry into the cell, protection of nucleic acid against degradation by nucleases, and release of the nucleic acid in functional form in the nucleus.<sup>259</sup> Nanoparticles, on the other hand, have outstanding therapeutic effects and are capable to deliver all kinds of oligonucleotides such as single stranded DNA (ssDNA), double stranded DNA (dsDNA), plasmids, and single stranded RNA (ssRNA). Gold nanoparticles such as

nanorods and nanospheres give protection to nucleic acid and prevent their degradation by nuclease. Oligonucleotide and siRNA-modified AuNPs conjugates are used in gene delivery and gene therapy as intracellular gene regulatory agents which are able to activate immune-related genes.<sup>260</sup>

AuNPs can be conjugated with oligonucleotides using both covalent and non-covalent interactions. Nucleic acid strands can be modified with thiols (-SH) for covalently grafting them onto nanoparticles. In one study, citrate-capped AuNPs were functionalized with antisense oligonucleotides using cyclic disulphides (DTPA) anchoring group and alkyl-thiol anchoring groups to produce tetra-thionate particles and mono-thiolated particles. The particles complexes were found to possess high affinity constant for the complementary nucleotide sequence and showed 99% higher cellular internalization without causing any cytotoxicity. When treated with DNase, AuNPs bound antisense oligonucleotides degraded at a much slower rate than the free antisense oligonucleotide duplexes.<sup>261</sup> The basic mechanism of the study is shown in [Figure 9A](#). A group has reported the synthesis of polyvalent nucleic acid and AuNPs conjugates by covalently bonding AuNPs with thiol modified nucleic acids. The resultant conjugate was resistant to any degradation by enzymes and showed high cellular internalization.<sup>262</sup> In another study, the same group has applied their "antisense particles" for tumor suppressing. They used mimics of tumor suppressive miRNA-miR-205 for functionalization of AuNPs and sense strand was linked to AuNPs through absorption of alkyl thiol linkage. These conjugates of miR-205 down-regulated the expression of miRNA target protein and successfully inhibited cancer cell proliferation as compared to non-targeted AuNPs.<sup>263</sup>

Nucleic acids can be conjugated with AuNPs via non-covalent interactions as well. Strongly anionic nucleic acids can interact with cationic AuNPs through electrostatic interactions. Gold clusters protected with mixed monolayer functionalized with quaternary ammonium salts were tested for their ability to transfect plasmid DNA. The results showed that various factors contribute to the successful transfection assemblies which include DNA:AuNPs and hydrophobicity.<sup>264</sup> Zhao et al<sup>265</sup> developed gold nanoparticle-based nano-carriers with polyallylamine hydrochloride (PAH) and poly-sodium 4-styrenesulfonate (PSS), and they were able to deliver a small interfering RNA (siRNA) targeting LSD1 gene to induce the differentiation of human mesenchymal stem cells (MSCs). The results of this research may contribute to





**Figure 9** (A) Citrate capped AuNPs conjugated with mono/tetrathiol modified antisense oligonucleotides treated with DNase. (B) Schematic illustration of preparation of insulin loaded AuNPs. Chitosan acts as a reducing and stabilizing agent during the formation of AuNPs. Insulin reacts with chitosan capped AuNPs through hydrogen bonding.

tissue regeneration therapy by delivering siRNA. An effective scaffold for binding of gold colloids with DNA can be produced by functionalizing AuNPs with amino acids. AuNPs functionalized with lysine dendron have been reported to be 28-fold more efficient in gene expression as compared to polylysine.<sup>206</sup>

## Nanocarriers for the Delivery of Protein

Gold nanoparticles can act as nanocarriers of proteins and peptides of interest. Verma et al<sup>266</sup> reported that cationic tetraalkyl ammonium functionalized gold nanoparticles identify the surface of an anionic protein  $\beta$ -galactosidase via complementary electrostatic interaction and restrain its activity which can be reversed by cellular concentrations of glutathione. The study showed that glutathione-mediated discharge of enzyme  $\beta$ -galactosidase from AuNPs, which depends on the chain length of monolayer, makes it a potential transporter of the protein. In an earlier study, gold nanoparticles functionalized by chitosan have been used to

deliver insulin. Chitosan is a non-toxic biopolymer used to synthesize and stabilize the nanoparticles. Chitosan functionalized insulin loaded AuNPs were found to lower the blood glucose level to 30.41% after 2 hours of oral administration.<sup>267</sup> The schematic illustration of this study is shown in Figure 9B. AuNPs conjugated with cell penetrating peptides and lysosomes sorting peptides were tested for their targeted localization into lysosomes. The results showed that these functionalized AuNPs can be efficiently delivered into lysosomes while causing minimum cytotoxicity. Schäffler et al<sup>268</sup> used gold nanoparticles for conjugation with human serum albumin (alb-AuNP) or apolipoprotein E prior to their intravenous injection. The outcome of the study demonstrated that protein conjugation extremely reduced the liver retention of AuNPs. This study suggests that the stable conjugation enhances the efficiency and specificity of nanoparticles in the target organ, therefore signifying a potential application in nanopharmacology and nanomedicine.

## Limitations of Delivery of Large Biomolecules Using AuNPs

The delivery of large biomolecules to the cells not only requires targeting to a site but also cellular internalization and sometimes intracellular release of the cargo. Therefore, various factors are to be considered before using gold nanoparticles in biomolecule delivery. As the size and shape of nanoparticles influence their cellular uptake, fabrication of gold nanoparticles of desired shape and size is critical. For the targeted drug delivery, the decoration of surface of AuNPs with specific ligands is very important. Moreover, lack of investigation on biodistribution and retention of these gene carriers is a major problem. In vivo investigations are needed to determine the response of living systems to these nanocarriers.

## Drug Release from AuNPs

### pH-Mediated Drug Release

One of the most appropriate conditions for the release of drug at site of the target over the surrounding tissues is pH.<sup>269</sup> The acidic environment with the pH range from 5.7–7.8 is present in human cancer cells or inside the cell organelles including endosomes and vesicles.<sup>270,271</sup> These specific pH conditions lead to the cleavage of acid sensitive bond and charge switching due to protonation and morphological alterations of carriers. For example, the acidic conditions (pH 5.0) in lysosomes or endosomes or both can cause the cleavage of the hydrazone bond which is an acid-sensitive bond.<sup>272</sup> This property of hydrazone bond has widely been used in the preparation of pH-responsive supramolecular fabrications for intracellular drug release. A study has reported the AuNPs modified with methyl thioglycolate (MTG) and thiolated methoxy polyethylene glycol (HS-mPEG) having a molar ratio of 1:1. When doxorubicin (DOX) was conjugated with MTG through hydrazone bond the resultant DOX-AuNPs conjugates showed higher pH-sensitive drug release under the pH 5.3 as compared to the normal pH 7. The results showed that after 28 hours of incubation the released DOX drug can be located in the perinuclear region and the nuclei of 4T1 cancer cells.<sup>273</sup> The schematic illustration of this study is presented in Figure 10A. A study has also shown the synthesis of AuNPs functionalized with PEG ligands terminated with DOX having hydrazone bond between PEG and DOX for the release of therapeutics under low pH. These particles were found to enter the cells through endocytosis. Apart from that, DOX-AuNPs

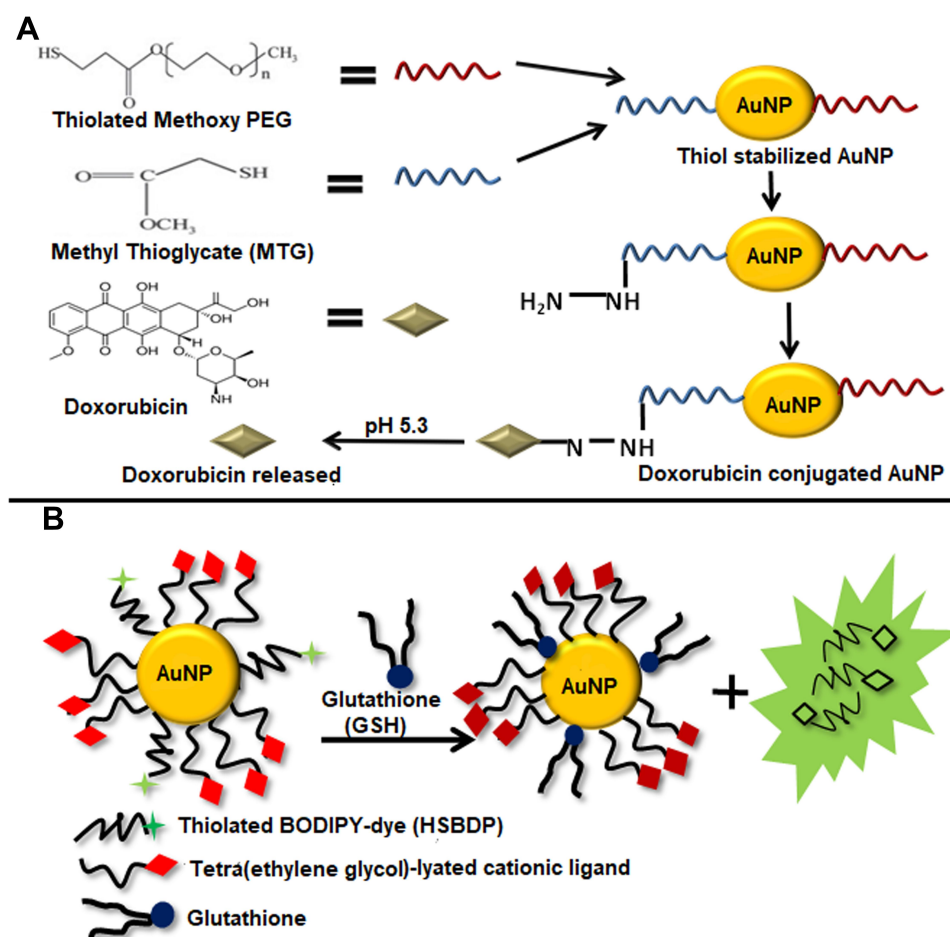
having hydrazone bond as compared to the free doxorubicin showed higher drug built-up and retention in MCF-7/ADR cancer cells which are multidrug resistant cells.<sup>274</sup>

## Glutathione (GSH)-Mediated Drug Release

Glutathione-mediated drug release characterizes an alternate non-enzymatic approach for the activation of prodrugs in the intracellular environment. The basic principle of this approach is based on the difference in the concentration of GSH in the intracellular environment (1–10 mM)<sup>275,276</sup> as compared to that in the extracellular conditions<sup>277</sup> and the major thiols present in the blood plasma are cysteine (8 μM) and glutathione (2 μM).<sup>278</sup> Previous approaches are based on the disulfide bond between the drugs and drug carriers.<sup>279,280</sup> Although this approach can be efficacious, modification of the reactivity of the disulfide bond is relatively difficult. Another limitation is that the thiol–disulfide exchange can take place in the presence of cysteines located on the surface of the blood proteins, thus giving rise to a protein–carrier conjugate with different bioaccumulation and pharmacokinetic profiles. In a recent study, hydrophobic dye was used as a model for demonstrating glutathione-mediated hydrophobic drug release using functionalized gold nanoparticles. A monolayer composed of PEGylated cationic ligands (TTMA) and thiolated bodipy fluorogenic ligands (HSBDP) was presented on the particles. The presence of cationic ligand enables the passage through the plasma membrane barrier. The release of BODIPY which was not observed when tripeptide was used instead of glutathione indicated that thiol linkage (present in GSH and absent in tripeptide) was required for the release of payload. When AuNPs are conjugated with the dye, BODIPY fluorescence does not occur because the gold core quenches fluorescence through energy and/or electron transfer mechanisms. The fluorescence is produced when AuNPs are triggered with glutathione in cuvette, or cellular thiols present in HepG2 human liver cells. The dye liberation from AuNPs could be controlled by treating embryonic fibroblast cells from mouse with various concentrations of glutathione monoester.<sup>196</sup> Figure 10B shows the schematic illustration of glutathione-mediated drug release from AuNPs.

## Light-Mediated Drug Release

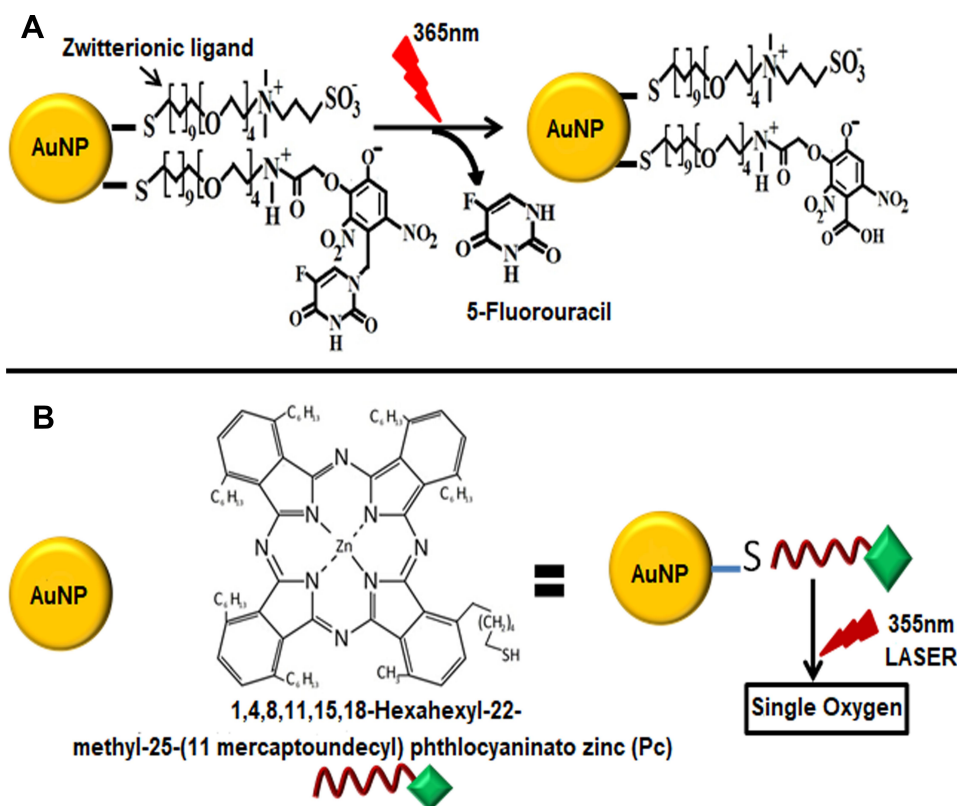
Studies have shown that gold nanoparticles having diverse shapes can exhibit very strong surface plasmon resonance



**Figure 10** (A) Schematic Illustration of pH-mediated release of Doxorubicin from Doxorubicin conjugated AuNPs. First thiol stabilized AuNPs were prepared using Thiolated Methoxy PEG and Methyl Thioglycate (MTG). Doxorubicin was conjugated to AuNPs through hydrazone bond. The acidic conditions (pH 5.4) in the tumor cells cause the breaking of hydrazone bond releasing doxorubicin. (B) Glutathione-mediated release of a drug analog (HSBDP) from AuNPs. TTMA and HSBDP functionalized AuNPs when treated with glutathione at 37°C cause the release of HSBDP which can be detected by the fluorescence it produces in the free form which was quenched when conjugated with AuNPs.

in the presence of light.<sup>281</sup> Due to this reason gold nanoparticles have been used for photothermal ablation and have been tested on various target cells including cancerous and bacterial cells.<sup>282–285</sup> Gold nanoparticles have also been used for photodynamic therapy during which light triggers the production of oxygen at the site of target. The heating of gold nanoparticles using light may also be used for releasing therapeutic drug attached to gold nanoparticles. This plasmon induced heating of gold nanoparticles has application in the delivery of therapeutics into the cells as well as into the nucleus of target cells. Drug release from gold nanoparticles using this property was first reported in 2000.<sup>286</sup> Similarly, later in time, Sreejivungsa et al<sup>287</sup> showed that AuNPs exhibiting a novel monolayer can be used for controlled discharge of a model drug by using the light. Hydrophobic parts of the drug are trapped in the portions of monolayers of AuNPs through non-

covalent linkages. When the light was irradiated it resulted in the cleavage of dinitrobenzyl linker causing the release of model drug entrapped in the compartments of monolayer of AuNPs. The results showed that more drug was released upon irradiation with UV light as compared to when no light was irradiated. Agasti et al<sup>288</sup> demonstrated light-mediated release of anticancer drug; 5-fluorouracil (5-FU) from gold nanoparticles. Zwitterionic ligand was used for enhanced solubility and controlled cellular internalization. The irradiation with light (365 nm) causes the cleavage of orthonitrobenzyl group releasing 5-FU from the conjugate. Figure 11A shows the schematic illustration of this study. In another study, Wang et al<sup>289</sup> used an amalgamation of chemotherapy and photothermal ablation to treat metastatic breast cancer using DNA-wrapped AuNRs loaded with DOX (GNR@DOX). Spherical gold nanoparticles display plasmon resonance in the central



**Figure 11 (A)** Light-mediated drug release from AuNPs. Zwitterionic ligand integrates 5-fluorouracil (5-FU) to AuNPs through the Orththonitrobenzyl group which when irradiated with UV light with 365 nm wavelength undergoes photolytic cleavage of bond liberating 5-FU. **(B)** Release of singlet oxygen from phthalocyanines (Pc) conjugated AuNPs. When these conjugates were irradiated at 355 nm using a Q-switched Nd:YAG laser they generated singlet oxygen.

region of visible light, yet this resonance peak can be shifted to wavelength range of  $\sim 800$ – $1,200$  nm in the infrared region when nanoparticles having complex shapes like rods and shells are used. As the body tissues are more transparent to NIR wavelength of light, nanoparticles with complex shapes can be used for *in vivo* therapy.<sup>290,291</sup> Another study has also reported the synthesis of hydrogel poly (Nisopropylacryamide) (PNIPAM) coated gold nanorods. When the NIR laser was irradiated onto these gold nanorods it resulted in the shrinkage of the hydrogels, thus causing the drug release.<sup>292,293</sup> As an extension of this basic principle, the surface of AuNPs was attached with light sensitive chemical species, spiropyran. Spiropyran can make complex structures in conjugation with amino acids which are sensitive to light. It can also reverse its conformational changes upon further irradiation with light. The complexes that spiropyran makes with amino acids in open form are destroyed upon irradiation with light and its conformation can be reversed to closed form thus causing the release of the amino acids.<sup>294</sup> These conjugates have a potential application as a basis for efficient light-

dependent controlled drug discharge systems for treatment of various conditions.

### Release of Atomic Oxygen or Nitric Oxide

Atomic oxygen ( $1/2O_2$ ) exhibit cytotoxic properties and can be used for photodynamic therapy of cancer.<sup>295</sup> AuNPs can act as delivery vehicles for the transport of such therapeutics as atomic oxygen, or nitric oxide. In an investigation, phthalocyanines (PCs) were attached on the surface of thiol-protected AuNPs, also called as monolayer protected clusters (MPCs), for the generation of atomic oxygen with good quantum yield.<sup>296</sup> The results indicated that as compared to the free PCs quantum yield was augmented by almost 50% when conjugated with MPCs. In addition to that, upon conjugation hydrophobic PCs became soluble in polar solvents such as ethanol and toluene. **Figure 11B** shows schematic illustration of this study for the release of singlet oxygen. On the other hand, nitric oxide (NO), controls numerous cellular processes including angiogenesis, vasodilation, as well as the immune response.<sup>297</sup> Controlled release of NO can prove



to be an efficient treatment strategy for hypoxic respiratory failure. Polizzi et al<sup>298</sup> reported that NO can be proficiently stored via covalent bonding with polyamine stabilized MPCs through the development of pH-sensitive N-diazoniumdiolate. The results showed that at a low pH of 3 there was an efficient liberation of NO from the nanocontainers. As the tumor and inflammatory tissues, endosomes etc. have mild acidic conditions these pH-sensitive materials can be efficiently used for drug delivery to these entities.<sup>299–301</sup>

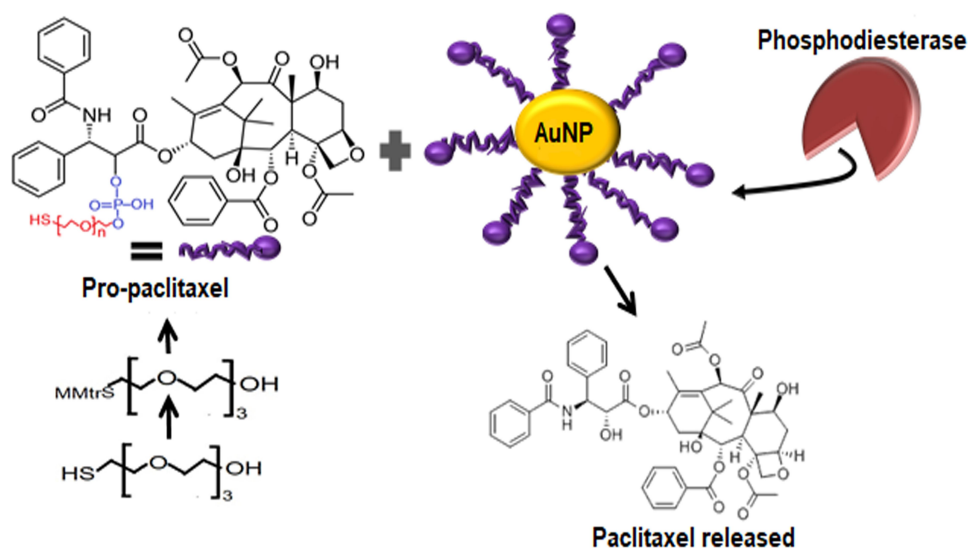
## Enzyme-Mediated Drug Release

Enzymes which are biological catalysts are responsible to sustain life as they catalyze millions of chemical reactions taking place in the living organism. They are substrate specific and not only increase the speed of chemical reactions but also control specificity of metabolic processes.<sup>302</sup> The characteristic degradation by enzyme can be employed for the selective and controlled release of therapeutics from gold nano-conjugates through enhanced permeability and retention effect (EPR).<sup>303</sup> In a study, Hwu et al<sup>304</sup> reported AuNPs and Fe<sub>3</sub>O<sub>4</sub> nanoparticles conjugated with Paclitaxel (PTX) through a phosphodiester bond between thiol-terminated tetraethylene glycol and the C-2' position of PTX. The phosphodiesterase enzyme present in cancer cells resulted in the cleavage of the phosphodiester linkage. The schematic illustration of this study is presented in Figure 12. Similarly, another method employed for the designing of prodrugs is esterification and acylation. Enzymes esterases and amidases

present in living cells can break the bonds between drugs and nanostructures resulting in liberation of drugs from their prodrugs.<sup>19</sup> Gibson et al,<sup>247</sup> in order to accurately measure the quantity of drug loading through thermogravimetric analysis efficaciously linked gold nanocrystals terminated with phenol to a linear derivative of paclitaxel. The mild esterification conditions were used for this reaction and resulted in the high yield of PTX payload.

## Advantages and Limitations in Drug Release Methods

The low pH value of cancer microenvironments can be used for effective drug release from nanocarrier by cleaving an acid sensitive bond between drug and nanoparticle. But with the evidence from new investigation which reported that cancer cells not only have acidic environment but also alkaline has limited the applications of pH sensitive nano drug carriers.<sup>305,306</sup> Moreover, the cleavage of pH responsive bond is not a rapid process making drug release time-consuming.<sup>307</sup> Light-mediated drug release is relatively new and an auspicious strategy to regulate the liberation of drugs and fluorescent dyes during in vitro and in vivo analysis. Even though initially this strategy has been very successful, the conventional mechanism which involves the use of light with short wavelength, ie, UV light and visible light has its inherited drawbacks. These drawbacks include inadequate tissue penetration, undesirable absorption, or scattering of light and possible phototoxicity to normal cells, etc. One possible solution is irradiation with NIR light which has



**Figure 12** Enzyme-mediated drug release from AuNPs. Pro-paclitaxel was produced by first protecting the thiol group of tetraethylene glycol using (mono-4-methoxy) trityl chloride (MMTrCl) and then reacting it with paclitaxel. The thiol terminal of pro-paclitaxel incorporated it onto AuNPs. When treated with enzyme Phosphodiesterase, the Phosphodiester moieties were hydrolyzed causing the liberation of free paclitaxel.



longer wavelength (700–1,000 nm) and demonstrates appropriate penetration of tissues and exhibits reduced phototoxicity in living systems. Although various NIR-sensitive compounds are being tested for clinical applications, several associated shortcomings include complex synthetic methodologies, low water solubility, and the capability of imperfectly aggregating in targeted areas which greatly hinders their applications in drug delivery to deep tissues.<sup>308</sup> Glutathione is considered as efficient stimuli for carrying out successful release of drug in vivo systems, yet there are certain limitations associated with this approach, as described in section 10.2. First, the modification of reactivity of disulphide bond is difficult and undesirable thiol-disulfide exchange reactions can take place with cysteines present on other proteins. Moreover, their instability in-vivo systems, challenges in the modifications of surface of AuNPs for multifunctioning and less blood circulation time period of gold nanoparticles protected with monolayer makes them unsuitable for applications in drug delivery.<sup>309</sup> In contrast, enzyme-responsive drug delivery structures are considered as a promising strategy to attain effective intracellular drug release,<sup>303,310</sup> since there are a plethora of enzymes comprising proteases, matrix metalloproteinases, and hyaluronidases located inside the lysosomes.<sup>307</sup> Complex formulation strategies of enzymes-responsive AuNP-drug carriers and severe conditions inside lysosomal environment causing the drug to degrade and reduce its practicability are major challenges of enzymes mediated drug release. It is anticipated that with the unceasing progress in formulation of materials science and cancer treatment, the difficulties cited above can be successfully resolved by the designing “smart” drug release systems.

## Conclusion

In this review article, we have discussed the approaches for the synthesis of gold nanoparticles, functionalization, and application in drug and biomolecule delivery. Due to their exclusive properties like tunable size and shape, ease of functionalization and fabrication, monodispersity, and low toxicity, AuNPs are considered as exceptionally suitable agents for drug delivery. Green synthesis of gold nanoparticle is rather an effortless and ecofriendly method which ousts the concerns associated with biomedical applications of chemically formulated gold nanoparticles. There are wide varieties of biological systems which have been tested for their potential to operate as reducing agents during synthesis reaction. Plants are contemplated to be the most dependable resource for this purpose. Not only do parts of plants (leaves, stems and roots) have reducing abilities, but also the waste

produced by plants, such as fruit peels etc. exhibit the same properties. Moreover, the process is quite simple and rapid. There are numerous possibilities for tuning the surface of AuNPs using different moieties, including PEG, amino acids and peptides, oligonucleotides, and antibodies to facilitate the loading of the drug and biomolecules. PEGylation of AuNPs is considered as the most suitable choice of functionalization for in vivo delivery of therapeutic agents as it is biocompatible and facilitates nano drug carriers to evade the body's immune system. Limitations associated with PEGylation such as loss of ability of AuNPs to bind with the target receptor can be addressed by decorating the surface of AuNPs with target specific ligands. Delivery of large biomolecules using AuNPs as a delivery vehicle is an innovative and interesting field and has received a lot of consideration over the past few years, but more investigations are still required to design structures capable of intracellular and intranuclear delivery of conjugates with minimal side effects. Another challenge related to the application of AuNPs in drug delivery is effective and efficient release of payloads at the target site. Various stimuli (both external and internal) have been reported to perform this function. Although each of these drug release strategies has its own limitations, designing a novel gold nano drug delivery system capable of flawlessly carrying and discharging payload at the target site is the subject of future research. Conclusively, gold nanoparticles offer a promising strategy, but in vivo delivery efficacy and clinical studies are critically needed.

## Disclosure

The authors report no conflicts of interest for this work.

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