

Gold nanoparticles: various methods of synthesis and antibacterial applications

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1. ABSTRACT

Colloidal gold is very attractive for several applications in biotechnology because of its unique physical and chemical properties. Many different synthesis methods have been developed to generate gold nanoparticles (AuNPs). Here, we will introduce these methods and discuss the differences between fabrication techniques. We will also discuss ecofriendly synthesis methods being developed to efficiently generate AuNPs without the use of toxic substrates. Finally, we will discuss the medical applications for AuNPs by highlighting the potential use of intact or functionalized AuNPs in combating bacterial infections.

2. INTRODUCTION

It is a well-known fact that all the matter in the universe is composed of atoms, regarded as the building blocks. Based on the arrangement and size of atoms, small localized matter, i.e. particles, are classified as coarse particles (10,000-2,500 nm), fine particles (2,500-100 nm) and ultrafine particles (1-100 nm) (Figure 1) (1) and the smallest of these categories, ultrafine particles, are also

commonly regarded as nanoparticles (Figure 2) (2). The branch of science dealing with nanoparticles, known as 'nanotechnology', has emerged as an area of intense scientific research and encompasses applications in the fields of medicine, electronics, biomaterials, energy storage production (3-6). This is clearly evident from the exponential growth (Figure 3) of research articles in the area of nanotechnology over last decade (7). The great potential of nanoparticles is due to the unique properties of elements when their size is reduced to nano-meter level. In general, the properties of particles above the nano-meter size do not differ significantly to their bulk counterparts. However, when particles are reduced to their nano-level, their physical and chemical properties (e.g., melting point, fluorescence, electrical conductivity, magnetic permeability and chemical reactivity) can drastically change. The changes in these properties are highly influenced by their size, shape and nature of surrounding environment (8). Lately, colloidal gold nanoparticles which have been known to mankind since the Roman times for their brilliant colors have gained much attention due to its potential for wide range of biological applications wherein the optical

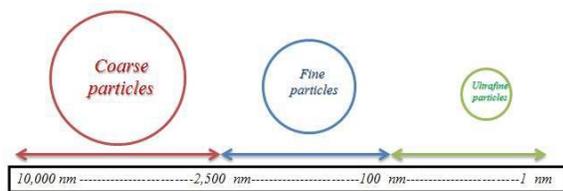


Figure 1. Classification of particles on the basis of their size distribution in the nanometer scale range.



Figure 2. A schematic illustration of some of the examples of size and their comparison at different size level. (Credit: National Nanotechnology Coordination Office).

properties of AuNPs are finely tuned by varying the morphological and reaction characteristics to yield AuNPs of desired shape and size (9). This review focuses on some of the basic aspects of AuNPs emphasizing on its history and existence since the ancient period, properties of AuNPs, various available synthetic routes and finally an overview of various AuNPs used either intact or conjugated with different functional moieties as an effective antibacterial agent along with their mechanism of action as found out from various individual studies all across the world.

3. GOLD NANOPARTICLES

3.1. A historical perspective

Historically, interest in nanotechnology is highlighted by the presentation entitled “There’s a Plenty of Room at the Bottom” by Richard P. Feynman during American physical society meeting at California in 1959 (10). Dr. Feynman described a process by which individual atoms and molecules could be manipulated, serving as potential candidates for future innovation and development. At present, nanoparticles can be broadly classified into five different categories viz. semiconductor quantum dots, magnetic nanoparticles, polymeric particles, carbon-based nanostructures and metallic nanoparticles (8). These are known as engineered nanoparticles as they do not occur naturally. Each of these nanostructures have characteristic properties and applications. For example, semiconductor quantum dots demonstrate fluorescent properties that are useful for biological labeling and imaging (11), and the magnetic properties of magnetic nanoparticles provide powerful tools for cell sorting, magnetic resonance induction, drug delivery and magnetic hyperthermia therapy (12). In contrast to the other categories, potential applications of metallic nanoparticles have proven to be the most flexible because of the ease in synthesis and control over size, shape, composition, structure and assembly. This results in fine tunability of their optical properties which forms the basis for various applications (13-15). Among all the metals that are commonly used for making nanoparticles, gold is the most widely used and studied metal for biological applications.

Gold is a soft, malleable, transition metal with the electronic configuration represented by $(Xe) 4f145d106s1$ and is one of the least chemically active elements (16). While commonly used as a precious metal for jewelry, colloidal gold, unlike bulk gold, is considered to be highly reactive, allowing for new applications. Gold salts have potent anti-inflammatory properties and have been administered to reduce pain and swelling associated with rheumatoid arthritis and tuberculosis (17). The applications of gold are further extended by colloidal gold which are submicrometer size particles of gold. Over the years, gold solutions were found to be used across different parts of the world for treating variety of ailments such as syphilis (18), alcoholism (19), and easing suffering in cancer patients (20). The capability of AuNPs for biomedical applications can be attributed to its plasmonic properties which is evident from a drastic color change from golden color in its bulk form to a variety of colors when reduced to its nanolevel (21). Currently, AuNPs are extensively used in molecular biology applications such as genomics, immunoassays and clinical chemistry, and medical applications such as detection and photothermolysis of microorganisms and cancer cells and targeted delivery of compounds such as peptides or DNA (22). The size of AuNPs governs the properties of the nanoparticles and the applications for which they are used. Small size AuNPs (2 nm-15 nm) are used in applications such as immunohistochemistry, microscopy (light and high magnification TEM) and biomarkers. Medium size AuNPs (20 nm-60 nm) are used in environmental detection and

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Table 1. Size dependent change in peak SPR wavelength of spherical AuNPs Source: Sigma-Aldrich

Size of gold nanoparticle	Peak SPR wavelength
5 nm	515-520 nm
10 nm	515-520 nm
20 nm	524 nm
30 nm	526 nm
40 nm	530 nm
50 nm	535 nm
60 nm	540 nm
80 nm	553 nm
100 nm	572 nm

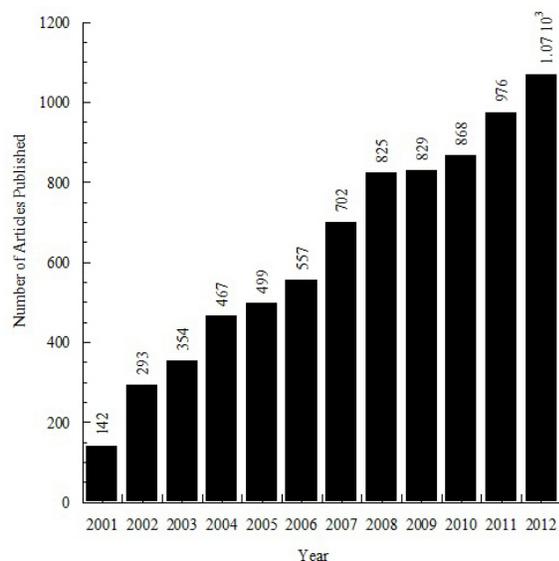


Figure 3. Evidence of exponential growth in nanotechnology research over the years. Graph shows number of articles published each year since 2001 in the ACS Nanoletters Journal (Source: pubs.acs.org).

purification, drug delivery, biomarkers, chemical sensors, DNA detection. Large size AuNPs (80 nm-250 nm) are used in forensic science, electronic device, manufacture, optical mammography etc. (23). AuNPs are generally preferred over other inorganic nanoparticles for biomedical applications due to its excellent biocompatibility with human cells (24-26), facile synthesis (8, 27), easy bioconjugation with various molecules such as polyethylene glycol (PEG), carboxyl groups, amine, DNA, RNA, antibodies, peptides etc. (28-48), chemical stability (49), tunable geometry (50, 51) etc. Moreover, gold salts are currently a choice of treatment for rheumatoid arthritis, psoriatic arthritis and bronchial asthma which proves their biosafety levels for clinical applications (52). The toxicity caused by AuNPs is a great topic of debate due to the ambiguity in the studies undertaken to determine its harmful effects. Some studies claim it to be non-toxic whereas other studies found AuNPs to be toxic to certain human cells (53-56).

3.2. Different nanostructures of gold

The intrinsic properties of AuNPs are greatly influenced by their size and shape (Table 1 and Figure 4) and have led to extensive research in finding efficient

fabrication techniques to obtain AuNPs of specific shapes and sizes (57). Surface plasmon resonance is a special phenomenon observed in AuNPs when the frequency of oscillating electrons present in the conduction band of the gold resonates with the frequency of incoming light radiation resulting in a plasmon band (58). Theoretical and experimental discussion about SPR can be found in earlier and recent literature (13, 14 and 59-64). The narrow frequency range of light radiation responsible for achieving SPR in AuNPs commonly fall in visible and near-infrared region (NIR). In general, a single plasmon band is observed for spherical AuNPs at ~520 nm. For anisotropic nanoparticles like nanorods, two plasmon bands are observed due to the electron oscillation along two axis viz. longitudinal (long) and transverse (short) respectively. The transverse plasmon band occurs at ~ 520 nm whereas the longitudinal plasmon band appears at a longer wavelength depending upon the aspect ratio of nanorod (ratio of length/width) (Figure 5) (8). SPR band intensity and peak depends on factors which mainly influences the electron charge density on the particle surface such as type of metal, particle size, shape, structure, composition and the dielectric constant of surrounding medium (22 and 65-67). SPR serves as an essential tool for monitoring the morphological property of AuNPs synthesized. In addition to the common spherical shape, AuNPs have also been synthesized into a variety of other shapes. A compilation of some of the more common shapes is shown in Figure 6. Different shapes can be achieved by using several synthesis methods and by varying multiple parameters such as the concentration of reactants, reaction conditions and the nature of solvent (7, 73-79).

4. SYNTHESIS OF GOLD NANOPARTICLES

4.1. Synthetic methods for gold nanoparticle

Techniques for making different AuNPs can be categorized into two principles, the “bottom up” method or “top down” methods (7). The bottom up method includes nanosphere lithography, chemical, photochemical, electrochemical, templating, sonochemical and thermal reduction techniques (80-85). This method involves assembly of atoms (produce by reduction of ions) into desired nanostructures. Top down methods such as photolithography and electron beam lithography (86, 87), requires the removal of matter from the bulk material to get the desired nanostructure. While both methods can generate AuNPs of desired shape and size, each have their own drawbacks, (e.g., poor monodispersity in case of bottom up methods, and extensive waste of material in top down methods). Some of the more commonly used techniques involving “bottom up” method for making AuNPs are reviewed below.

4.1.1. Turkevich method

The Turkevich method was first described in 1951 (90) and is one of the most commonly used methods for synthesis of spherical AuNPs in the size range of 10 nm-20 nm (Figure 7). The principle of this method involves reduction of gold ions (Au^{3+}) to gold atoms (Au^0) in the presence of reducing agents like citrate (90-92), amino acids, ascorbic acid or UV light (93-96). Size of AuNPs is

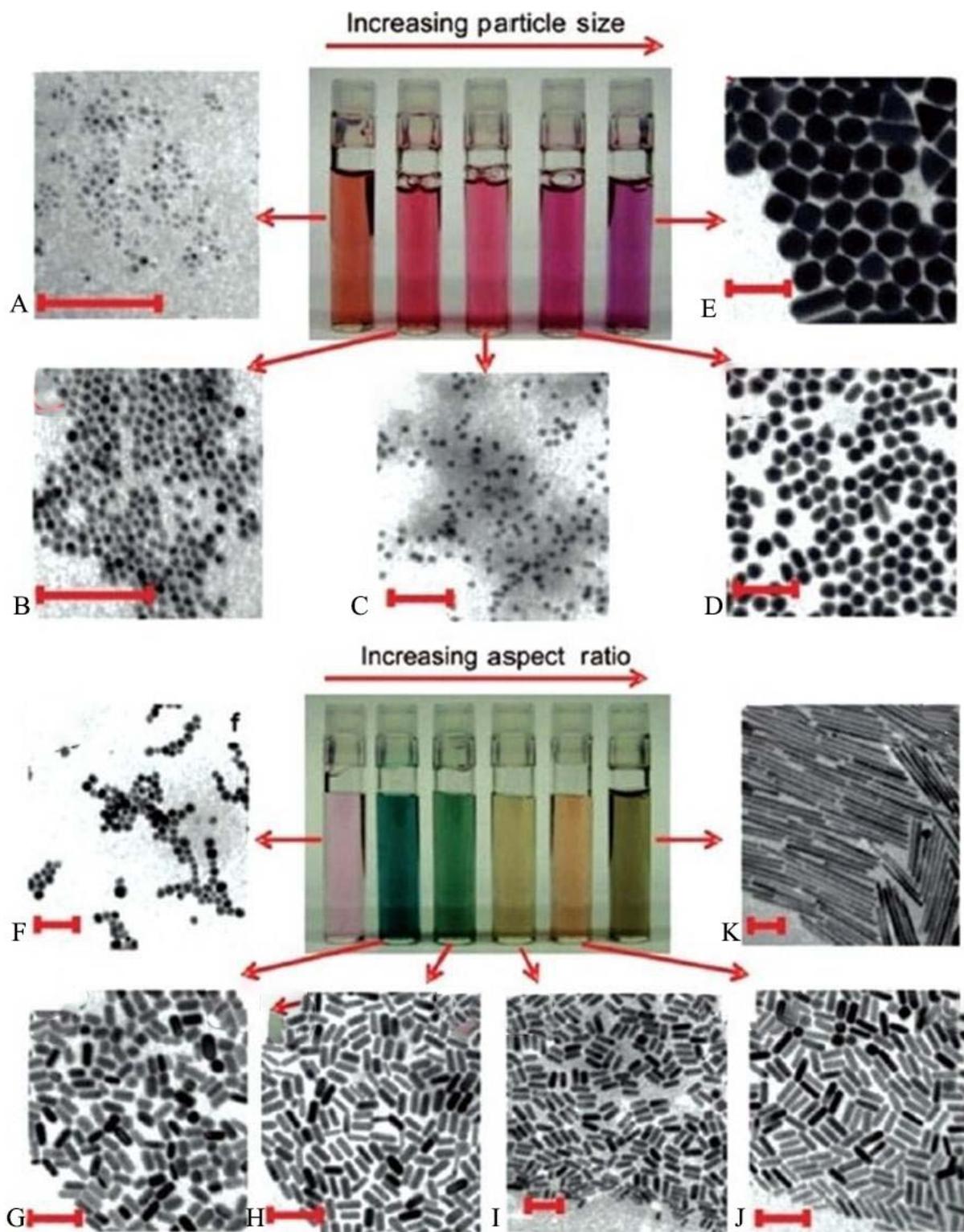


Figure 4. TEM images of gold spheres and gold nanorods in increasing order of dimensions with a scale bar of 100 nm for all. Solution appears in different color based on size or aspect ratio (length/width ratio) which is more significant for nanorods when compared to spheres as evident from the intensity of color change. Size for gold spheres (A-E) varies from 4-40 nm whereas for gold nanorods (F-K), the aspect ratio varies from 1.5. to 20. Reproduced with permission from reference (22).

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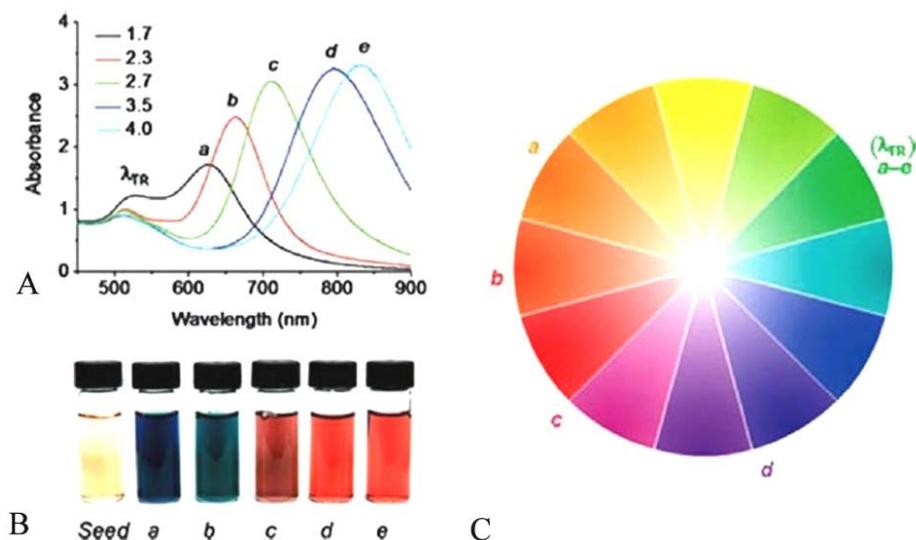


Figure 5. (A) Shows tunable optical absorbance of gold nanorods; (B) with different aspect ratios from visible to near-infrared wavelength regions; (C) Shows a color wheel with different colors concomitant with the gold nanorod labeled a-e. Reproduced with permission from reference (22).

further stabilized using various capping/stabilizing agents. Initially the Turkevich method was limited by the narrow range of AuNPs that could be generated by this method. However, several advances in the original method have allowed for researchers to expand the size range of particles that can be generated via this method. In 1973, Frens found that by varying the ratio of reducing to stabilizing agents, AuNPs of specific size, ranging from 16 nm-147 nm can be achieved (97-99). Later, the roles of pH, temperature and sodium citrate concentration were better understood, allowing for the generation of a particle growth model (100-103).

4.1.2. Brust method

The Brust method was first described in 1994 (105). This method is a two phase process to generate 1.5 nm-5.2 nm AuNPs using organic solvents (Figure 8) and by varying the ratio of thiol to gold. The Brust method was inspired from Faraday's two phase system. The method involves transfer of gold salt from aqueous solution to an organic solvent (e.g. toluene) using a phase transfer agent (e.g., tetraoctylammonium bromide (TOAB)). The gold is then reduced using sodium borohydride in presence of an alkanethiol. The alkanethiols stabilize the AuNPs (107), resulting in a color change of the reaction from orange to brown (105, 106). Purification of AuNPs stabilized with dodecanethiol from TOAB was reported by Schriffin (108). Seeding Growth Method

4.1.3. Seeded growth method

While the Turkevich and Brust methods can generate spherical AuNPs, AuNPs can also exist in variety of nanostructures (110-114) such as rods (73, 74, 96), cubes (75, 109), tubes (115) etc. The most widely preferred technique to obtain AuNPs in other shapes is seed mediated growth (79) (Figure 9). The basic principle of this technique is to first produce seed particles by reducing gold

salts with a strong reducing agent like sodium borohydride. The seed particles are then added to a solution of metal salt in presence of a weak reducing agent (ascorbic acid) and structure directing agent to prevent further nucleation and accelerate the anisotropic growth of AuNPs. Geometry of gold nanostructures can be altered by varying the concentration of seeds, reducing agents and structure directing agents.

4.1.4. Miscellaneous methods

Digestive ripening (78) has proven to be a convenient method to generate monodisperse gold nanoparticles from polydisperse nanoparticles by using excessive ligands (digestive ripening agents). The process involves heating a colloidal suspension at high temperatures (~138 0C) for 2 minutes followed by 110 0C for 5 hour in presence of alkanethiols. Temperature plays an important role in controlling the size distribution of the gold colloids produced. Various ligands that are used for digestive ripening process include thiols, amines, silanes, phosphines etc. (117-120). In addition, other utilized methods involving ultrasonic waves (121-123), microwaves (124, 125), laser ablation (126, 127), solvothermal method (128), electrochemical and photochemical reduction (129, 130) etc. have also been explored for making AuNPs (Figure 10 and 11).

4.2. Biosynthesis of gold nanoparticles

While the methods described above can efficiently generate AuNPs, a major drawback of these methods is a requirement for, and generation of, toxic byproducts that may prove to have environmental consequences during large scale production (53, 131). Furthermore, the use of toxic chemicals and solvents in these methods may prove to be problematic for downstream biological applications of AuNPs. In response to these concerns, new strategies to generate AuNPs without toxic

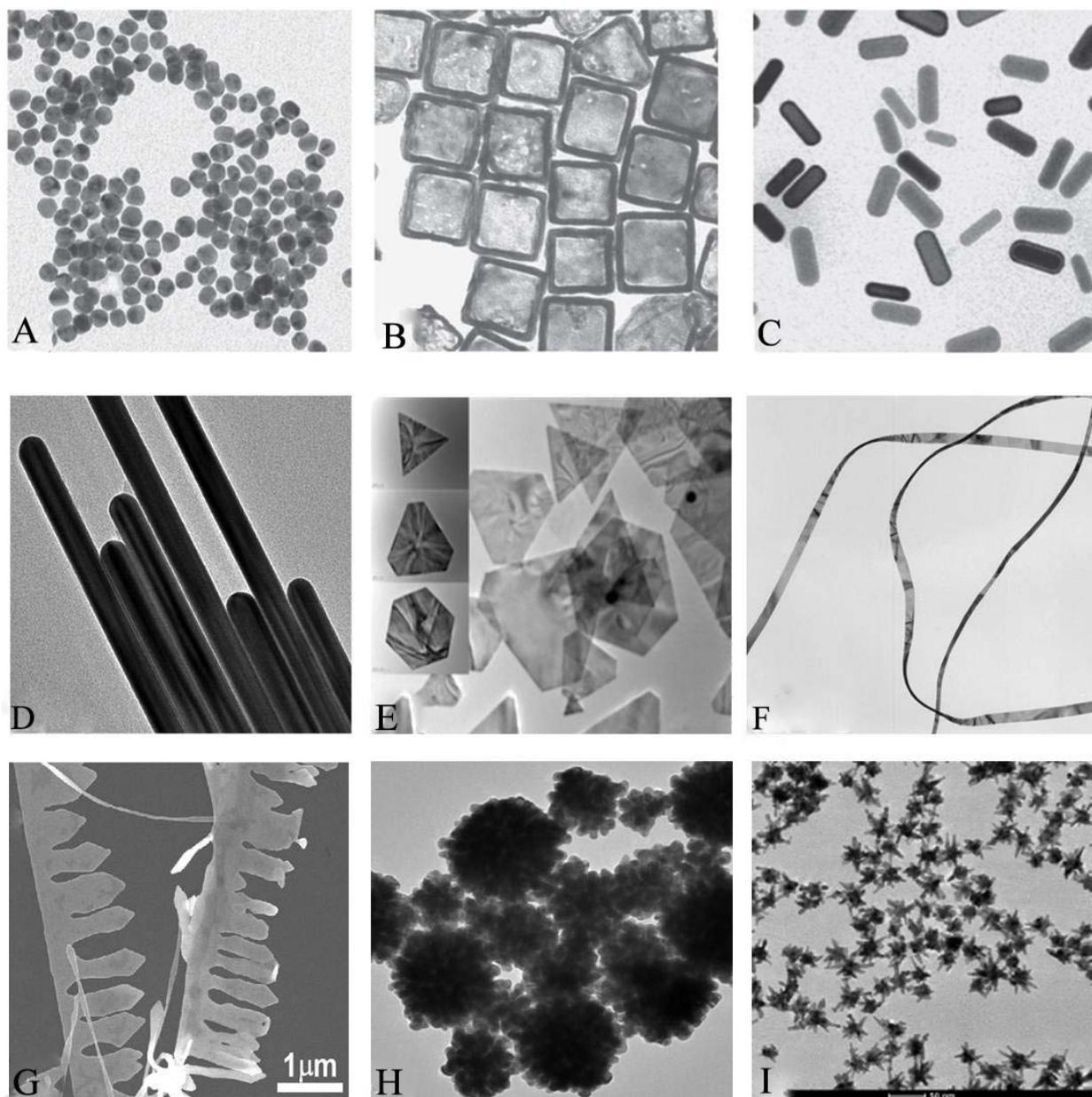


Figure 6. Compilation of TEM images of different shapes of gold nanostructures synthesized by different methods. (A-I) represents TEM images of gold nanospheres (50), gold nanocages (50), gold nanorods (50), gold nanowires (68), gold nanoplates (69), gold nanobelts (70), gold nanocombs (70), gold nanoflowers (71) and gold nanostars (72) respectively. Reproduced with permission from respective sources.

chemicals are being actively developed. The development of these non-toxic methods have embraced the principles of green chemistry, such as the use of rapidly biodegradable reagents, limiting waste products, synthesis at ambient temperature and pressure, and low toxicity of chemical products (132). Biological synthesis of AuNPs, using components like carbohydrates, lipids, nucleic acids or proteins produced in nature (Figure 12), is fast growing area of research to synthesize AuNPs in a clean, eco-friendly, non-toxic method. In addition to decreasing the toxicity issues associated with AuNPs synthesis,

biocomponents have additional advantages such as wide availability, low cost of production, ease of synthesis, and environmental safe. To date, many studies have been published involving AuNPs of different sizes and shapes using the above biological sources (Figure 13).

4.2.1. Using plant constituents

Plants are proven to be excellent candidates for the biosynthesis of AuNPs in a clean, reliable and biofriendly way. There are many articles which report biosynthesis of AuNPs using different plants or plant

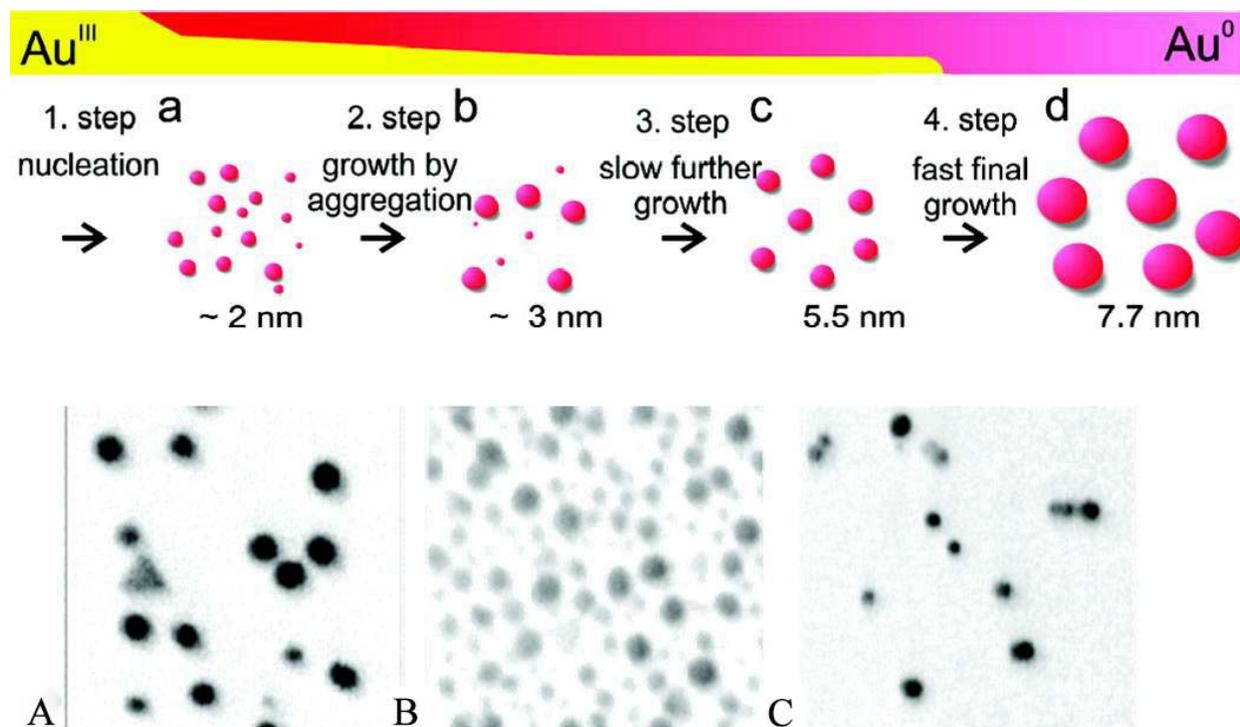


Figure 7. Upper panel shows a schematic illustration of the mechanism involved in the synthesis of gold nanoparticles using the common citrate reduction method. Reprinted with permission from reference (88). Lower panel shows SEM images of gold nanoparticles (A-C) synthesized using various chemical reduction methods such as citrate reduction at 100 °C, UV irradiation and ascorbic acid reduction respectively. Reproduced with permission from reference (89).

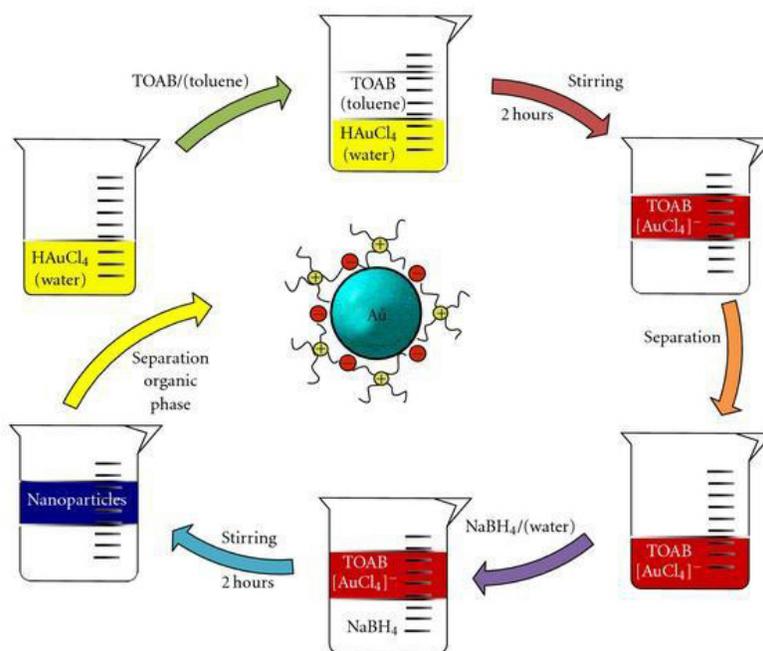


Figure 8. A detailed schematic representation of the steps involved in the synthesis of gold nanoparticles using Brust method. In this process the metal (Au^{3+}) precursor which is $HAuCl_4$ is transferred to an organic phase (toluene) with the help of TOAB followed by its reduction to Au^0 in presence of $NaBH_4$ to yield 5 ± 1 nm nanoparticles. Reproduced with permission from reference (104).

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Table 2. A list of some of the different plants which have been used as whole or a part of it for synthesis of AuNPs

Plant	Part of plant used	Size of gold nanoparticle (diameter)	Reference
Sapindus mukorossi	Fruit pericarp	9 nm -19 nm	133
Prunus domestica	Fruit	14 nm -26 nm	134
Magnolia kobus	Leaf	5 nm- 300 nm	135
Diopyros kaki	Leaf	5 nm – 300 nm	135
Coleus amboinicus lour	Leaf	9.0.5. nm – 31.9.5. nm	136
Cassia auriculata	Leaf	15 nm – 25 nm	137
Abelmoschus esculentus	Seed	45 nm – 75 nm	138
Zingiber officinale	Root	5nm – 15 nm	139
Rosa hybrid	Petal	10 nm	140
Cicer arietinum	Bean extract	N/A	141
Beta vulgaris	Sugar beet pulp	N/A	142
Nyctanthes arbortristis	Flower extract	19.8. nm	143
Gnidia glauca	Flower extract	50 nm – 150 nm	144

Table 3. A compilation of different microorganisms which have been used for synthesis of AuNPs of different sizes and shapes

Microorganism	Genus	Size of gold nanoparticle (diameter)	Reference
<i>Pseudomonas fluorescens</i>	<i>Bacterium</i>	50 nm – 70 nm	146
<i>Shewanella algae</i>	<i>Bacterium</i>	10 nm – 20 nm	147
<i>Geobacillus stearothermophilus</i>	<i>Bacterium</i>	-	148
<i>Escherichia coli DHSa</i>	<i>Bacterium</i>		149
<i>Marinobacter Pelagius</i>	<i>Bacterium</i>	10 nm	150
<i>Stenotrophomonas maltophilia</i>	<i>Bacterium</i>	40 nm	151
<i>Rhodopseudomonas capsulate</i>	<i>Bacterium</i>	10 nm – 20 nm	152
<i>Micrococcus luteus</i>	<i>Bacterium</i>	-	153
<i>Yarrowia lipolytica</i>	<i>Yeast</i>	-	154
<i>Acanthella elongate</i>	<i>Sponge</i>	7 nm – 20 nm	155
<i>Stoechospermum marginatum</i>	<i>Algae</i>	18.7. nm – 93.7. nm	156
<i>Sargassum wightii Greville</i>	<i>Algae</i>	8 nm – 12 nm	157
<i>Streptomyces viridogens</i>	<i>Bacterium</i>	18 nm – 20 nm	158
<i>Candida albicans</i>	<i>Fungi</i>	20 nm – 80 nm	159

extracts. Some of the green benefits of using plant or plant extracts for making AuNPs include use of nontoxic biocomponents for reducing and capping AuNPs, limiting the waste formation, cutting down the need for extra purification steps and ease of availability. Various biocomponents present in plants such as flavanoids, phytosterols, quinones etc. are involved in synthesis of AuNPs as they possess functional groups which catalyze the reduction and stabilization of AuNPs. The procedure involves mixing the gold salt with extracts of plant for definite amount of time under varied reaction conditions like pH, incubation time and temperature to obtain specific shapes and sizes of AuNPs. (Table 2) shows different part of plants which have been exploited by researchers for making AuNPs.

4.2.2. Using microorganisms

Bacteria and yeast are widely known for their interaction with inorganic metals and are commonly used in bioleaching of minerals such as gold, zinc and silver from their ores (145). Lately, variety of microorganisms has been used as factories for making AuNPs both intracellular and extracellular. Microbial cells upon treatment with gold salts synthesize gold nanostructures which are then isolated and purified using various techniques to obtain AuNPs. Control over the size and shape of AuNPs can be achieved by manipulating the important growth parameters. Table 3 shows a variety of microbes along with their genus which were used to make AuNPs of different size range.

4.2.3. Using biomolecules

Molecules produced by living organisms to catalyze biological functions of the body are known as

biomolecules (160). Biomolecules include amino acids, nucleic acids, carbohydrates and lipids. These molecules possess hydroxyl and carbonyl functional groups which can reduce Au^{3+} ions to Au^0 neutral atoms. Au^0 are then capped to form stabilized AuNPs. This method can overcome the problem of biosafety of the reactants used for the synthesis of AuNPs. Table 4 shows different biomolecule mediated synthesis of AuNPs.

5. ROLE OF GOLD NANOPARTICLES AS ANTIBACTERIAL AGENTS

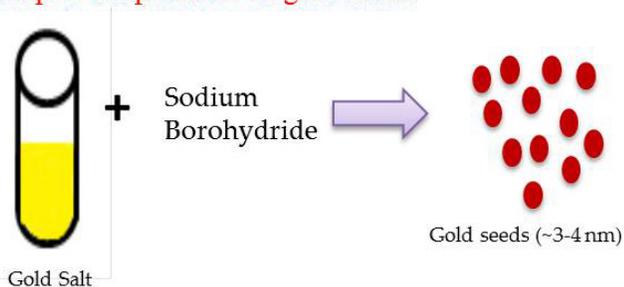
Antibiotics revolutionized the field of medicine by allowing the treatment of most bacterial infections which were once considered incurable. Unfortunately, many bacteria have evolved to evade killing currently used antibiotics. The most common antibiotic resistant bacteria are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*, which as a group are referred to as “ESKAPE” organisms (Figure 14) (176). Bacteria have developed resistance to antibiotics by various mechanisms including removing the antibiotics from cell through efflux pumps, modifying the target site of antibiotic, inactivation of antibiotic through enzymes, alteration to metabolic pathway (177-180) (Figure 15). The current rate of resistance development and lack of new antibiotics in the drug pipeline suggests that bacterial infections that were once easy to treat will no longer be treated in the clinic (181). It is evident that the time and capital required for developing new antibiotics is great (182-185). In the absence of new classes of antibiotics, one approach to improve treatment options is to modify the

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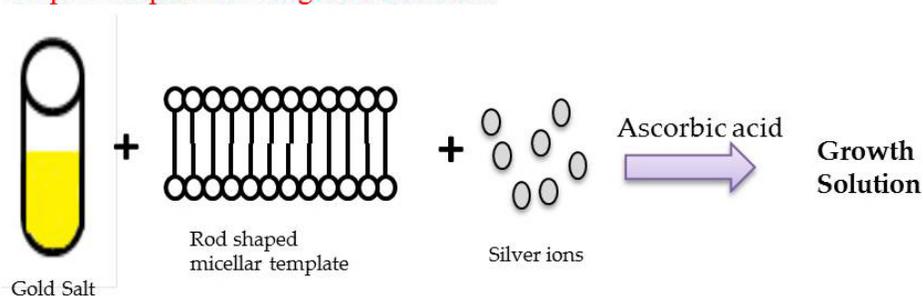
Table 4. List of various biomolecules involved in synthesis of AuNPs

Biomolecule	Type	Size of gold nanoparticle (diameter)	Reference
Linoleic acid	Fatty acid	10 nm	161
Tannic acid	Fatty acid	8 nm – 12 nm	162
NADPH-dependent enzyme	Enzyme	25 nm	163
Aminodextran	Polysaccharide	18 nm – 40 nm	164
Chitosan	Polysaccharide	N/A	165
Glucose	Carbohydrate	22 nm – 38 nm	166
Sucrose	Carbohydrate	4 nm – 16 nm	166
Raffinose	Carbohydrate	30 nm – 48 nm	166
Dextrose	Carbohydrate	25 nm, 60 nm, 120 nm	167
Starch	Polysaccharide	11 nm – 15 nm	168
Bovine serum albumin	Protein	N/A	169
Serrapeptase	Protein	20 nm -200 nm	170
Trypsin	Enzyme	N/A	72
Glycosaminoglycans	Mucopolysaccharides	N/A	171
Serratiopeptidase	Enzyme	N/A	172
DNA	Nucleotide	45 nm – 80 nm	173
Aspartate	Amino acid	30 nm	174
Phospholipid	Lipids	5 nm	175

Step 1: Preparation of gold seeds



Step 1: Preparation of growth solution



Step 3: Preparation of gold nanorods

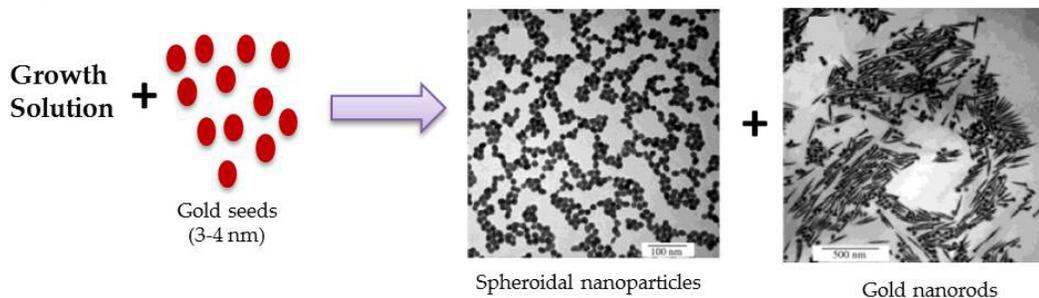


Figure 9. A step wise diagrammatic representation for the synthesis of gold nanorods and spheroidal nanoparticles using seeding growth method and TEM images of resulting particles. Step 1 involves synthesis of gold seeds which are then mixed with growth solution synthesized in step 2 to yield spheroidal gold nanoparticles and gold nanorods. TEM images reproduced with permission from reference (116).

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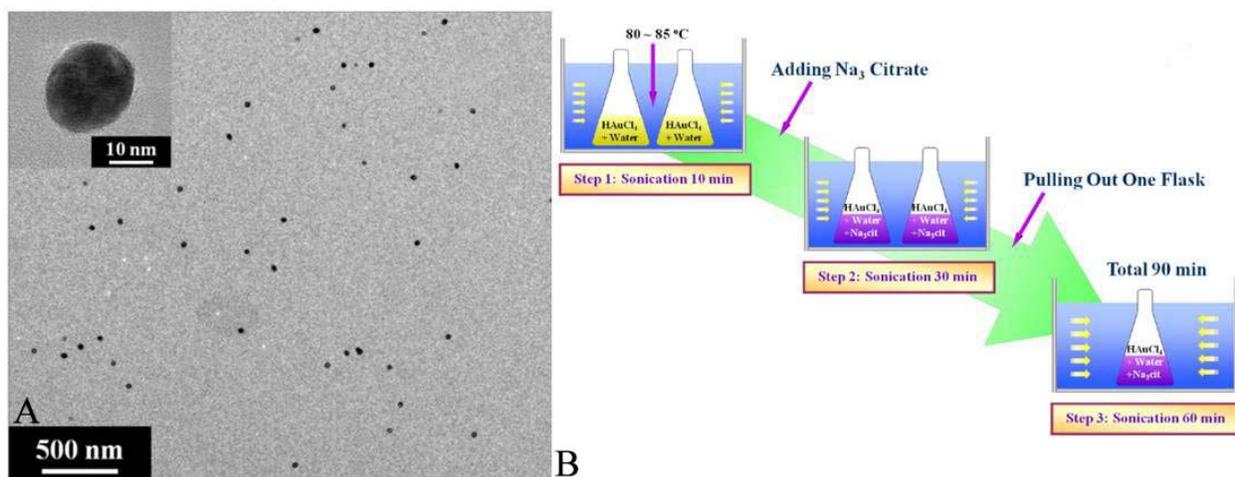


Figure 10. (A) Shows TEM image of gold nanoparticles approximately 20 nm in diameter, synthesized using 135 W ultrasonic powers; (B) Shows various Steps involved in ultrasonic mediated synthesis of gold nanoparticles. Reproduced with permission from reference (121).

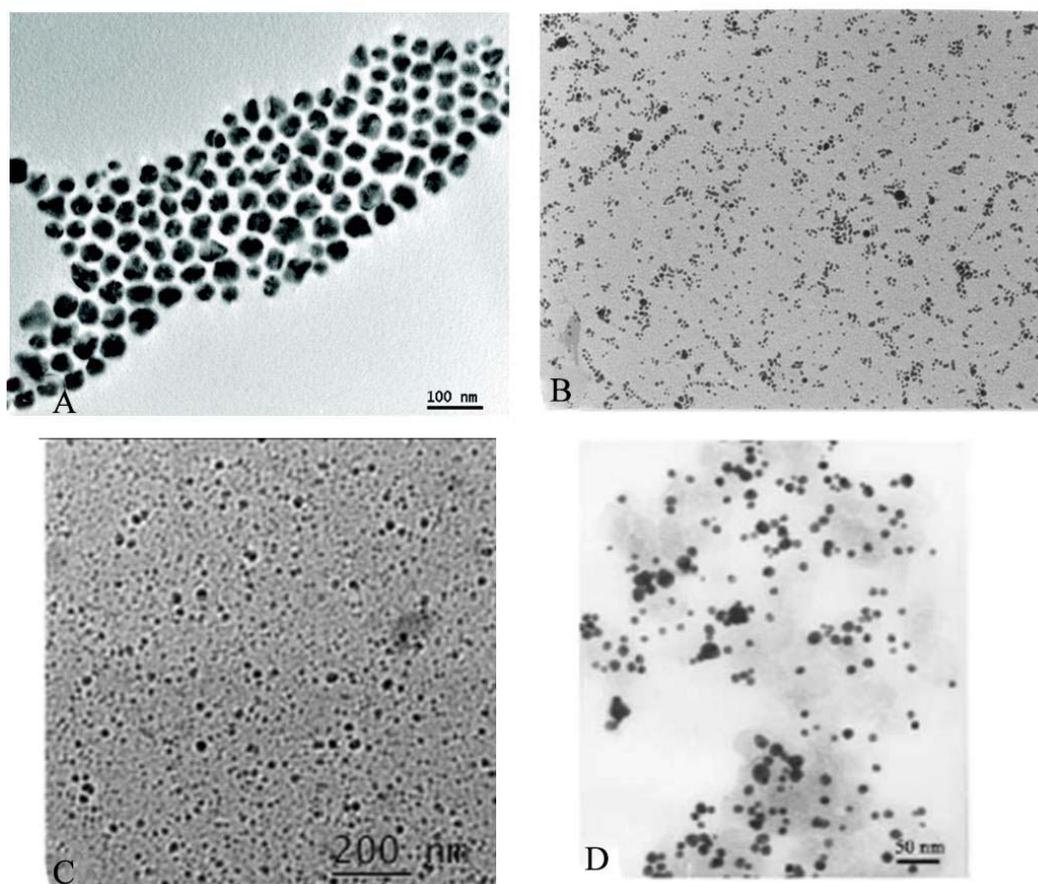


Figure 11. TEM images of gold nanoparticles (A-D) which are synthesized by employing miscellaneous techniques. (A) Represents TEM picture of microwave mediated synthesis of gold nanoparticles (124); (B) Shows gold nanoparticles synthesized using laser ablation technique (126); (C) TEM of gold nanoparticles using solvothermal technique (128). (D) Shows gold nanoparticles synthesized by electrochemical reduction method (129). Reproduced with permission from respective sources.

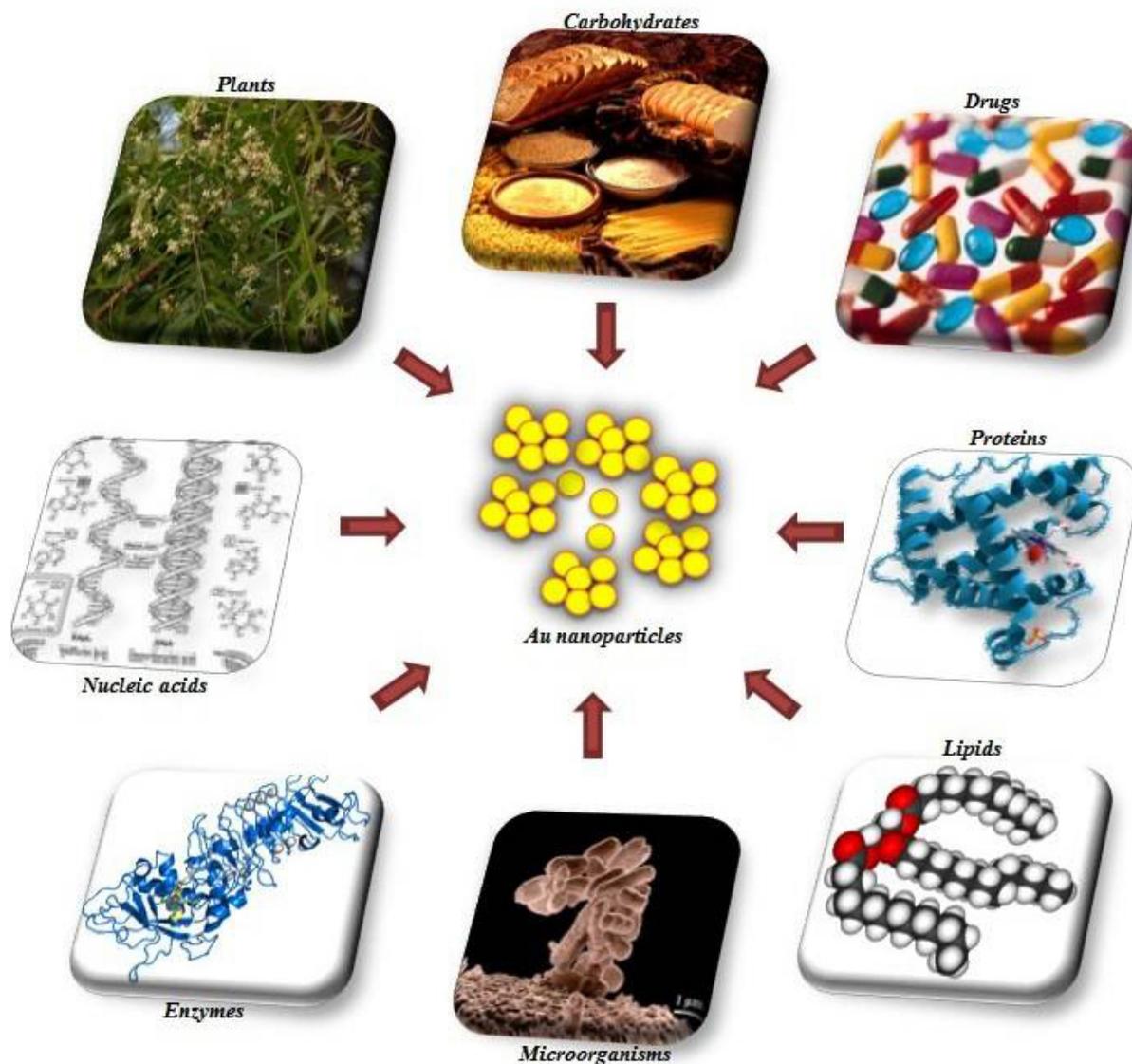


Figure 12. An illustration showing different biological sources ranging from biomolecules (carbohydrates, lipids, enzymes, nucleic acids and proteins), drugs, plants and microorganisms which are used in green synthesis of gold nanoparticles due to the combine reducing and capping property of different biocomponents present in them (Image credit: wikipedia.org).

current antibiotics to improve their efficacy against the drug resistant bacteria. Strategies which are generally employed include changing the molecular structure of antibiotic by adding/removing functional moieties, improving the drug delivery or combining multiple drugs in the treatment plan.

AuNPs conjugated with or without antibiotics can be used to improve antibiotic delivery, target specificity, dosage and bioavailability etc. (186) In addition to improving the efficacy of the conjugated antibiotic, AuNPs have been evaluated by many researchers for antibacterial activity against various bacterial strains. (Reference). There is still an ambiguity regarding the antibacterial activity of naked AuNPs which are not capped with any antibiotics. A research group lead by William *et*

al. showed that AuNPs themselves does not show any antibacterial activity (187). There are numerous findings about the antibacterial activity of AuNPs which are usually synthesized and functionalized by different methods. AuNPs are synthesized using different synthetic methods which are described earlier in this review followed by their evaluation for antibacterial activity either intact or conjugated with different molecules such as antibiotics, zeolites, lysozymes etc. Table 5 summarizes various AuNPs which are synthesized and evaluated for antibacterial activity by different research groups.

AuNPs conjugated with functional moieties have been used for photothermal killing of bacteria. Similarly,

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Table 5. A summary of various AuNPs synthesized by different methods for their role as antibacterial agents

Method of Synthesis	Reducing agent	Capping agent	Size diameter of gold nanoparticle	Bacteria type used	Reference
Biosynthesis Polysaccharide	Starch	Starch	120 nm	Gram negative Gram positive	167
Chemical reduction	Citric acid	CTAB	1 nm -22 nm	Gram negative	188
	Citric acid	Polyallylamine Hydrochloride PAH	~ 22 nm	Gram negative	189
Biosynthesis Grape fruit extract	Polyphenols	Polyphenols	N/A	Gram positive Gram negative	190
Biosynthesis Soybean extract	Polyphenols	Polyphenols	7 nm – 12 nm	Gram positive Gram negative	191
Biosynthesis Plant	Phytochemicals	Phytochemicals	15 nm – 35 nm	Gram positive Gram negative	192
Biosynthesis Antibiotic	Cefaclor	Cefaclor	22 nm – 52 nm	Gram positive Gram negative	193
	Ampicillin	Ampicillin	< 20 nm		194
	Gentamicin	Gentamicin	16 nm		195
	Streptomycin	Streptomycin	< 20 nm		194
	Kanamycin	Kanamycin	< 20 nm		195
	Cephalexin	Cephalexin	50 nm – 200 nm		196
	Vancomycin	Vancomycin	N/A		197
Quinolone	Quinolone	N/A	198		
Zeolites	N/A	N/A	5 nm	Gram negative	199
Chemical reduction	Sodium Borohydride	Pyrimidine	3 nm	Gram negative	200

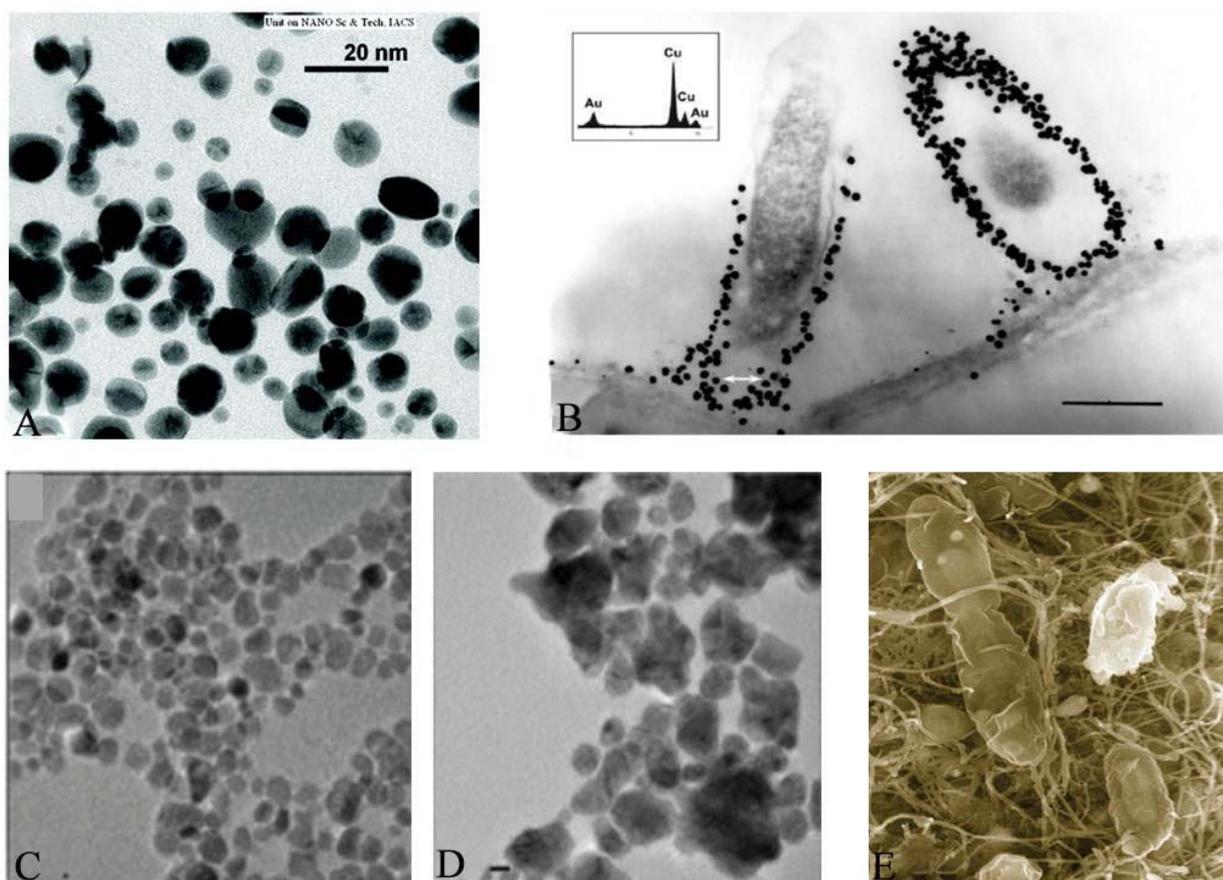


Figure 13. TEM images of gold nanoparticles synthesized using biological sources. (A-E) Represents TEM images of gold nanoparticle synthesized using fungi (206), plants (205), chloroplast (204), DNA (173) and bacteria (207) respectively. Reproduced with permission from respective sources.

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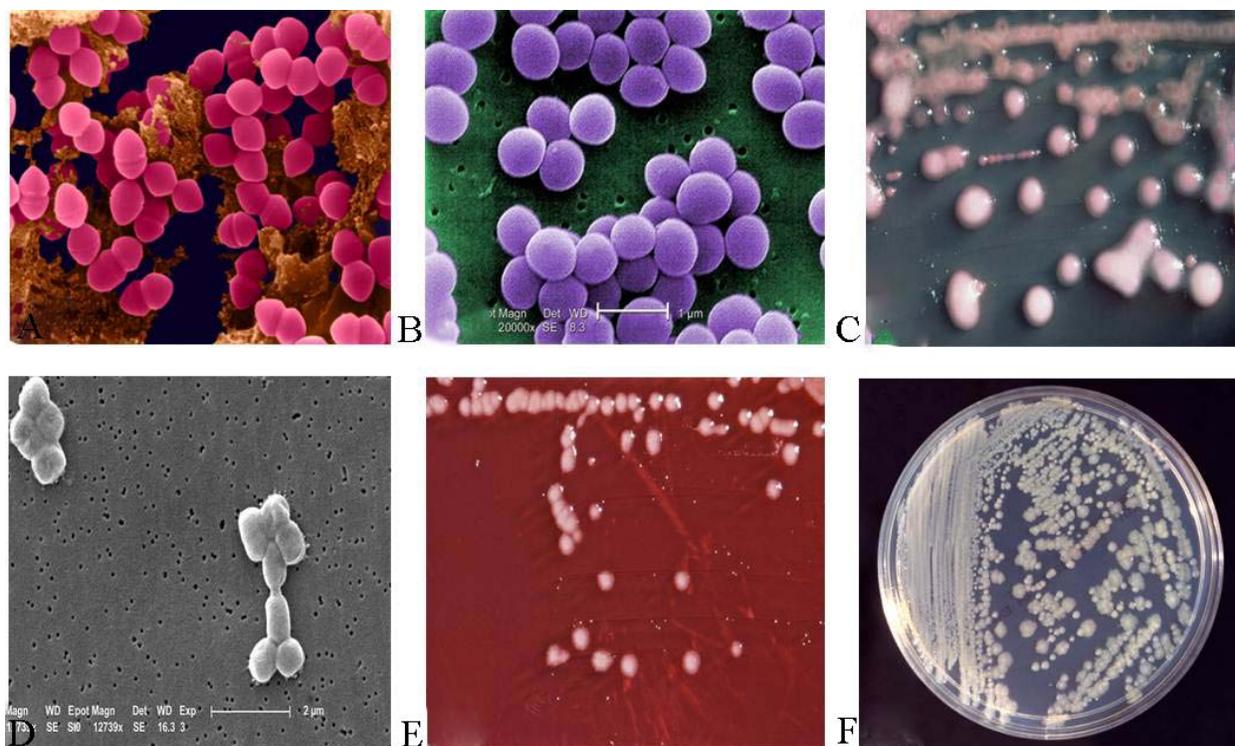


Figure 14. (A-F) are the images which represents “ESKAPE” organisms viz. *Enterococcus faecium**, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* which have developed resistance against a number of front line antibiotics ranging from β -lactam, cephalosporins, tetracyclines, aminoglycosides etc. (*Image copyright Dennis Kunkel Microscopy, Inc.; Source: wikipedia.org).

W.C Huang and group (201) used polygonal AuNPs attached with vancomycin for photothermal killing of Gram-positive, Gram-negative and antibiotic-resistant bacteria. Results of the study showed successful attachment of vancomycin-gold nanoparticles to the D-Ala-D-Ala moieties of the peptides on pathogen cell wall and destroyed more than 99% of bacteria after illuminating with NIR light. In another study, different sizes of AuNPs conjugated with anti-protein A antibodies were used for selective killing of *Staphylococcus aureus* by passing laser pulses (202). Intact AuNPs have also been used in many studies for evaluating antibacterial activity. A team of researchers from India synthesized AuNPs in the size range of 1 nm-22 nm using citric acid and CTAB as reducing agent and tested against *E.coli* bacterium which showed high antibacterial potency with the zone of inhibition of ~22 mm (188). Researchers from Texas A&M University, USA evaluated and determined the potent antibacterial activity and mechanism of AuNPs against *E.coli*. Two different AuNPs stabilized using citrate and PAH respectively were synthesized of roughly 22 nm in diameter (189). In a quest to improve the antibacterial efficiency of AuNPs, scientist have tried to conjugate AuNPs with different antibiotics and evaluated for antibacterial activity. Various methods have been employed for coating antibiotics onto AuNPs such as citrate reduction method wherein AuNPs of desired size are first produced by reducing gold salts using sodium borohydride to yield 14 nm AuNPs followed by

their functionalization with different antibiotics like ampicillin, streptomycin and kanamycin. Efficiency of antibiotic conjugated AuNPs was evaluated by comparing the zone of inhibition against *E. coli*, *M. luteus* and *S. aureus* with free antibiotics. Results revealed a higher inhibitory zone for antibiotics conjugated AuNPs when compared to free antibiotics against all the three strains. (194). In 2003, B. Xu *et al* (197) modified vancomycin antibiotic into bis(vancomycin) cystamide followed by its conjugation to 4 nm-5 nm AuNPs. The attachment was successful due to formation of Au-S bonds which linked AuNP with vancomycin cystamide. Enhanced antimicrobial activity against *vancomycin-resistant enterococci* was observed for above modified AuNPs. Lately, biological synthesis of AuNPs for their use as antibacterial agents have gained an immense interest of research. In 2003, for the first time P. Poddar and team synthesized gold nanostructures using a broad-spectrum antibiotic cephalixin (196). Researchers claim this process as eco-friendly synthesis of AuNPs, excluding the need for using toxic chemicals during the synthesis as seen in conventional methods This study also highlighted the combine reducing and capping ability of antibiotics. Similarly, In 2010 scientists from India and UK utilized the combined reducing and capping ability of a cephalosporin antibiotic cefaclor for the synthesis of 22 nm- 52 nm spherical AuNPs (193). Antimicrobial assays against *S. aureus* and *E. coli* showed potent

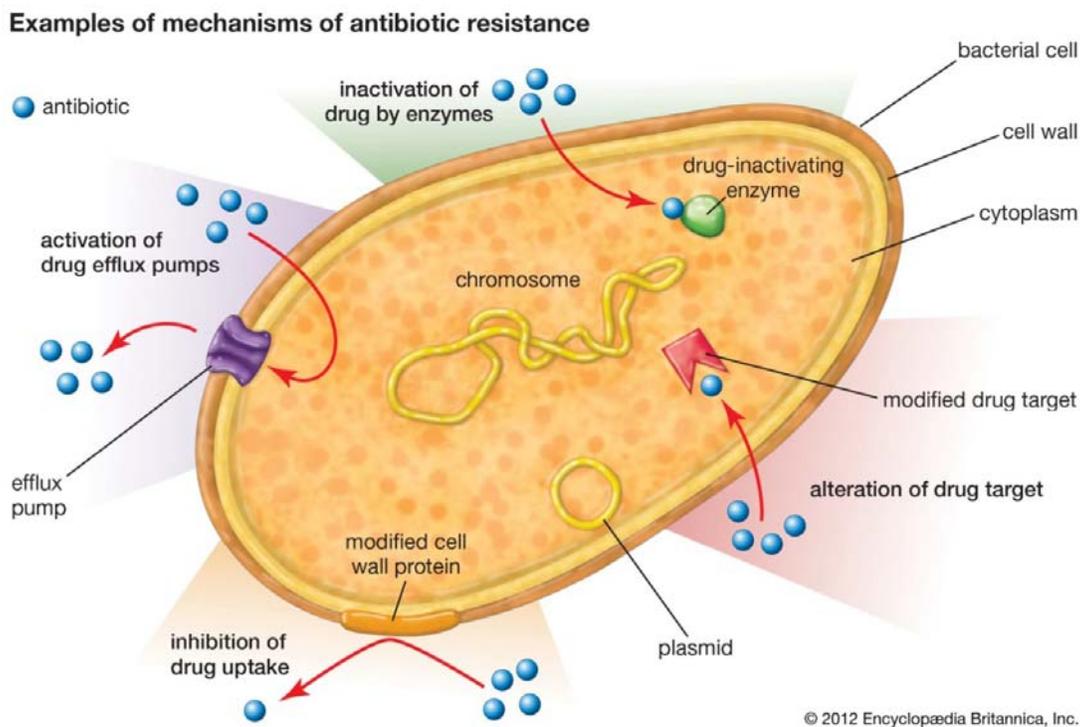


Figure 15. An illustration showing some of the different mechanisms by which a bacteria develops resistance towards the antibiotic drug (Credit: Encyclopedia Britannica, Inc., copyright 2012).

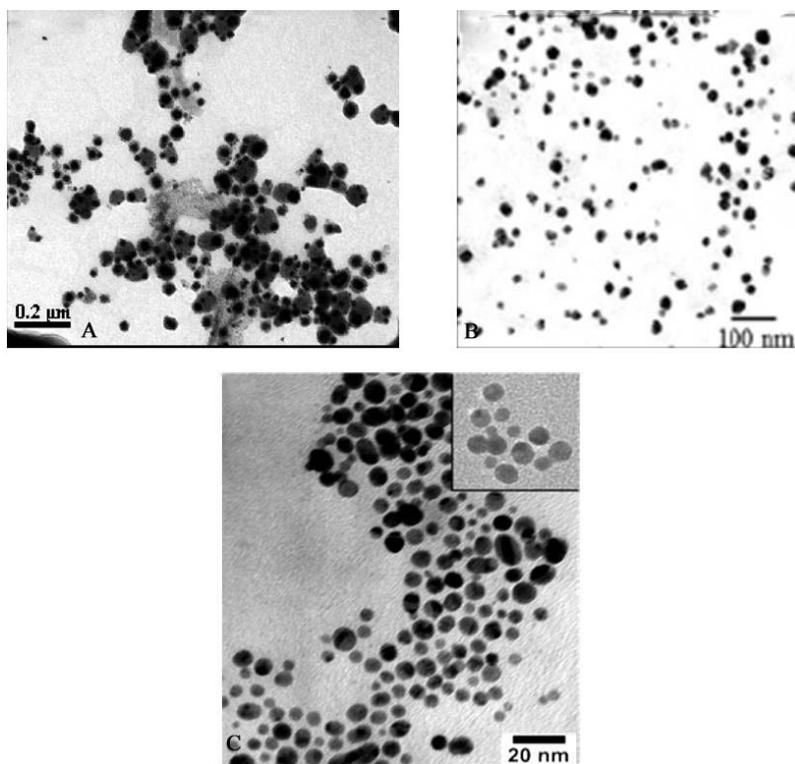


Figure 16. TEM images of gold nanoparticles which have been synthesized using different antibiotics. (A-C) Represents TEM image of gold nanoparticles synthesized using cephalixin (196), ampicillin (203) and vancomycin (197) antibiotic respectively. Reproduced with permission from respective sources.

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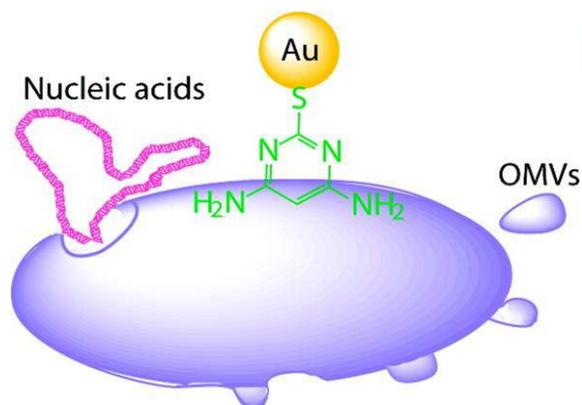


Figure 17. An illustration showing the antibacterial action of gold nanoparticles capped with amino-substituted pyrimidine groups via sequestration of magnesium or calcium ions disrupting bacterial cell membrane resulting in leakage of cytoplasmic contents and also by interaction with DNA and inhibition of protein synthesis. Reproduced with permission from reference (200).

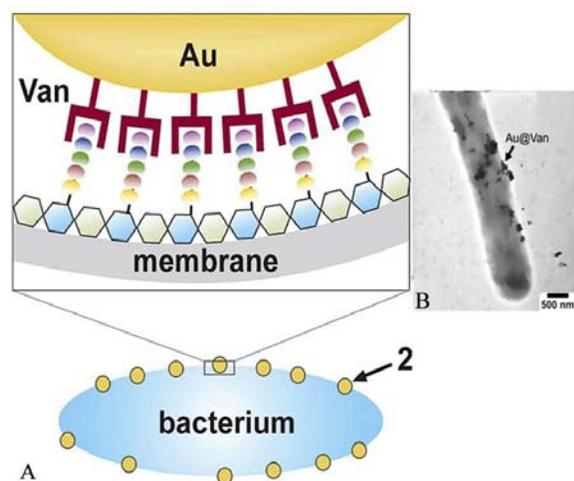


Figure 18. (A) Illustration showing interaction of vancomycin capped gold nanoparticles with VanA genotype VRE strain (hexagons: glycosides; ellipses represent the amino acid residues of the glycanpeptidyl precursor with different colors: L-Ala (yellow), D-Glu (orange), L-Lys (green), D-Ala (blue), and D-Lac (purple)); and (B) TEM image of interaction of *E.coli* with Van-capped gold nanoparticle. Reproduced with permission from reference (197).

antimicrobial activity of efactor-AuNPs when compared to free cefaclor (219). The MIC for cefaclor-AuNPs against *S.aureus* and *E.coli* was 10 $\mu\text{g mL}^{-1}$ and 100 $\mu\text{g mL}^{-1}$ respectively. The researchers claimed that the primary amine group in cefaclor helped in reducing and capping of AuNPs leaving the β -lactam ring unchanged for antimicrobial activity. In another independent study, ampicillin was used for single step synthesis of AuNPs (205). The TEM images of AuNPs synthesized in above studies are shown in (Figure 16). It is still unclear about the

exact mechanism by which the antibiotic forms AuNPs. Apart from antibiotics, various other biological molecules have also been used to synthesize AuNPs with potent antibacterial activity. R. Dakshinamurthy *et al* (167) synthesized different sizes of AuNPs capped with carbohydrate by green process wherein the sugar was used as both reducing and capping agent. Antimicrobial assay revealed size dependent antimicrobial activity of carbohydrate-AuNPs against both Gram-positive and Gram-negative bacteria. Biocomponents from plants have also been used for making AuNPs. In a recent finding, Murdoch University researchers have created antibacterial AuNPs from the leaves of *Eucalyptus macrocarpa* (210). Results showed increased zone of inhibition against *B.subtilis* and *E.coli*. This finding can be used as a new tool to combat the antibiotic-resistant strains of microorganisms.

Understanding the exact mechanism involved in enhanced antibacterial activity of AuNPs capped with antibiotics or other functional groups is a challenging task for scientists. Various hypotheses have been proposed from multiple studies in this arena but very few of them are in common conclusion. More likely, it is believed that the antibacterial activity of AuNPs is dependent on the nature of the capping agents present on the surface of AuNPs. In one study, the researchers compared the antibacterial activity and mechanism involved for AuNPs which were capped with citrate and PAH respectively (189). It was found that both had different mechanism of antibacterial activity. Citrate capped AuNPs which is a weak capping agent lead to more aggregation of AuNPs thereby reducing the surface area resulting in reduce interactions between nanoparticles and bacteria whereas PAH which is a strong capping agent caused the AuNPs to self-assembled into 4-5 micron long chains preventing further aggregation (189).

These chains were disturbed after entering the bacterial cell, resulting in more scattered AuNPs causing cell lysis. The above finding justified the claim that cationic coated AuNPs are more toxic than anionic coated AuNPs (203). For antibiotics which belong to β -lactam group such as penicillin and cephalosporin which show antibacterial activity by inhibiting the cell wall synthesis shows quite similar mechanism when they are coated on AuNPs (193). The activity of antibiotic is not hindered as the active group i.e. the β -lactam is not altered or modified during fabrication of antibiotic-AuNPs. Against gram positive microorganism, the antibiotic conjugated on the surface of AuNPs first reacts with the outer peptidoglycan layer of gram positive microbes which results in increase membrane porosity. Subsequently AuNPs penetrate the membrane and get bound to bacterial DNA, preventing it from unwinding thereby stopping transcription (193). AuNPs have positive charge on their surface which reacts with negative charge on phosphate groups of DNA making a strong interaction. In addition, the antibiotic destroys the cell wall which results in leakage of cellular contents ultimately leading to death of microorganism (193). In comparison against Gram-negative organisms, the effect is quite slow due to the fact that AuNPs have to first diffuse through the membrane and then the antibiotic reacts with peptidoglycan layer making perforations in the membrane followed by

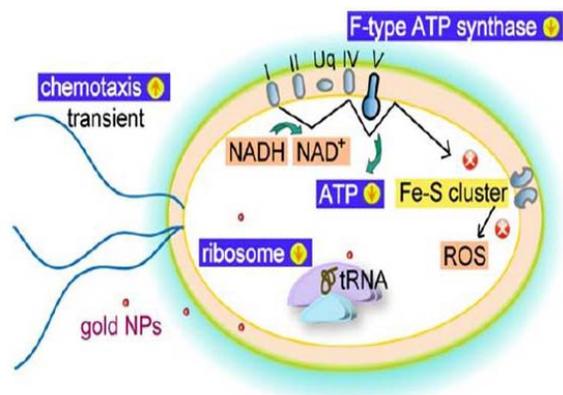


Figure 19. A diagrammatic representation showing some of the mechanisms believed to be involved in the antibacterial action of gold nanoparticles against *E.coli* such as down- regulation of oxidative phosphorylation pathway (F-type synthase and ATP level) and ribosome pathways along with up-regulation of chemotaxis of gold nanoparticles. No reactive oxygen species were involved in the antibacterial action. Reproduced with permission from reference (210).

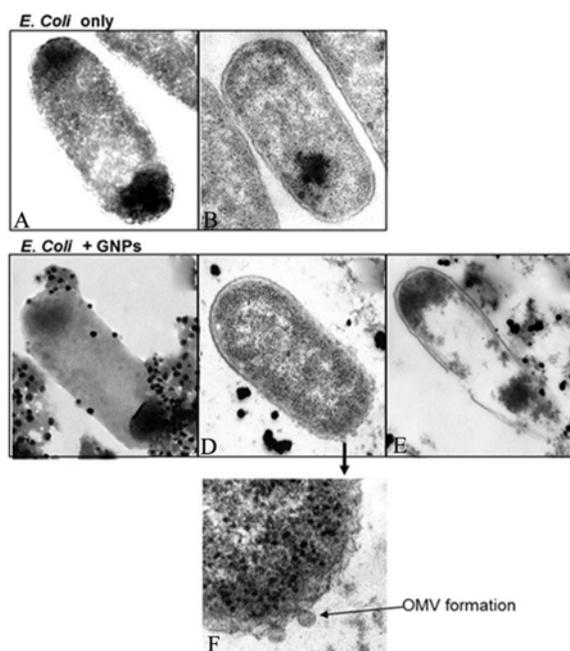


Figure 20. TEM images showing interaction of *E.coli* with dextrose capped gold nanoparticles (D-GNPs) before and after their exposure. (A-B) show TEM of *E.coli* before interaction with D-GNPs; (C-F) shows interaction of *E.coli* with D-GNPs at different time intervals. D-GNPs induced cell disruption of bacterial cell which is justified from the outer membrane vesicles (OMV) formation after 12 hour treatment. Reproduced with permission from reference (167).

AuNPs:DNA interaction (193). Overall, AuNPs have found to increase the half-life of antibiotic thereby enhancing the longevity of drug by showing synergistic effect which

might explain the reason for increase in potency of antibiotic-AuNPs over free antibiotic (193-198). In one study, a research group tried to cap amino-substituted pyrimidine groups onto AuNPs and tested them for antibacterial activity which resulted in enhanced antibacterial effect (200). The mechanism involved behind the antibacterial effect was found to be sequestration of magnesium or calcium ions leading to disruption of bacterial cell membrane which ultimately resulted in leakage of cytoplasmic contents. Interaction with DNA and inhibition of protein synthesis was also believed to be involved in the antibacterial activity (200) (Figure 17). Vancomycin when capped onto AuNPs showed enhanced activity against *vancomycin-resistant enterococci* but the exact mechanism behind this activity is yet to be discovered (197) (Figure 18). Another study showed the bactericidal mechanism of AuNPs is mainly by two methods which involves 1) collapse of membrane potential, inhibiting ATPase activity to decrease the ATP level and 2) inhibiting a subunit of ribosome from binding to tRNA thereby preventing translation (204). They increase chemotaxis in the early phase reaction. Reactive oxygen species (ROS) were believed to be not involved in the antibacterial effect (204) (Figure 19). In some studies, TEM images of interaction of bacterial strain with AuNPs capped with different moieties showed the bacterial cell wall disruption and formation of outer membrane vesicle (OMV) after an incubation time of 10 hours (167) (Figure 20). There is still a lot to know and understand about the various mechanisms involved in antibacterial activity of AuNPs capped with antibiotics or other antibacterial agents. Better technology and sophisticated instruments in future might give us some lead in understanding the mechanism at the molecular and cellular level.

6. SUMMARY AND PERSPECTIVE

AuNPs have truly revolutionized the field of bionanomedicine which is evident from its numerous existing applications and many more important applications can be expected in near future. AuNPs existed since a long time but lately, since last two decades, the applications of AuNPs skyrocketed due to advancement of nanoscale analytical tools. This article discusses some of the most commonly used chemical synthetic methods for AuNPs of different shapes and sizes. Environmental and biological toxicity associated with the above methods due to use of toxic chemicals and solvents sown seed for developing biological methods which can be more ecofriendly and biofriendly, utilizing the combine reduction and capping ability of various biological sources such as plants, microorganisms and biomolecules for making AuNPs. Although, due to poor efficiency and moderate control on polydispersity of synthesized AuNPs, further development in the field is required. An efficient antibacterial agent can be formulated by combining the antibiotic with AuNPs.

Improved antibacterial properties of such AuNPs seems to be an optimistic solution to combat several dreadful diseases caused by various MDR bacteria which is spreading rapidly throughout the world. Many strategies of using modified AuNP as an antibiotic, have been developed

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recently and some of them as mentioned in this review, have shown very promising results and given high hopes for its commercial availability in market. In addition to its nontoxicity, use of AuNP in humans, as an antibacterial agent warrants an undisputable knowledge about the mechanisms of antibiotic action of AuNP, so as to avoid any risk that may cause to normal tissue/organ. Although, many efforts have been taken so far to explore the mechanism of action, at present the lack of consistent conclusions from various studies necessitates further development in this area.

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Abbreviations: AuNPs:Gold nanoparticles, ATP:Adenine tri-phosphate, CTAB:Cetyl trimethylammonium bromide, dGNP:Dextrose encapsulated gold nanoparticles , MIC: Minimum inhibitory concentration, MDR:Multi-drug resistant, nm:nanometer, NIR: near-infrared region, OMV:Outer membrane vesicles, PAH:Polyallylamine hydrochloride, PCR:Polymerase chain reaction, ROS:Reactive oxygen species , SPR:Surface plasmon resonance, SERS: Surface enhanced Raman spectroscopy, SEM: Scanning electron microscope, TEM:Transmission electron microscope , TOAB:Tetraoctylammonium bromide , tRNA:Transfer ribonucleic acid, UV:Ultra-Violet

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