Chapter 3
Gold Nanoparticle-Mediated Delivery of Therapeutic Enzymes for Biomedical Applications

Madan L. Verma, Pankaj Kumar, Sneh Sharma, Karuna Dhiman, Deepka Sharma, and Aruna Verma

Contents

Abstract Nanobiotechnology application, at the interface of nanocarrier and therapeutic enzyme, holds great promises in the nanomedicine. In this direction, gold nanocarriers contribute a plethora of nanobiotechnological applications due to their unique properties. The salient features of gold nanoparticle include high catalytic activity, unique optical properties, ease of surface functionalization, biocompatibility and long-period stability. The potential use of gold nanoparticle in conjunction with therapeutic enzymes can be further extended for curing many dreadful diseases.

M. L. Verma (✉)
Centre for Chemistry and Biotechnology, Deakin University, Melbourne, VIC, Australia

P. Kumar · S. Sharma · K. Dhiman · D. Sharma
Department of Biotechnology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India

A. Verma
Department of Biosciences, Himachal Pradesh University, Shimla, Himachal Pradesh, India

H. K. Daima et al. (eds.), *Nanoscience in Medicine Vol. 1*, Environmental Chemistry for a Sustainable World 39,
https://doi.org/10.1007/978-3-030-29207-2_3

We reviewed the suitability of gold nanocarrier-bound therapeutic enzyme delivery in biomedical modality, in particular to therapeutic application. The major health issues such as cancer, cardiovascular disease and brain disease are regulated with the intervention of gold nanoparticle-bound therapeutic enzyme delivery. Gold nanocarrier-bound therapeutic enzyme has increased the pharmacokinetic and pharmacodynamic correlation in drug delivery. Therapeutic fungal asparaginase covalently immobilized on the surface of gold nanoparticles demonstrated higher cytotoxicity effect against lung cancer and ovarian cell lines. It is further demonstrated that the gold nanoparticle-bound asparaginase has increased its bioavailability up to 85% more against lung cancer. The serratiopeptidase-bound gold nanoparticle has considerably increased anti-inflammatory response. The present chapter is concluded with recent literature discussion that gold nanoparticle-bound therapeutic enzyme has broadened the scope of traditional therapeutics to effective therapeutic enzyme delivery.

Keywords Nanogold · Biogenic methods · Therapeutic enzyme · Enzyme as a drug · Bioconjugation · Stability · Applications · Cell lines · Drug delivery · Anti-inflammatory · Cancer

3.1 Introduction

Nanomaterials, in particular gold nanoparticles, have gained attention due to the simplicity in its mode of action, ease of surface modifications, a plethora of applications such as data storage, environment, especially in medical biotechnology as nanocarrier for enzyme immobilizations and for drug delivery (Chamundeeswari et al. 2018; Golchin et al. 2018; Kaphle et al. 2018; Dykman and Khlebtsov. 2017; Verma 2017a, b, c, d; Gupta et al. 2016; Shankar et al. 2015; Kumar et al. 2014a, b; Sharma et al. 2014a, b; Verma et al. 2013a, b, c, d). Drug delivery is a fascinating field of scientific research in nanobiotechnology. Drug delivery is defined as the process for the release of biologically active medicament at a definite speed and at a destined location (Xin et al. 2017). Functionalized gold nanocarriers present huge probabilities for multiple, locus-specific drug delivery to the disease locus as their diminutive size can effectively penetrate across obstacles through small capillaries into individual cells. Specifically, gold nanoparticles have revealed great capacity to be used as drug delivery platforms (Pelaz et al. 2017). Gold nanoparticles have tremendous potential to deliver multiple drug molecules, recombinant proteins, vaccines and nucleotides into their targets effectively. Targeted/localized drug delivery is possibly achieved through active or passive approaches. Active targeting is based on conjugating the therapeutic agent or carrier system to a cell- or tissue-specific ligand, whereas passive targeting is based on a therapeutic agent that passively reaches out to a localized organ for efficient biomedical application such as target tumours by incorporation in the macromolecule or nanoparticle (Daraee et al. 2016).

Gold nanoparticles have always been considered as potential target for localized drug delivery applications in the field of biomedicine (Baskar et al. 2018). Nanocarriers have unique physicochemical characteristics such as definite size, surface area to mass ratio, chemical stability with high reactivity and functionalized structure with admirable biocompatibilities (Kong et al. 2017). Today, nanocarriers can serve as drug depots exhibiting prolonged-release kinetics and long persistence at the target site. Nanotechnology-based biomedicines have improved the pharmacokinetic and pharmacodynamic potential of different drug molecules which are capable of targeted/localized drug delivery applications such as early detection of cancer lesions, determination of molecular signatures of the tumour by non-invasive imaging and, most importantly, molecular-targeted cancer therapy and cardiovascular and neurodegenerative disease treatments (Pietro et al. 2017). Biocompatibility of gold nanoparticles, with ease of their biological and chemical nature, mimics the function of some enzymes including superoxide dismutase, esterase, peroxidase and glucose oxidase for various therapeutic applications such as tissue regeneration (Golchin et al. 2018). Localized delivery of drug-coated nanoparticles and emergence of such nanotherapeutics/diagnostics based on therapeutic enzymes provides the way for deeper understanding of human longevity and human ills that include genetic disorders, cancer and cardiovascular disease (Peer et al. 2007).

The present article is focussed on the applications of gold nanoparticle-mediated therapeutic enzyme delivery. Various physicochemical and biological methods of gold nanoparticle synthesis, biotechnology of therapeutic enzyme production, strategies of robust nanocarrier-enzyme bioconjugate development and biomedical applications of the gold nanocarrier-bound therapeutic enzyme are critically discussed.

3.2 Synthesis of Gold Nanoparticles

Various methods such as the Turkevich method, Brust-Schiffrin method, seeding growth method and biological method have been employed for the synthesis of gold nanoparticles (Herizchi et al. 2016; Rawat et al. 2016; Abdulghani and Hussain 2014; Singh et al. 2013; Siti et al. 2013; Bisker et al. 2012; Chithrani et al. 2010; Akbarzadeh et al. 2009; Mohanpuria et al. 2008; Brust et al. 1994; Turkevich et al. 1951).

Various chemical and physical methods of gold nanoparticle synthesis are most commonly used. However, these chemical methods involve the use of expensive and hazardous chemicals under extreme reaction conditions (Ahmed et al. 2015a; Ahmed et al. 2015b; Krishnaswamy et al. 2014; Kumar et al. 2011a, b). In addition, these nanoparticles may have harmful effects in biomedical applications (Noruzi et al. 2011; Shankar et al. 2004a, b). To overcome these problems, green synthesis of nanoparticles is an emerging field of research in the current era (Kulkarni and Muddapur 2014; Mittal et al. 2013). Hence, there is a growing need to develop eco-friendly and cost-effective procedures for the synthesis of nanoparticles. The inherent,

clean, nontoxic and environment-friendly ability of microorganisms and plant systems to synthesize the gold nanoparticles is particularly important in the advancement of nanobiotechnology (Mohanpuria et al. 2008).

Recently, plants are commonly employed for the synthesis of gold nanoparticle (Table 3.1A). The biosynthesis of gold nanoparticles using plants and plant extracts is a very important aspect due to lack of pathogenicity and their diversity (Chandran et al. 2014). Green synthesis of nanoparticles uses extracts of various plants such as *Aloe vera* (Chandran et al. 2006), *Pogostemon benghalensis* (Paul et al. 2015), *Salix alba* (Ul et al. 2015), *Solanum nigrum* (Muthuvel et al. 2014), *Terminalia arjuna*

Table 3.1A List of different plants employed for the synthesis of gold nanoparticles

Name of plants	Size of gold nanoparticles	References
Gymnocladus assamicus	4–22 nm	Tamuly et al. (2013a, b)
Cacumen platycladi	Variable	Wu et al. (2013)
Pogostemon benghalensis	13 nm	Paul et al. (2015)
Mangifera indica	6–18 nm	Yang et al. (2014)
Coriandrum sativum	6–57 nm	Narayanan and Sakthivel (2008)
Nerium oleander	2–10 nm	Tahir et al. (2015)
Butea monosperma	10–100 nm	Patra et al. (2015)
Arachis hypogaea	110–130 nm	Raju et al. (2014)
Solanum nigrum	50 nm	Muthuvel et al. (2014)
Hibiscus cannabinus	10–13 nm	Bindhu et al. (2014)
Sesbania grandiflora	7–34 nm	Das and Velusamy (2014)
Salix alba	50–80 nm	Ul et al. (2015)
Eucommia ulmoides	NA	Guo et al. (2015)
Galaxaura elongata	3–77 nm	Abdel-Raouf et al. (2017)
Ocimum sanctum	30 nm	Philip et al. (2011)
Torreya nucifera	10–125 nm	Kalpana et al. (2014)
Olea europaea	50–100 nm	Khalil et al. (2012)
Rosa indica	23–60 nm	Manikandan et al. (2014)
Pistacia integerrima	20–200 nm	Islam et al. (2015)
Terminalia arjuna	60 nm	MohanKumar et al. (2013)
Euphorbia hirta	6–71 nm	Annamalai et al. (2013)
Morinda citrifolia	12–38 nm	Suman et al. (2014)
Ziziphus mauritiana	20–40 nm	Sadeghi (2015)
Aloe vera	2–8 nm	Chandran et al. (2006)
Cassia auriculata	15–25 nm	Kumar et al. (2011a, b)
Hibiscus rosa-sinensis	16–30 nm	Philip (2010)
Ananas comosus	10–11 nm	Bindhu et al. (2014)
Sapindus mukorossi	9–19 nm	Reddy et al. (2013)
Prunus domestica	14–26 nm	Dauthal and Mukhopadhyay (2012)
Magnolia kobus	5–300 nm	Song et al. (2009)
Coleus amboinicus lour	9–31 nm	Narayanan and Sakthivel (2010)
Gnidia glauca	50–150 nm	Ghosh et al. (2012)

NA: not available

(MohanKumar et al. 2013), *Piper pedicellatum* (Sujitha and Kannan 2013), *Terminalia chebula* (Tamuly et al. 2013a, b), *Citrus reticulata* and *Citrus sinensis* (Mittal et al. 2013), *Mangifera indica* (Philip et al. 2011), *Murraya koenigii* (Das et al. 2011), *Zingiber officinale* (Kumar et al. 2011a, b), *Cymbopogon citratus* (Parida et al. 2011; Smithaa et al. 2009), *Coriandrum sativum* (Narayanan and Sakthivel 2008), *Azadirachta indica* (Shankar et al. 2004a, b) and *Medicago sativa* (Gardea-Torresdey et al. 2002). Plant extracts may act as both reducing agent and stabilizing agent in the synthesis of nanoparticles. In view of its simplicity, the use of plant extract for reducing metal salts to nanoparticles has attracted considerable attention (Mittal et al. 2013). Large-scale biosynthesis of nanoparticles is a main factor in green syntheses in which suitability of the reagents plays an important role (Chandran et al. 2014). Gold nanoparticles are rapidly synthesized using aqueous leaf extracts of *Acalypha indica* and *Azadirachta indica* as novel sources of bio-reductants (Krishnaraj et al. 2014). Biosynthesis of gold nanoparticles using leaf extracts of *Zingiber officinale,* which acted as a reducing and capping agent, was also reported (Singh et al. 2011). The use of plants and plant extracts for the preparation of gold nanoparticles is more advantageous. It does not require elaborate processes such as intracellular synthesis and multiple purification steps.

The biological method for the synthesis of nanoparticles by using microbes like bacteria, fungi, actinomycetes, yeast and algae is providing a wide range of resources for the synthesis of nanoparticles (Table 3.1B). Use of diverse microorganisms such as *Bacillus marisflavi* (Nilofar and Shivangi 2016), *Bacillus subtillus* (Reddy et al. 2010),

Table 3.1B List of different microorganisms employed for the synthesis of gold nanoparticles with different sizes

Type	Name	Size	References
Bacteria	*Bacillus subtilis*	5–25 nm	Reddy et al. (2010)
	Pseudomonas aeruginosa	5–30 nm	Husseiny et al. (2007)
	Escherichia coli	25–33 nm	Du et al. (2007)
	Rhodopseudomonas capsulata	10–20 nm	Shiying et al. (2007)
	Stenotrophomonas maltophilia	40 nm	Nangia et al. (2009)
	Brevibacterium casei	10–50 nm	Kalishwaralal et al. (2010)
	Bacillus licheniformis	10–100 nm	Kalishwaralal et al. (2009)
	Pseudomonas veronii	5–25 nm	Baker and Satish (2015)
	Klebsiella pneumoniae	35–65 nm	Malarkodi et al. (2013)
	Marinobacter pelagius	20 nm	Sharma et al. (2012)
	Geobacillus sp.	5–50 nm	Correa-Llantén et al. (2013)
	Bacillus marisflavi	14 nm	Nilofar and Shivangi (2016)
Fungi	*Rhizopus oryzae*	9–10 nm	Mukherjee et al. (2002)
	Fusarium oxysporum	8–40 nm	Das et al. (2012)
Algae	*Shewanella algae*	10 nm	Ogi et al. (2010)
	Sargassum wightii	8–12 nm	Singaravelu et al. (2007)
	Chlorella vulgaris	NA	Xie et al. (2007)
	Galaxaura elongate	3–77 nm	Abdel-Raouf et al. (2017)

NA: not available

Bacillus licheniformis (Kalishwaralal et al. 2009), *Pseudomonas veronii* (Baker and Satish 2015), *Galaxaura elongata* (Abdel-Raouf et al. 2017), *Chlorella vulgaris (*Xie et al. 2007), *Trichoderma asperellum* and *Trichoderma reesei* (Vahabi et al. 2011; Mukherjee et al. 2008), *Fusarium oxysporum* (Das et al. 2012), endophytic fungus *Verticillium* sp. (Bharde et al. 2006) and *Rhizopus oryzae* (Mukherjee et al. 2002) was employed for the synthesis of gold nanoparticles. It is a relatively new area of research with considerable prospects that can be used either extracellularly or intracellularly due to their innate potential. Mukherjee et al. (2002) also demonstrated that fungi secrete a significantly higher amount of proteins than bacteria; this would amplify the productivity of nanoparticle synthesis. Further, it is environmentally acceptable, economic, time saving and easily scaled up. Due to this ability to adapt to extreme conditions, these fungi can be used as a potential resource for biosynthesis of nanoparticles.

It can be inferred from the above-stated various methods of gold nanoparticle synthesis that the biological route provides an attractive possibility for the scale-up of gold nanoparticle production.

3.3 Biotechnology of Therapeutic Microbial Enzymes

Enzymes are the excellent biocatalysts that catalyse complex chemical reactions under appropriate physiological conditions. Enzymes possess a unique chiral-selective property, a prerequisite step for enantiomerically pure pharmaceutical drug production (Mane and Tale 2015; Bankar et al. 2009; Underkofler et al. 1957). Use of enzymes as drug target exhibits advantages over conventional drugs due to their unique target specificity and multiple substrate conversion (SKumar and Abdulhameed 2017). Therapeutic enzymes are obtained from bacteria, fungi and yeast (Table 3.2). Microbial enzyme production offers cost-effective technology that has a potential profitable market (Mane and Tale 2015; Gurung et al. 2013; Teal and Wymer 1991). Nowadays therapeutic enzymes are used for treating a diverse spectrum of life-threatening diseases such as cancer and gastrointestinal disorders and enzyme replacement therapy. Thus, therapeutic enzymes served as oncolytics, thrombolytics or anticoagulants and anti-inflammatory agents (Mane and Tale 2015; Gurung 2013; Gurung et al. 2013; Vellard 2003; Ozcan et al. 2002; Gonzalez and Isaacs 1999).

3.3.1 Different Types of Therapeutic Enzymes

Specificity of therapeutic enzymes makes them the most desirable therapeutic agents for the treatment of various diseases. Digestive and metabolic enzymes can be used either alone or in combination with other therapies for treating a variety of

Table 3.2 List of therapeutically important microbial enzymes employed for drug delivery

Microbial enzyme/source	Applications	References
Nattokinase/*Bacillus subtilis*	Cardiovascular disorder treatment	Dabbagh et al. (2014)and Hsia et al. (2009)
Uricase/Aspergillus flavus	Gout treatment	Terkeltaub (2009)
Superoxide dismutase/*Mycobacterium* sp., *Nocardia* sp.	Anti-inflammatory action	Ethiraj and Gopinath (2017) and Kaur and Sekhon (2012)
Serratiopeptidase/*Serratia marcescens*		
Glucosidase/*Aspergillus niger*	Cancer treatment	Ahmed et al. (2017), Dubey et al. (2015), Sharma et al. (2014), Yu et al. (2013), Kaur and Sekhon (2012), Jain et al. (2012), Para et al. (1984), Spiers and Wade (1976)and Peterson and Ciegler (1969)
L-Methionase/*Pseudomonas* sp.		
Arginase/*Bacillus subtilis*, *E. coli*		
Asparaginase/*E. coli*		
Glutaminase/*E. coli*, *Bacillus subtilis*		
Tyrosinase/*Streptomyces glaucescens, Erwinia herbicola*		
Staphylokinase/*Staphylococcus aureus, Streptococci* sp.	Anticoagulant action	Vakili et al. (2017), Kaur and Sekhon (2012), Zaitsev et al. (2010) and Banerjee et al. (2004)
Streptokinase/*Streptococci* sp.		
Urokinase/*Bacillus subtilis*		

diseases safely (Mane and Tale 2015; Kaur and Sekhon 2012; Sabu 2003; Vellard 2003; Cooney and Rosenbluth 1975).

Demands of therapeutic enzymes are growing rapidly due to massive biomedical applications. At present, the most prominent medical uses of microbial enzymes are the removal of dead skin and burns by proteolytic enzymes and clot busting by fibrinolytic enzymes (Singh et al. 2016). For example, a good agent for thrombosis therapy is nattokinase, a potent fibrinolytic enzyme (Sumi et al. 1987). Enzymes, namely, L-asparaginase, L-glutaminase, L-tyrosinase and galactosidase, are used as antitumour agents, and streptokinase and urokinase act as anticoagulants. Acid protease, dextranase and rhodanase may be used to treat alimentary dyspepsia, tooth decay and cyanide poisoning, respectively (Okafor, 2007). Microbial lipases and polyphenol oxidases are involved in the synthesis of diltiazem intermediate (2R,3S)-3-(4-methoxyphenyl)methyl glycidate and 3,4-dihydroxylphenyl alanine (DOPA, for treatment of Parkinson's disease), respectively (Faber 1997). Tyrosinase, an important oxidase enzyme, is involved in melanogenesis and in the production of L-DOPA. Dopamine, a potent drug to control the myocardium neurogenic injury and for the treatment of Parkinson's disease, is produced using L-DOPA as a precursor (Zaidi et al. 2014; Ikram-ul-Haq and Qadeer 2002). Chitosanase catalyses hydrolysis of chitosan to biologically active chitosan oligosaccharides, which are used as antimicrobial and antioxidant, in lowering blood cholesterol and high blood pressure, controlling arthritis, protecting against infections and improving antitumour

properties (Thadathil and Velappan 2014; Zhang et al. 2012; Ming et al. 2006; Kim and Rajapakse 2005).

3.3.2 Therapeutic Enzyme Production

In the pharmaceutical industry, bioprocessing of enzymes for use as drugs is an important aspect that is now being capitalized at every research and development centre across the globe (Cassileth 1998). Microbial therapeutic enzymes offer economic feasibility. That is why the use of microbial enzymes is increasing day by day (Gurung et al. 2013). Various methods involving fermentation technology are available for the production of microbial enzymes (Sabu et al. 2000). These include solid-state fermentation and submerged fermentation. On commercial scale, these methods are utilized for mass production of therapeutic enzymes than liquid cultures in huge bioreactors (Lozano et al. 2012). These important enzymes can be produced by different methods of fermentation. On an industrial scale, liquid cultures in huge bioreactors are preferred for producing therapeutic enzymes in bulk. Other processes like solid-state fermentations and submerged fermentations are also widely used for the production of therapeutic enzymes (Sabu 2003). Large-scale productions of microbial therapeutic enzymes using various production techniques and downstream processing have been reported (Sabu et al. 2005; Sabu 2003).

Gold nanoparticle was employed for some enzyme deliveries such as superoxide dismutase, esterase, peroxidase and glucose oxidase for various therapeutic applications (Golchin et al. 2018). It is very pertinent that only a few therapeutic enzymes have been explored for gold nanoparticle-mediated drug delivery so far. Thus, it can be inferred that many therapeutic enzymes have to be employed for nanocarrier-mediated drug delivery.

3.4 Methods for Developing Robust Gold Nanocarrier for Therapeutic Enzyme Delivery

Therapeutic enzymes are susceptible to denaturation under harsh environmental conditions (Abraham et al. 2014; Puri et al. 2013; Verma and Kanwar 2010, 2012; Verma et al. 2009, 2011, 2012). In order to make a robust and biocatalytic stable enzyme, enzymes need protection and cost-effective recyclability by immobilizing the suitable inert carrier (Verma et al. 2016). Nanomaterials possess many physicochemical advantages over their bulk materials. Immobilization of enzymes on the nanoparticles holds a great promise to improve their functionality and biocatalytic potentials. Nano-immobilization methods are generally categorized into four types, namely, (1) electrostatic adsorption, (2) conjugation of the ligand on the nanoparticle surface, (3) conjugation to a small cofactor molecule that the protein can recognize and bind to and (4) direct conjugation to the gold nanoparticle surface (Fig. 3.1;

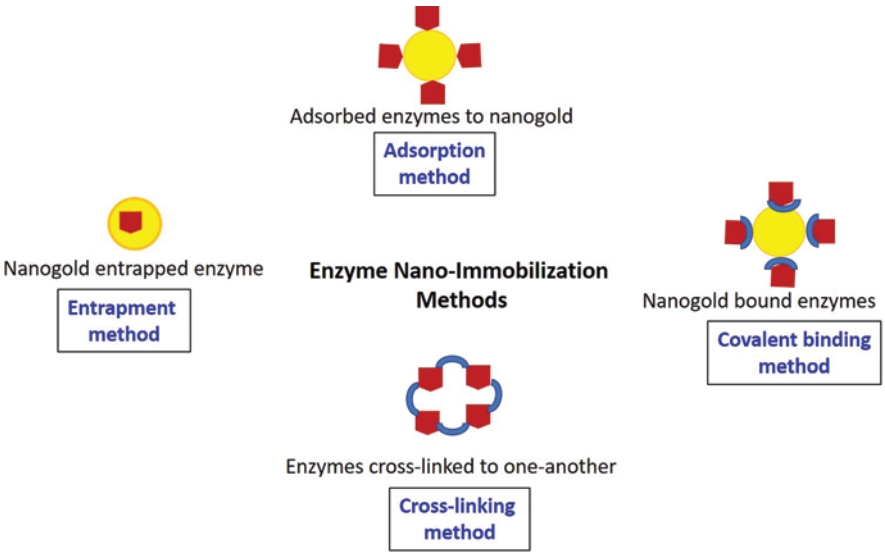

Fig. 3.1 Schematic of four methods of enzyme nano-immobilization with gold nanoparticle

Table 3.3 Pros and cons of the enzyme nano-immobilization methods

Type of immobilization method	Advantages	Disadvantages	References
Adsorption method	Simple and chemical free method, no confirmation of the enzyme	Weak bonding may cause enzyme leakage (desorption) from the nanocarrier	Verma et al. (2016), Kanwar and Verma (2010), Kanwar et al. (2007a, b)
Entrapment method	Enzyme protection, ease of separation	Possibility of enzyme leakage, low enzyme loading	Kadri et al. (2018) and Verma et al. (2016)
Cross-linking method	High enzyme loading, strong binding	Possibility of alteration in enzyme active site, loss of enzyme activity	Velasco-Lozano et al. (2016) and Verma and Barrow (2015)
Covalent binding method	Strong enzyme binding, leakage-free enzyme binding	Chemical modification of enzymes, enzyme denaturation	Kumar et al. (2014a, b), Abraham et al. (2014) and Verma et al. (2013a, b, c, d)

Verma et al. 2016; Verma and Barrow 2015; Puri et al. 2013; Yeh et al. 2012; Ackerson et al. 2010; Aubin and Hamad 2008). Each of these techniques has its pros and cons (Table 3.3; Kanwar and Verma 2010; Kanwar et al. 2008; Kanwar et al. 2007a,b; Kanwar et al. 2006; Kanwar et al. 2005). Thus, sometimes a combination of these nano-immobilization techniques is employed in order to get robust gold nanoparticle conjugates.

Among different nanoparticles (fullerenes, single-walled and multiwalled carbon nanotubes, magnetic nanoparticles, modified silicon nanowires, dendrimers and quantum dots), only gold nanoparticles can be marked as the most used and widespread for biomedical applications (Zhang et al. 2015). Besides the common properties typical for nanomaterials, the main specific characteristics of gold nanostructures are its stability for a long period of time, easy surface functionalization, biocompatibility, unique optical properties and high catalytic activity providing the successful use of gold nanoparticles (Yu et al. 2016). Gold nanoparticles can be attached to those functional groups which have positive charge because of negative charge on their surface. Likewise, the presence of six free electrons in the conduction band of gold nanoparticles makes them potential candidates to bind with reactive functional groups like thiols and amines. Silica, aluminium oxide and titanium oxides facilitate the attachment of different functional groups on the surface of gold nanoparticles (Sharma et al. 2015; Sharma et al. 2010; Kim et al. 2010; Sun et al. 2008; Tkachenko et al. 2004). Thus, gold nanoparticles can be easily tagged with various proteins and biomolecules that are rich in amino acids (Giljohann et al. 2010; Eustis and El-Sayed 2005). Therapeutic and diagnostic efficiency can strongly be influenced by changing the surface characteristics of nanoparticles such as size, shape and surface charge which in turn change cellular uptake and functional surface area (Jazayeri et al. 2016).

The conjugation of different functionalized groups to nanoparticles is prerequisite for improving stability, functionality and biocompatibility (Delong et al. 2010). It has also been reported that it is possible to control the interactions of gold nanoparticles with cell membranes in order to improve their cellular uptake while minimizing their toxicity by rigid change of the surface charge densities (Lin et al. 2010). Physical and chemical interactions are used for attaching functional groups (DNA, RNA, enzymes, peptides, bovine serum albumin, polyethylene glycol and proteins) to gold nanoparticles' surface (Cho et al. 2012; Lee et al. 2008). Noncovalent interaction between functional groups and gold nanoparticles depends on three phenomena: (a) ionic attraction between the negatively charged gold and the positively charged functional group, (b) hydrophobic attraction between the functional group and the gold surface and (c) dative binding between the gold conducting electrons and functional group. Covalent interactions between functional groups and nanoparticle surface are achieved in a number of ways like (i) through chemisorption via thiol derivatives, (ii) through the use of bifunctional linkers and (iii) through the use of adapter molecules like streptavidin and biotin (Delong et al. 2010). Other functional groups like citrate, tannic acid and polyvinylpyrrolidone can be capped to gold nanoparticles (Marcelo et al. 2015; Senoudi et al. 2014; Mirza and Shamshad 2011).

Gold nanoparticles are useful for important biomedical applications including targeted drug delivery, cellular imaging and biosensing (Hwang et al. 2012; Hong et al. 2012; Giljohann et al. 2010; Huang and El-Sayed 2010). In a recent study, therapeutic fungal asparaginase was covalently immobilized on the surface of gold nanoparticles or nanoporous gold nanoparticles (Baskar et al. 2018). Immobilized gold nanoparticle was further targeted for drug delivery with respect to cancer treat-

ment. It has been demonstrated that the synthesized gold nanobiocomposite of asparaginase can be used as an effective anticancer drug with increased bioavailability against lung cancer.

Gold nanoparticles proved robust nanocarriers for neurotrophin peptides (Patrizia et al. 2017). The immobilization of neurotrophin peptide was achieved by direct physisorption and lipid bilayer-mediated adsorption methods. The nanobioconjugates were characterized by UV-vis spectroscopy, X-ray photoelectron spectroscopy, dynamic light scattering, zeta-potential analyses and atomic force microscopy. Both peptide- and lipid-dependent features were identified to have a modulation in the peptide coverage of nanoparticles as well as in the cellular uptake of nerve growth factors and brain-derived neurotrophic factors. Robust hybrid gold peptide nanointerface demonstrated a promising approach to neurotrophin for crossing blood-brain barriers. Gold nanocarrier provided **new multipotential therapeutic nanoplatform for the treatment of** central nervous system **disorders.**

Gupta et al. (2016) reported a new generation of surface ligands based on a combination of short oligo(ethylene glycol) chains and zwitterions capable of providing non-fouling characteristics while maintaining colloidal stability and functionalization capabilities. Moreover, conjugation of gold nanoparticles with avidin helped in the development of a universal toolkit for further functionalization of nanomaterials.

Muthurasu and Ganesh (2016) prepared glucose oxidase-stabilized gold nanoparticles by changing the pH and showed feasibility of employing such nanocarrier as an ideal sensor for dual-mode sensing of glucose. Gold nanoparticles were able to detect glucose at a low concentration with high sensitivity, good stability and reproducibility suggesting promising applications in the field of nanobiosensors.

Malda et al. (2010) developed a conjugate of gold nanoparticle and therapeutically important superoxide dismutase at specific physiochemical reaction condition. Binding of enzyme-nanoparticle was confirmed by gel electrophoreses. Superoxide dismutase is a metalloenzyme that catalysed the dismutation of superoxide radicals into hydrogen peroxide and oxygen. Reactive oxygen species, such as superoxide radicals, are the root cause to pathogenesis of several diseases, such as familial amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, Down syndrome and several neurological disorders (Halliwell and Gutteridge 2012; Pissuwan et al. 2007). Gold nanoparticle-superoxide dismutase enzyme conjugates proved its therapeutic potential in the prevention of oxidative damage from superoxide radicals (He et al. 2013; Zhao et al. 2012).

Synthesis of gold nanoparticles using the therapeutic enzyme serratiopeptidase was done at 25 °C and physiological pH 7 (Venkatpurwar and Pokharkar 2010). The formation of serratiopeptidase-reduced gold nanoparticles was confirmed by UV-visible spectroscopy, transmission electron microscopy, X-ray diffraction and Fourier transform infrared spectroscopy. This study successfully demonstrated that physiological condition is an important process parameter for the controlled synthesis of highly stable gold nanoparticles with respect to retention of biocatalyst activity. Researchers further confirmed use of gold nanoparticle as a carrier for

serratiopeptidase led to an improved anti-inflammatory response (Venkatpurwar and Pokharkar 2010).

It is inferred from the above-stated studies that the binding of gold nanocarrier either non-covalently or covalently to therapeutically important enzyme depends on the immobilization reaction conditions and enzyme stability. This is a very critical step to immobilize fragile enzyme on the non-functionalized surface of gold nanoparticle. Robust gold nanocarrier immobilized enzyme successfully demonstrated various biomedical applications such as neurological and inflammatory issues.

3.5 Potential Applications of Gold Nanocarriers in Enzyme-Mediated Drug Delivery

Gold nanoparticle-based targeted drug deliveries have considerable applications to overcome the limitations in traditional therapeutics (Daraee et al. 2016). For example, antineoplastics, antiviral drugs and various other types of drugs are manifestly stuck due to their inability to cross the blood-brain barrier. Nanoparticle application to deliver drugs across this barrier is enormously promising. Researchers have reported that nanoparticles can cross several biological barriers for sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumours (Nazir et al. 2014; Hainfeld et al. 2013).

The potential of nanomedicine with respect to targeted drug delivery has improved with the ease of nanoformulation technique and widened the scope of delivering a range of drugs. Nanomedicine has developed novel diagnostic and screening techniques that have extended the scope of molecular diagnostics. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and deliver the drug at a controlled and sustained rate to the site of action, minimizing undesirable side effects of the drug and allowing for more efficient use of the drug (Bosio et al. 2016).

Today, therapeutic enzymes are considered as one of the most promising applications in the pharmaceutical field. It has been reported by various researchers that enzymatic biocatalyst properties improved considerably by enzyme immobilization on nanomaterials, thereby increasing its stability and reusability and most importantly enhancing their targeting/localization to specific cell and tissues (Golchin et al. 2018; Xin et al. 2017). Gold nanoparticle-based therapeutic biocatalyst provides new tools for the diagnosis and treatment of old and newly emerging pathologies and presents distinctive modality for therapeutic delivery (Table 3.4; Golchin et al. 2018). Thus, gold nanoparticle-based therapeutic enzymes represent a highly promising alternative for treating a variety of pathologies by localized drug delivery approach.

Asparaginase obtained from *Aspergillus terreus* is a potent drug for the treatment of cancer and has antineoplastic or cytotoxic chemotherapy effect (Baskar and

Table 3.4 List of gold nanoparticle-immobilized therapeutic enzymes

Nanocarrier	Therapeutic enzyme/peptide	Type of immobilization method	Application	References
Gold nanoparticle	Asparaginase	Covalent binding method	Anticancerous activity	Baskar et al. (2018)
Gold nanoparticle	Neurotrophin peptides	Adsorption method	Promising drugs in neurodegenerative disorders	Patrizia et al. (2017)
Gold nanoparticle	Glucose oxidase		Biosensing	Muthurasu and Ganesh (2016)
Gold nanoparticle	Superoxide dismutase	Adsorption method	Prevention of oxidative damage from superoxide radicals	Malda et al. (2010)
Gold nanoparticle	Serratiopeptidase	Adsorption method	Strong anti-inflammatory response	Venkatpurwar and Pokharkar (2010)
Gold nanoparticle	Serratiopeptidase	Covalent binding method	Anti-inflammatory activity	Venkatpurwar and Pokharkar (2010)
Silica-coated gold nanoparticles	Oxidase and peroxidase	Adsorption method	Antibacterial properties	Tao et al. (2015)
Gold nanoparticle nanocomposite	Peroxidase	Covalent binding method	Anticancerous activity	Maji et al. (2015)

Renganathan 2012). Malignant cells lack asparagine synthase and employ the free circulating asparagine for its growth. Asparaginase converts the free circulating asparagine into aspartic acid and ammonia thereby lacking the asparaginase and leading to the death of tumour cells. So, research have been carried out across globe to target better asparaginase delivery system by immobilizing asparaginase on to gold nanoparticles followed by procedure of asparaginase gold nano-bioconjugate as potential drug candidate for curbing cancer, by testing against lung cancer cell line and ovarian cancer cell line.

Researchers studied gold nanoparticle-mediated delivery of fungal asparaginase against cancer cells (Baskar et al. 2018). The fungal asparaginase immobilized on gold nanoparticles showed efficient drug delivery in cancer treatment. Fourier transform infrared spectroscopy and nuclear magnetic resonance analysis of the synthesized asparaginase gold nano-bioconjugate showed that primary amines, secondary amines and allylic carbon are the main functional groups concerned with binding of asparaginase onto gold nanoparticles. Increment in the specific enzyme activity of asparaginase was recorded from crude (252.05 U/mg) to gold nano-bioconjugate (364 U/mg). Protein concentration was also increased from 0.018 mg/ml in crude asparaginase to 0.332 mg/ml in gold nano-bioconjugate. Nano-bioconjugate cytotoxicity effect was also observed to be higher against lung cancer cell line A549

than ovarian cancer cell line A2780. Finally, authors demonstrated that synthesized gold asparaginase nano-bioconjugate can be used as an effective anticancer drug and for targeted drug delivery with its increased bioavailability against lung cancer cell line (A549), given that toxicity is 84.51% (Baskar et al. 2018).

Serratiopeptidase, a proteolytic endopeptidase bioenzyme, is recognized as one of the most important therapeutic enzymes having anti-inflammatory activity (Salamone and Wodzinski 1997). Traditionally, therapeutic enzyme delivery is limited due to their poor uptake and vulnerability to degradation inside the gastrointestinal tract. For efficient drug delivery, nanoparticles such as gold nanoparticle complex have immense potential in the therapeutic perspective of biomedicine formulation. With this, the prerequisite is the nanocarrier which plays an important role in the bioavailability of the pharmaceutically active compound, efficiently improving absorption across the gastrointestinal mucosa (Dykman and Khlebtsov 2017).

Venkatpurwar and Pokharkar (2010) have reported the synthesis of gold nanoparticle using a therapeutic enzyme serratiopeptidase at physiological conditions which retained enzyme activity, and serratiopeptidase-capped gold nanoparticle complex led to improved therapeutic benefit. Characterization of synthesized gold nanoparticles has been reported using UV-visible spectroscopy, transmission electron microscopy, X-ray diffraction and Fourier transform infrared spectroscopy. Synthesized nanoparticle stability was assessed at ambient temperature up to 6 months. The retention of enzymatic activity was confirmed by in vitro enzymatic activity and in vivo anti-inflammatory activity of synthesized serratiopeptidase-capped gold nanoparticle complex. The tri-functional role of serratiopeptidase was reported, such as reduction, stabilization and therapeutic activity, finally demonstrating the gold nanoparticles as a nanocarrier for the immobilization and efficient and improved delivery of a therapeutic enzyme for an oral administration with improved therapeutic benefit (Venkatpurwar and Pokharkar 2010).

Tao et al. (2015) studied the bifunctionalized mesoporous silica-supported gold nanoparticles that showed intrinsic oxidase and peroxidase catalytic activities for antibacterial applications for their targeted delivery. Gold nanoparticles have exhibited both oxidase and peroxidase mimicking activities imparting end reactions as reactive oxygen species (ROS). Antibacterial properties proved against both Gram-negative and Gram-positive bacteria.

Superoxide dismutase is an important metalloenzyme and antioxidant defence against free radicals. It catalyses the dismutation of superoxide radicals into hydrogen peroxide and oxygen. Also, catalase is classified under a therapeutic enzymatic group supporting the cell from oxidative damage by reactive oxygen species (Golchin et al. 2018). Reactive oxygen species, such as superoxide radicals, have received great attention due to their involvement in the pathogenesis of various diseases, such as Alzheimer's disease, Down syndrome, cataract, familial amyotrophic lateral sclerosis, Parkinson's disease, cardiac myocytes and several neurological disorders. Superoxide dismutase enzymes have vast physiological importance and therapeutic benefit in the prevention of the oxidative damage from superoxide radicals (He et al. 2013; Zhao et al. 2012). Malda et al. (2010) have synthesized gold

nanoparticle-iron-bound enzyme that demonstrated vast efficacy of gold colloid nanoparticle-bound superoxide dismutase protein.

Maji et al. (2015) have developed the new nanostructured hybrid as a mimetic enzyme for in vitro detection and therapeutic treatment of cancer cells. For targeted drug delivery application in the emerging field of nanobiotechnology, an artificial therapeutic enzyme conjugate was prepared by the immobilization of gold nanoparticles on mesoporous silica-coated nanosized reduced graphene oxide conjugated with folic acid, a cancer cell-targeting ligand. In vitro experiments with bioconjugate hybrid using human cervical cancer cells led to an enhanced cytotoxicity to Henrietta Lacks (HeLa) cells. In the case of normal cells (human embryonic kidney HEK 293 cells), the treatment with the hybrid and H_2O_2 showed no obvious damage, proving selective killing effect of the hybrid to cancer cells. Hybrid therapeutic enzyme bioconjugate with peroxidase activity has dual applications: firstly, detection (selective quantitation and colorimetric) of cancer cells and, secondly, cancer therapy by activating oxidative stress. Both detection and therapeutic processes are selective to cancer cells, indicating high specificity and robustness of the hybrid (gold nanoparticle) conjugate proved as a promising candidate for clinical cancer diagnostics and treatment and their targeted drug delivery approach (Nasrabadi et al. 2016).

It can be inferred from few of the above-discussed studies of nanocarrier-bound therapeutic enzyme delivery that nanocarrier-based approach such as gold nanoparticle-immobilized enzymes represents an important modality within therapeutic and diagnostic biomedical applications including cancer, cardiovascular diseases and brain diseases.

3.6 Conclusion

Gold nanoparticles offer an excellent platform for biomedical applications due to their unique physical and chemical properties. Amongst the various physicochemical and biological methods of gold nanoparticle syntheses, the biological route has become most fascinating due to total avoidance of toxic chemical and ambient reaction conditions and more biocompatibity of the gold nanoparticles, since delivery of enzyme as drug along with the antimicrobial property of gold nanocarrier adds additional double effects on various health ailments. Very few therapeutic microbial enzymes are used till date, and more research on gold nanocarrier-bound therapeutic important enzyme is the need of the hour.

The selection of the most appropriate methods for robust gold nanocarrier design needs a thorough understanding of non-covalent and covalent interactions at the interface of different types of therapeutic enzymes and different functionalized gold nanoparticles. It is inferred that gold nanocarrier-bound limited therapeutic enzyme has shown promising results in the treatment of central nervous system disorders. To sum up, gold nanocarrier-mediated delivery of therapeutic enzymes holds a great potential for biomedical applications.

Acknowledgement The authors would like to thank the director of the Indian Institute of Information Technology Una for providing the necessary facility to carry out nanobiotechnology work.

References

Abdel-Raouf N, Al-Enazi NM, Ibraheem IBM (2017) Green biosynthesis of gold nanoparticles using *Galaxaura elongata* and characterization of their antibacterial activity. Arab J Chem 10:S3029–S3039. https://doi.org/10.1016/j.arabjc.2013.11.044

Abdulghani J, Hussain RK (2014) Synthesis of gold nanoparticles via chemical reduction of Au (III) Ions by isatin in aqueous solutions: ligand concentrations and pH effects. J Baghdad Sci 11:1201–1216. https://www.iasj.net/iasj?func=fulltext&aId=93354

Abraham RE, Verma ML, Barrow CJ, Puri M (2014) Suitability of ferrite nanoparticles immobilised cellulases in enhancing enzymatic saccharification of pretreated hemp biomass. Biotechnol Biofuels 7:90. https://doi.org/10.1186/1754-6834-7-90

Ackerson CJ, Powell RD, Hainfeld JF (2010) Site-specific biomolecule labeling with gold clusters. Methods Enzymol 481:195–230. https://doi.org/10.1016/S0076-6879(10)81009-2

Ahmed S, Ahmad M, Swami BL, Ikram S (2015a) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J Adv Res 7:17–28. https://doi.org/10.1016/j.jare.2015.02.007

Ahmed S, Ullah S, Ahmad M, Swami BL (2015b) Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J Rad Res App Sci 9:1–7. https://doi.org/10.1016/j.jrras.2015.06.006

Ahmed A, Nasim FH, Batool K, Bibi A (2017) Microbial β-glucosidase: sources, production and applications. J Appl Environ Microbiol 5:31–46. https://doi.org/10.12691/jaem-5-1-4

Akbarzadeh A, Zare D, Farhangi A, Mohammad RM, Norouzian D, Tangestaninejad S, Moghadam M, Bararpour N (2009) Synthesis and characterization of gold nanoparticles by tryptophane. Am J Appl Sci 6:691–695. https://doi.org/10.3844/ajassp.2009.691.695

Annamalai A, Christina VLP, Sudha D, Kalpana M, Lakshmi PTV (2013) Green synthesis, characterization and antimicrobial activity of AuNPs using *Euphorbia hirta* L. leaf extract. Colloids Surf B Biointerfaces 108:60–65. https://doi.org/10.1016/j.colsurfb.2013.02.012

Aubin-Tam ME, Hamad-Schifferli K (2008) Structure and function of nanoparticle-protein conjugates. Biomed Mater 3:034001. https://doi.org/10.1088/1748-6041/3/3/034001

Baker S, Satish S (2015) Biosynthesis of gold nanoparticles by *Pseudomonas veronii* AS41G inhabiting *Annona squamosa* L. Spectrochim. Acta Part A Mol Biomol Spectrosc 15:691–695. https://doi.org/10.1016/j.saa.2015.05.080

Banerjee A, Chisti Y, Banerjee UC (2004) Streptokinase-A clinically useful thrombolytic agent. Biotechnol Adv 22:287–307. https://doi.org/10.1016/j.biotechadv.2003.09.004

Bankar SB, Bule MV, Singhal RS, Ananthanarayan L (2009) Glucose oxidase an overview. Biotechnol Adv 27:489–501. https://doi.org/10.1016/j.biotechadv.2009.04.003

Baskar G, Renganathan S (2012) Optimization of L-asparaginase production by *Aspergillus terreus* MTCC 1782 using response surface methodology and artificial neural network-linked genetic algorithm. Asia Pac J Chem Eng 7:212–220. https://doi.org/10.1002/apj.520

Baskar G, Garrick BG, Lalitha K, Chamundeeswari M (2018) Gold nanoparticle mediated delivery of fungal asparaginase against cancer cells. J Drug Delivery Sci Technol 44:498–504. https://doi.org/10.1016/j.jddst.2018.02.007

Bharde A, Rautaray D, Bansal V, Ahmad A, Sarkar I, Yusuf SM, Sanyal M, Sastry M (2006) Extracellular biosynthesis of magnetite using fungi. Small 2:135–141. https://doi.org/10.1002/smll.200500180

Bindhu MR, Umadevi M (2014) Antibacterial activities of green synthesized gold nanoparticles. Funct Mater Lett 120:122–125. https://doi.org/10.1016/j.matlet.2014.01.108

Bindhu MR, Vijaya Rekha P, Umamaheswari T, Umadevi M (2014) Antibacterial activities of *Hibiscus cannabinus* stem-assisted silver and gold nanoparticles. Mater Lett 131:194–197. https://doi.org/10.1016/j.matlet.2014.05.172

Bisker G, Yeheskely-Hayon D, Minai L, Yelin D (2012) Controlled release of Rituximab from gold nanoparticles for phototherapy of malignant cells. J Control Release 162:303–309. https://doi.org/10.1016/j.jconrel.2012.06.030

Bosio VE, German A, Yanina N, Martinez ND, Guillermo R (2016) Nanodevices for the immobilization of therapeutic enzymes. Crit Rev Biotechnol 36:447–464. https://doi.org/10.3109/07388551.2014.990414

Brust M, Walker M, Bethell D, Schiffrin DJ, Whyman RJ (1994) Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid-liquid system. Chem Soc Chem Commun 7:801–802. https://doi.org/10.1039/C39940000801

Cassileth B (1998) The alternative medicine handbook. Norton WW & Co., New York. https://www.publishersweekly.com/978-0-393-04566-6

Chamundeeswari M, Jeslin J, Verma ML (2018) Nanocarriers for drug delivery applications. Environ Chem Lett. https://doi.org/10.1007/s10311-018-00841-1

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M (2006) Synthesis of gold nanotriangles and silver nanoparticles using *Aloe Vera* plant extract. Biotechnol Prog 22:577–583. https://doi.org/10.1021/bp0501423

Chandran K, Song S, Yun S (2014) Effect of size and shape controlled biogenic synthesis of gold nanoparticles and their mode of interactions against food borne bacterial pathogens. Arabian J Chem (article in press). https://doi.org/10.1016/j.arabjc.2014.11.041

Chithrani DB, Dunne M, Stewart J, Allen C, Jaffray DA (2010) Cellular uptake and transport of gold nanoparticles incorporated in a liposomal carrier. Nanomed Nanotechnol Biol Med 6:161–169. https://doi.org/10.1016/j.nano.2009.04.009

Cho WS, Cho M, Jeong J, Choi M, Han BS, Shin HS, Hong J, Chung BH, Jeong J, Cho MH (2012) Size dependent tissue kinetics of PEG-coated gold nanoparticles. Toxicol Appl Pharmacol 245:116–123. https://doi.org/10.1016/j.taap.2010.02.013

Cooney DA, Rosenbluth RJ (1975) Enzymes as therapeutic agents. Adv Pharmacol Chemother 12:185–289. https://www.ncbi.nlm.nih.gov/pubmed/168755

Correa-Llantén DN, Muñoz-Ibacache SA, Castro ME, Muñoz PA, Blamey JM (2013) Gold nanoparticles synthesized by *Geobacillus* sp. strain ID17 a thermophilic bacterium isolated from Deception Island, Antarctica. Microb Cell Fact 12:1–6. https://doi.org/10.1186/1475-2859-12-7

Dabbagh F, Negahdaripour M, Berenjian A, Behfar A, Mohammadi F, Zamani M, Irajie C, Ghasemi Y (2014) Nattokinase: production and application. Appl Microbiol Biotechnol 98:9199–9206. https://doi.org/10.1007/s00253-014-6135-3

Daraee H, Eatemadi A, Abbasi E, Fekri AS, Kouhi M, Akbarzadeh A (2016) Application of gold nanoparticles in biomedical and drug delivery. Artif Cells Nanomed Biotechnol 44:410–422. https://doi.org/10.3109/21691401.2014.955107

Das J, Velusamy P (2014) Catalytic reduction of methylene blue using biogenic gold nanoparticles from *Sesbania grandiflora* L. J Taiwan Inst Chem Eng 45:2280–2285. https://doi.org/10.1016/j.jtice.2014.04.005

Das RK, Gogoi N, Bora U (2011) Green synthesis of gold nanoparticles using *Nyctanthes arbortristis* flower extract. Bioprocess Biosyst Eng 34:615–619. https://doi.org/10.1007/s00449-010-0510-y

Das SK, Dickinson C, Lafir F, Brougham DF, Marsili E (2012) Synthesis, characterization and catalytic activity of gold nanoparticles biosynthesized with *Rhizopus oryzae* protein extract. Green Chem 14:1322–1334. https://doi.org/10.1039/C2GC16676C

Dauthal P, Mukhopadhyay M (2012) *Prunus domestica* fruit extract- mediated synthesis of gold nanoparticles and its catalytic activity for 4-nitrophenol reduction. Ind Eng Chem Res 51:13014–13020. https://doi.org/10.1021/ie300369g

DeLong RK, Reynolds CM, Malcolm Y, Schaeffer A, Severs T, Wanekaya A (2010) Functionalized gold nanoparticles for the binding, stabilization, and delivery of therapeutic DNA, RNA, and

other biological macromolecules. Nanotechnol Sci Appl 3:53–63. https://doi.org/10.2147/NSA.S8984

Dolynchuk K, Keast D, Campbell K (2000) Best practices for the prevention and treatment of pressure ulcers. Ostomy/Wound Manag 46:38–53. https://www.ncbi.nlm.nih.gov/pubmed/11889736

Du L, Hong J, Xiaohua L, Erkang W (2007) Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5α and its application on direct electrochemistry of haemoglobin. Electrochem Commun. 9:1165–1170. https://doi.org/10.1016/j.elecom.2007.01.007

Dubey R, Paul A, Prity N (2015) Isolation, production & screening of anti-cancer enzyme L-glutaminase from *Bacillus subtilis*. Int J Pharm Bio Sci 5:96–105. https://ijpbs.com/ijpbsadmin/upload/ijpbs_55941b093bbed.pdf

Dykman LA, Khlebtsov NG (2017) Immunological properties of gold nanoparticles. Chem Sci 8:1719–1735. https://doi.org/10.1039/C6SC03631G

Ethiraj S, Gopinath S (2017) Production, purification, characterization, immobilization, and application of Serrapeptase: a review. Front Biol 12:333–348. https://doi.org/10.1007/s11515-017-1461-3

Eustis S, El-Sayed M (2005) Aspect ratio dependence of the enhanced fluorescence intensity of gold nanorods: experimental and simulation study. J Phys Chem B 109:16350–16356. https://doi.org/10.1021/jp052951a

Faber K (1997) Biotransformations in organic chemistry: a textbook. Springer, Berlin. https://www.springer.com/in/book/9783642173936

Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Troiani HE, Santiago P, Jose Yacaman M (2002) Formation and growth of Au nanoparticles inside live alfalfa plants. Nano Lett 2:397–401. https://doi.org/10.1021/nl015673+

Ghosh S, Patil S, Ahire M, Kitture R, Gurav D, Jabgunde AM, Kale S, Pardesi K, Shinde V, Bellare V, Dhavale DD, Chopade BA (2012) *Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. J. Nanobiotec 10:17. https://doi.org/10.1186/1477-3155-10-17

Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA (2010) Gold nanoparticles for biology and medicine. Angew Chem Int Ed 49:3280–3294. https://doi.org/10.1002/anie.200904359

Golchin K, Golchin J, Ghaderi S, Alidadiani N, Eslamkhah S, Eslamkhah M, Davaran S, Akbarzadeh A (2018) Gold nanoparticles applications: from artificial enzyme till drug delivery. Artif Cells Nanomed Biotechnol. 46:250–254. https://doi.org/10.1080/21691401.2017.1305393

Gonzalez NJ, Isaacs LL (1999) Evaluation of pancreatic proteolytic enzyme treatment of adenocarcinoma of the pancreas with nutrition and detoxification support. Nutr Cancer 33:117–124. https://doi.org/10.1207/S15327914NC330201

Guo M, Li W, Yang F, Liu H (2015) Controllable biosynthesis of gold nanoparticles from a *Eucommia ulmoides* bark aqueous extract. Spectrochim Acta Part a Mol Biomol Spectrosc 142:73–79. https://doi.org/10.1016/j.saa.2015.01.109

Gupta A, Moyano DF, Parnsubsakul A, Papadopoulos A, Wang LS, Landis RF, Das R, Rotello VM (2016) Ultrastable and biofunctionalizable gold nanoparticles. ACS Appl Mater Interfaces 8:14096–14101. https://doi.org/10.1021/acsami.6b02548

Gurung N, Ray S, Bose S, Rai V (2013) A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. BioMed Res Int 2013:329121, 18 pages. https://doi.org/10.1155/2013/329121

Hainfeld JF, Smilowitz HM, O'Connor MJ, Dilmanian FA, Slatkin DN (2013) Gold nanoparticle imaging and radiotherapy of brain tumors in mice. Nanomedicine (Lond) 8:1601–1609. https://doi.org/10.2217/nnm.12.165

Halliwell B, Gutteridge JMC (2012) Free radicals in biology and medicine, 4th edn. Oxford University Press, Oxford. https://global.oup.com/academic/product/free-radicals-in-biology-and-medicine-9780198717485?cc=us&lang=en&

He W, Zhou Y-T, Wamer WG, Hu X, Wu X, Zheng Z, Boudreau MD, Yin JJ (2013) The Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging. Biomaterials 34:765–773. https://doi.org/10.1016/j.biomaterials.2012.10.010

Herizchi R, Abbasi E, Milani M, Akbarzadeh A (2016) Current methods for synthesis of gold nanoparticles. Artif Cells Nanomed Biotechnol 44:596–602. https://doi.org/10.3109/21691401.2014.971807

Hong Y, Huh YM, Yoon DS, Yang J (2012) Nanobiosensors based on localized surface plasmon resonance for biomarker detection. J Nanomater 2012:759830, 13 pages. https://doi.org/10.1155/2012/759830

Hsia CH, Shen MC, Lin JS, Wen YK, Hwang KL, Cham TM (2009) Nattokinase decreases plasma levels of fibrinogen, factor VII, and factor VIII in human subjects. Nutr Res 29:190–196. https://doi.org/10.1016/j.nutres.2009.01.009

Huang X, El-Sayed MA (2010) Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. J Adv Res 1:13–28. https://doi.org/10.1016/j.jare.2010.02.002

Husseiny MI, El-Aziz MA, Badr Y, Mahmoud MA (2007) Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. Spectrochim. Acta A Mol Biomol Spectrosc 67:1003–1006. https://doi.org/10.1016/j.saa.2006.09.028

Hwang WS, Truong PL, Sim SJ (2012) Size-dependent plasmonic responses of single gold nanoparticles for analysis of biorecognition. Anal Biochem 421:213–218. https://doi.org/10.1016/j.ab.2011.11.001

Ikram-ul-Haq AS, Qadeer MA (2002) Biosynthesis of l-DOPA by *Aspergillus oryzae*. Bioresour Technol 85:25–29. https://doi.org/10.1016/S0960-8524(02)00060-3

Islam NU, Jalil K, Shahid M, Muhammad N, Rauf A (2015) *Pistacia integerrima* gall extract mediated green synthesis of gold nanoparticles and their biological activities. Arab J Chem (article in press). https://doi.org/10.1016/j.arabjc.2015.02.014

Jain R, Zaidi KU, Verma V, Saxena P (2012) L-Asparaginase: a promising enzyme for treatment of acute lymphoblastic leukemia. People's J Sci Res 5:29–35. https://www.researchgate.net/publication/267688669

Jazayeri MH, Hamed A, Ali AP, Hamidreza P, Bijan S (2016) Various methods of gold nanoparticles (GNPs) conjugation to antibodies. Sens Biosensing Res 9:17–22. https://doi.org/10.1016/j.sbsr.2016.04.002

Kadri T, Cuprys A, Rouissi T, Brar SK, Daghrir R, Lauzon JM (2018) Nanoencapsulation and release study of enzymes from *Alkanivorax borkumensis* in chitosan-tripolyphosphate formulation. Biochem Eng J 137:1–10. https://doi.org/10.1016/j.bej.2018.05.013

Kalishwaralal K, Deepak V, Pandian SRK, Gurunathan S (2009) Biological synthesis of gold nanocubes from *Bacillus licheniformis*. Bioresour Technol 100:5356–5358. https://doi.org/10.1016/j.biortech.2009.05.051

Kalishwaralal K, Deepak V, Pandian SBRK, Kottaisamy M, BMK S, Kartikeyan B, Gurunathan S (2010) Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. Colloids Surf B 77:257–262. https://doi.org/10.1016/j.colsurfb.2010.02.007

Kalpana D, Han JH, Park WS, Lee SM, Wahab R, Lee YS (2014) Green biosynthesis of silver nanoparticles using torreya nucifera and their antibacterial activity. Arab J Chem (article in press). https://doi.org/10.1016/j.arabjc.2014.08.016

Kanwar SS, Verma ML (2010). Lipases, In Encyclopedia of Industrial Biotechnology, Wiley Publishers, USA, pp 1–16. https://doi.org/10.1002/9780470054581.eib387

Kanwar SS, Kaushal RK, Verma ML, Kumar Y, Chauhan GS, Gupta R, Chimni SS (2005) Synthesis of ethyl laurate by hydrogel immobilized lipase of *Bacillus coagulans* MTCC-6375. Indian J Microbiol 45:187–193: http://dro.deakin.edu.au/view/DU:30047962

Kanwar SS, Verma HK, Pathak S, Kaushal RK, Kumar Y, Verma ML, Chimni SS, Chauhan GS (2006) Enhancement of ethyl propionate synthesis by poly (AAc-co-HPMA-clMBAm)-

immobilized *Pseudomonas aeruginosa* MTCC-4713 exposed to Hg^{2+}, and NH_4^+ ions. Acta Microbiol Immunol Hung 53:195–207. https://doi.org/10.1556/AMicr.53.2006.2.6

Kanwar SS, Verma ML, Maheshwari C, Chauhan S, Chimni SS, Chauhan GS (2007a) Properties of poly (AAc-co-HPMA-cl-EGDMA) hydrogel-bound lipase of *Pseudomonas aeruginosa* MTCC-4713 and its use in synthesis of methyl acrylate. J Appl Polym Sci 104:183–191. https://doi.org/10.1002/app.25315

Kanwar SS, Kaushal RK, Verma ML, Kumar Y, Azmi W, Gupta R, Chimni SS, Chauhan GS (2007b) Synthesis of ethyl oleate employing synthetic hydrogel-immobilized lipase of *Bacillus coagulans* MTCC-6375. Indian J Biotechnol 6:68–73. http://hdl.handle.net/123456789/3015

Kanwar SS, Gehlot S, Verma ML, Gupta R, Kumar Y, Chauhan GS (2008) Synthesis of geranyl butyrate employing poly (AAc-co-HPMA-cl-EGDMA) hydrogel-immobilized lipase of *Pseudomonas aeruginosa* MTCC-4713. J Appl Polym Sci 110:2681–2692. https://doi.org/10.1002/app.28241

Kaphle A, Nagaraju N, Daima HK (2018) Contemporary developments in nanobiotechnology: applications, toxicity, sustainability, and future perspective. In: Dhawan A, Singh S, Kumar A (eds) Nanobiotechnology: Human Health and the Environment. CRC Press, Boca Raton, pp 1–34. https://doi.org/10.1201%2F9781351031585-1

Kaur R, Sekhon BS (2012) Enzymes as drugs: an overview. J Pharm Educ Res 3:29–41. Enzymes-as-Drugs-10.36.03-AM

Khalil MMH, Ismail EH, El-Magdoub F (2012) Biosynthesis of Au nanoparticles using olive leaf extract. Arab J Chem 5:431–437. https://doi.org/10.1016/j.arabjc.2010.11.011

Kim SK, Rajapakse N (2005) Enzymatic production and biologicalactivities of chitosan oligosaccharides (COS): a review. Carbohydr Polym 62:357–368. https://doi.org/10.1016/j.carbpol.2005.08.012

Kim JH, Jang HH, Ryou SM, Kim S, Bae J, Lee K, Han MS (2010) A functionalized gold nanoparticles assisted universal carrier for antisense DNA. Chem commun 46:4151–4153. https://doi.org/10.1039/C0CC00103A

Kong FY, Zhang JW, Li RF, Wang ZX, Wang WJ, Wang W (2017) Unique roles of gold nanoparticles in drug delivery, targeting and imaging applications. Molecules 22:1445–1451. https://doi.org/10.3390/molecules22091445

Krishnaraj C, Muthukumaran P, Ramachandran R, Balakumaran MD, Kalaichelvan PT (2014) *Acalypha indica* Linn: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. Biotechnol Reports 4:42–49. https://doi.org/10.1016/j.btre.2014.08.002

Krishnaswamy K, Vali H, Orsat V (2014) Value-adding to grape waste: Green synthesis of gold nanoparticles. J Food Eng 142:210–220. https://doi.org/10.1016/j.jfoodeng.2014.06.014

Kulkarni N, Muddapur U (2014) Biosynthesis of metal nanoparticles: a review. J Nanotechnol:1–8. https://doi.org/10.1155/2014/510246

Kumar KP, Paul W, Sharma CP (2011a) Green synthesis of gold nanoparticles with *Zingiber officinale* extract: characterization and blood compatibility. Proc Biochem 46:2007–2013. https://doi.org/10.1016/j.procbio.2011.07.011

Kumar VG, Gokavarapu SD, Rajeswari A, Dhas TS, Karthick V, Kapadia Z, Shrestha T, Barathy IA, Roy A, Sinha S (2011b) Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent *Cassia auriculate*. Colloids Surf B Biointerfaces 87:159–163. https://doi.org/10.1016/j.colsurfb.2011.05.016

Kumar S, Jana AK, Dhamija I, Maiti M (2014a) Chitosan-assisted immobilization of serratiopeptidase on magnetic nanoparticles, characterization and its target delivery. J Drug Target 22:123–137. https://doi.org/10.3109/1061186X.2013.844157

Kumar S, Jana AK, Maiti M, Dhamija I (2014b) Carbodiimide-mediated immobilization of serratiopeptidase on amino-, carboxyl-functionalized magnetic nanoparticles and characterization for target delivery. J Nanopart Res 16:2233. https://doi.org/10.1007/s11051-013-2233-x

Lee SH, Bae KH, Kim SH, Lee KR, Park TG (2008) Amine-functionalized gold nanoparticles as noncytotoxic and efficient intracellular siRNA delivery carriers. Int J Pharma 364:94–101. https://doi.org/10.1016/j.ijpharm.2008.07.027

Lin J, Zhang H, Chen Z, Zheng Y (2010) Penetration of lipid membranes by gold nanoparticles: insights into cellular uptake, cytotoxicity, and their relationship. ACS Nano 4:5421–5429. https://doi.org/10.1021/nn1010792

Lozano SV, Sepulveda TV, Torres EF (2012) Lipases production by solid fermentation: the case of *Rhizopusho mothallicus* in perlite. Methods Mol Biol 861:227–237. https://doi.org/10.1007/978-1-61779-600-5_14

Maji SK, Mandal AK, Nguyen KT, Borah P, Zhao Y (2015) Cancer cell detection and therapeutics using peroxidase-active nanohybrid of gold nanoparticle-loaded mesoporous silica-coated graphene. ACS Appl Mater Interfaces 7:9807–9816. https://doi.org/10.1021/acsami.5b01758

Malarkodi C, Rajeshkumar S, Vanaja M, Paulkuman K, Gnanajobitha G, Annadurai G (2013) Eco-friendly synthesis and characterization of gold nanoparticles using *Klebsiella pneumoniae*. J Nanostruct Chem 3:1–7. https://doi.org/10.1186/2193-8865-3-30

Malda ET, Olangua L, Asensio AC, Arzamendi G, Gandía LM, Moran JF (2010) Gold nanoparticle-sod enzyme conjugates for therapeutic applications. NanoSpain 2010, 23-26 March, 2010 Malaga-Spain, Poster presentation. http://www.nanospainconf.org/2010/Posters/Nanospain2010_Tellechea.pdf

Mane P, Tale V (2015) Overview of microbial therapeutic enzymes. Int J Curr Microbiol Appl Sci 4:17–26. https://www.ijcmas.com/Archives-29.php

Manikandan R, Manikandan B, Raman T, Arunagirinathan K, Prabhu NM, Basu MJ, Perumal M, Palanisamy S, Munusamy A (2014) Biosynthesis of silver nanoparticles using ethanolic petals extract of *Rosa indica* and characterization of its antibacterial, anticancer and anti-inflammatory activities. Spectrochim Acta A Mol Biomol Spectrosc 138C:120–129. https://doi.org/10.1016/j.saa.2014.10.043

Marcelo G, Kaplan E, Tarazona MP, Mendicuti F (2015) Interaction of gold nanoparticles with doxorubicin mediated by supramolecular chemistry. Colloids Surf B Biointerfaces 128:237–244. https://doi.org/10.1016/j.colsurfb.2015.01.041

Ming M, Kuroiwa T, Ichikawa S et al (2006) Production of chitosan oligosaccharides by chitosanase directly immobilized on an agar gel coated multi disk impeller. Biochem Eng J 28:289–294. https://doi.org/10.1016/j.bej.2005.11.015

Mirza AZ, Shamshad H (2011) Preparation and characterization of doxorubicin functionalized gold nanoparticles. Eur J Med Chem 46:1857–1860. https://doi.org/10.1016/j.ejmech.2011.02.048

Mittal AK, Chisti Y, Banerjee UC (2013) Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv 31:346–356. https://doi.org/10.1016/j.biotechadv.2013.01.003

MohanKumar K, Mandal BK, Kiran Kumar HA, Maddinedi SB (2013) Green synthesis of size controllable gold nanoparticles. Spectrochim Acta-Part A Mol Biomol Spectrosc 116:539–545. https://doi.org/10.1016/j.saa.2013.07.077

Mohanpuria P, Rana NK, Yadav SK (2008) Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res 10:507–517. https://doi.org/10.1007/s11051-007-9275-x

Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M (2002) Extracellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*. Chem Biol Chem 3:461–463. 10.1002/1439-7633(20020503)3:5<461::AID-CBIC461>3.0.CO;2-X

Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, Tyagi AK, Kale SP (2008) Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. Nanotechnology 19:075103. https://doi.org/10.1088/0957-4484/19/7/075103

Muthurasu A, Ganesh V (*2016*) *Glucose oxidase* stabilized fluorescent gold nanoparticles as an ideal sensor matrix for dual mode sensing of glucose. RSC Advances 6:7212–7223. https://doi.org/10.1039/C5RA22477B

Muthuvel A, Adavallan K, Balamurugan K, Krishnakumar N (2014) Biosynthesis of gold nanoparticles using *Solanum nugrum* leaf extract and screening their free radical scavenging and antibacterial Properties. Biomed Prev Nutr 4:325–332. https://doi.org/10.1016/j.bionut.2014.03.004

Nangia Y, Nishima W, Nisha G, Shekhawat G, Suri CR (2009) A novel bacterial isolate *Stenotrophomonas maltophilia* as living factory for synthesis of gold nanoparticles. Microb Cell Fact 8:39–46. https://doi.org/10.1186/1475-2859-8-39

Narayanan KB, Sakthivel N (2008) Coriander leaf mediated biosynthesis of gold nanoparticles. Mater Lett 62:4588–4590. https://doi.org/10.1016/j.matlet.2008.08.044

Narayanan K, Sakthivel N (2010) Phytosynthesis of gold nanoparticles using leaf extract of *Coleus amboinicus* Lour. Mater Charact 61:1232–1238. https://doi.org/10.1016/j.matchar.2010.08.003

Nasrabadi HT, Abbasi E, Davaran S, Kouhi M, Akbarzadeh A (2016) Bimetallic nanoparticles: preparation, properties, and biomedical applications. Artif Cells Nanomed Biotechnol 44:376–380. https://doi.org/10.3109/21691401.2014.953632

Nazir S, Hussain T, Ayub A, Rashid U, MacRobert AJ (2014) Nanomaterials in combating cancer: therapeutic applications and developments. Nanomed Nanotechnol Biol Med 10:19–34. https://doi.org/10.1016/j.nano.2013.07.001

Nilofar YN, Shivangi SK (2016) Biosynthesis of gold nanoparticles by *Bacillus marisflavi* and its potential in catalytic dye degradation. Arabian J Chem (article in press). https://doi.org/10.1016/j.arabjc.2016.09.020

Noruzi M, Zare D, Khoshnevisan K, Davoodi D (2011) Rapid green synthesis of gold nanoparticles using *Rosa hybrida* petal extract at room temperature. Spectrochim Acta A Mol Biomol Spectrosc 79:1461–1465. https://doi.org/10.1016/j.saa.2011.05.001

Ogi T, Saitoh N, Nomura T, Konishi Y (2010) Room-temperature synthesis of gold nanoparticles and nanoplates using *Shewanella* algae cell extract. J. Nanopart Res 12:2531–2539. https://doi.org/10.1007/s11051-009-9822-8

Okafor N (2007) Biocatalysis: Immobilized enzymes and immobilized cells. Modern Ind Microbiol Biotechnol:398. http://site.iugaza.edu.ps/mwhindi/files/Modern-Industrial-MicrobiologyBiotechnology.pdf

Ozcan C, Ergun O, Celik A, Corduk N, Ozok G (2002) Enzymatic debridement of burn wound with collagenase in children with partial-thickness burns. Burns 28:791–794. https://doi.org/10.1016/S0305-4179(02)00191-2

Para G, Rifai S, Baratti J (1984) Production of L-DOPA from pyrocatechol and DL-serine by bioconversion using immobilized *Erwinia herbicola* cells. Biotechnol Lett 6:703–708. https://doi.org/10.1007/BF00133060

Parida UK, Bindhani BK, Nayak P (2011) Green synthesis and characterization of gold nanoparticles using onion (*Allium cepa*) extract. World J Nano Sci Eng 1:93–98. https://doi.org/10.4236/wjnse.2011.14015

Patra S, Mukherjee S, Barui AK, Ganguly A, Sreedhar B, Patra CR (2015) Green synthesis, characterization of gold and silver nanoparticles and their potential application for cancer therapeutics. Mater Sci Eng C Mater Biol Appl 53:298–309. https://doi.org/10.1016/j.msec.2015.04.048

Patrizia DP, Nunzia C, Carmelina DA, Lupo G, Antonio M, Diego La M, Cristina S (2017) Immobilization of neurotrophin peptides on gold nanoparticles by direct and lipid-mediated interaction: a new multipotential therapeutic nanoplatform for CNS Disorders. *ACS Omega* 2:4071–4079. https://doi.org/10.1021/acsomega.7b00458

Paul B, Bhuyan B, Dhar Purkayastha D, Dey M, Dhar SS (2015) Green synthesis of gold nanoparticles using *Pogestemon benghalensis* (B) O. Ktz. leaf extract and studies of their photocatalytic activity in degradation of methylene blue. Mater Lett 148:37–40. https://doi.org/10.1016/j.matlet.2015.02.054

Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R (2007) Nanocarriers as an emerging platform for cancer therapy. Nature Nanotechnol 2:751–760. https://doi.org/10.1038/nnano.2007.387

Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, Ashraf S et al (2017) Diverse applications of nanomedicine. ACS Nano 11:2313–2381. https://doi.org/10.1021/acsnano.6b06040

Peterson RE, Ciegler A (1969) L-Asparaginase production by various bacteria. Appl Microbiol. 17:929-930. DOI: applmicro00006-0167

Philip D (2010) Green synthesis of gold and silver nanoparticles using *Hibiscus rosa sinensis*. Phys E Low Dimens Syst Nanostruct 42:1417–1424. https://doi.org/10.1016/j.physe.2009.11.081

Philip D, Unni C (2011) Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (*Ocimum sanctum*) leaf. Physica E Low dimens Syst Nanostruct 43:1318–1322. https://doi.org/10.1016/j.physe.2010.10.006

Philip D, Unni C, Aromal SA, Vidhu VK (2011) *Murraya Koenigii* leaf assisted rapid green synthesis of silver and gold nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc 78:899–904. https://doi.org/10.1016/j.saa.2010.12.060

Pietro PD, Caporarello N, Anfuso CD, Lupo G, Magrì A, Mendola DL, Satriano C (2017) Immobilization of neurotrophin peptides on gold nanoparticles by direct and lipid-mediated interaction: a new multipotential therapeutic nanoplatform for CNS disorders. ACS Omega 2:4071–4079. https://doi.org/10.1021/acsomega.7b00458

Pissuwan D, Cortie CH, Valenzuela SM, Cortie MB (2007) Gold nanosphere-antibody conjugates for hyperthermal therapeutic applications. Gold Bulletin 40:121–129. https://doi.org/10.1007/BF03215568

Puri M, Barrow CJ, Verma ML (2013) Enzyme immobilization on nanomaterials for biofuel production. Trends Biotechnol 31:215–216. https://doi.org/10.1016/j.tibtech.2013.01.002

Raju D, Vishwakarma RK, Khan BM, Mehta UJ, Ahmad A (2014) Biological synthesis of cationic gold nanoparticles and binding of plasmid DNA. Mater Lett 129:159–161. https://doi.org/10.1016/j.matlet.2014.05.021

Rawat P, Rajput YS, Bharti MK, Sharma R (2016) A method for synthesis of gold nanoparticles using 1-amino-2- naphthol-4-sulphonic acid as reducing agent. Curr Sci 110:2297–2300. https://www.currentscience.ac.in/Volumes/110/12/2297.pdf

Reddy AS, Chen CY, Chen CC, Jean JS, Chen HR, Tseng MJ, Fan CW, Wang JC (2010) Biological synthesis of gold and silver nanoparticles mediated by the bacteria *Bacillus subtilis*. J Nanosci Nanotechnol 10:6567–6574. https://www.ncbi.nlm.nih.gov/pubmed/21137763

Reddy V, Torati RS, Oh S, Kim CG (2013) Biosynthesis of gold nanoparticles assisted by *Sapindus mukorossi* Gaertn. Fruit pericarp and their catalytic application for the reduction of p-nitroaniline. Ind Eng Chem Res 52:556–564. https://doi.org/10.1021/ie302037c

Sabu A (2003) Sources, properties and applications of microbial therapeutic enzymes. Indian J Biotechnol 2:334–341. http://nopr.niscair.res.in/handle/123456789/11329

Sabu A, Chandrasekaran M, Pandey A (2000) Biopotential of microbial glutaminases. Chem Today 18:21–25. https://www.researchgate.net/publication/283410584

Sabu A, Nampoothiri KM, Pandey A (2005) L-glutaminase as a therapeutic enzyme of microbial origin. Microbial enzymes and biotransformations. Series: Methods Biotechnol 17:75–90. https://doi.org/10.1385/1-59259-846-3:075

Sadeghi B (2015) *Zizyphus mauritiana* extract-mediated green and rapid synthesis of gold nanoparticles and its antibacterial activity. J Nanostruct Chem 5:265–273. https://doi.org/10.1007/s40097-015-0157-y

Salamone P, Wodzinski R (1997) Production, purification and characterization of a 50-kDa extracellular metalloprotease from *Serratia marcescens*. Appl Microbiol Biotechnol 48:317–321. https://doi.org/10.1007/s002530051056

Senoudi AR, Chabane Sari SM, Hakem IF (2014) Analysis of the evolution of tannic acid stabilized gold nanoparticles using mie theory. Int J Anal Chem 2014:832657, 6 pages. https://doi.org/10.1155/2014/832657

Shankar SS, Rai A, Ahmad A, Sastry M (2004a) Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. J Colloid Interface Sci 275:496–502. https://doi.org/10.1016/j.jcis.2004.03.003

Shankar SS, Rai A, Ankamwar B, Singh A, Ahmad A, Sastry M (2004b) Biological synthesis of triangular gold nanoprisms. Nat Mater 3:482–488. https://doi.org/10.1038/nmat1152

Shankar S, Soni SK, Daima HK, Selvakannan PR, Khire JM, Bhargava SK, Bansal V (2015) Charge-switchable gold nanoparticles for enhanced enzymatic thermostability. Phys Chem Chem Phys 17:21517–21524. https://doi.org/10.1039/C5CP03021H

Sharma A, Matharu Z, Sumana G, Solanki PR, Kim GC, Malhotra BD (2010) Antibody immobilized cysteamine functionalized-gold nanoparticles for aflatoxin detection. Thin Solid Films 159:1213–1218. https://doi.org/10.1016/j.tsf.2010.08.071

Sharma N, Pinnaka AK, Raje M, Ashish FN, Bhattacharyya MS, Choudhury AR (2012) Exploitation of marine bacteria for production of gold nanoparticles. Microb Cell Fact 11:86. https://doi.org/10.1186/1475-2859-11-86

Sharma B, Singh S, Kanwar SS (2014a) L-methionase: a therapeutic enzyme to treat malignancies. BioMed Res Inter 2014:506287, 13 pages. https://doi.org/10.1155/2014/506287

Sharma TK, Ramanathan R, Weerathunge P, Mohammadtaheri M, Daima HK, Shukla R, Bansal V (2014b) Aptamer-mediated 'turn-off/turn-on' nanozyme activity of gold nanoparticles for kanamycin detection. Chem Commun 50:15856–15859. https://doi.org/10.1039/C4CC07275H

Sharma N, Bhatt G, Kothiyal P (2015) Gold nanoparticles synthesis, properties, and forthcoming applications-a review. Indian J Pharm Biol Res 3:13–27. 138e/80a3b1e9d936325a8cb50ef8338cf0e544eb

Shiying H, Zhirui G, Zhang Y, Zhang S, Wang J, Ning G (2007) Biosynthesis of gold nanoparticles using the bacteria *Rhodopseudomonas capsulate*. Mater Lett 61:3984–3987. https://doi.org/10.1016/j.matlet.2007.01.018

Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K (2007) A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii Greville*. Colloids Surf. B: Biointerfaces 57:97–101. https://doi.org/10.1016/j.colsurfb.2007.01.010

Singh C, Sharma V, Naik PKR, Singh H (2011) A green biogenic approach for synthesis of gold and silver. Dig. J Nanomater Biostructures 6:535-542. DOI: 4c1a/58006bd0ce11a8d3ed4de0b1d634b48b7507

Singh M, Kalaivani R, Manikandan S, Sangeetha N, Kumaraguru AK (2013) Facile green synthesis of variable metallic gold nanoparticle using *Padina gymnospora*, a brown marine macroalga. Appl Nanosci 3:145–151. https://doi.org/10.1007/s13204-012-0115-7

Singh R, Kumar M, Mittal A, Mehta PK (2016) Microbial enzymes: industrial progress in 21st century. 3 Biotech 6:174. https://doi.org/10.1007/s13205-016-0485-8

Siti RM, Khairunisak AR, Azlan AZ, Rahmah N (2013) Green synthesis of 10 nm gold nanoparticles via seeded-growth method and its conjugation properties on lateral flow immunoassay. Adv Mater Res 686:8–12. https://doi.org/10.1088/2053-1591/aaa562

Skumar S, Abdulhameed S (2017) Therapeutic Enzymes. Biores bioprocess Biotechnol 2:45–73. https://doi.org/10.2174/1389201018666170808150742

Smithaa SL, Philip D, Gopchandrana KG (2009) Green synthesis of gold nanoparticles using *Cinnamomum zeylanicum* leaf broth. Spectrochim Acta A Mol Biomol Spectrosc 74:735–739. https://doi.org/10.1016/j.saa.2009.08.007

Song JY, Jang HK, Kim BS (2009) Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. Process Biochem 44:1133–1138. https://doi.org/10.1016/j.procbio.2009.06.005

Spiers ASD, Wade HE (1976) Bacterial glutaminase in treatment of acute leukaemia. Br Med J 1:1317–1319. https://www.ncbi.nlm.nih.gov/pubmed/773514

Sujitha MV, Kannan S (2013) Green synthesis of gold nanoparticles using citrus fruits *Citrus limon*, *Citrus reticulata* and *Citrus sinensis* aqueous extract and its characterization. Spectrochim Acta A Mol Biomol Spectrosc 102:15–23. https://doi.org/10.1016/j.saa.2012.09.042

Suman TY, Rajasree SRR, Ramkumar R, Rajthilak C, Perumal P (2014) The Green synthesis of gold nanoparticles using an aqueous root extract of *Morinda citrifolia* L. Spectrochim. Acta A Mol Biomol Spectrosc 118:11–16. https://doi.org/10.1016/j.saa.2013.08.066

Sumi H et al (1987) A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. Experientia 43:1110–1111. https://doi.org/10.1007/BF01956052

Sun L, Liu K, Wang Z (2008) Functional gold nanoparticlepeptide complexes as cell-targeting agents. Langmuir 24:10293–10297. https://doi.org/10.1021/la8015063

Tabata K, Ikeda H, Hashimoto S (2005) ywfE in *Bacillus subtilis* codes for a novel enzyme, L-amino acid ligase. J Bacteriol 187:5195–5202. https://doi.org/10.1128/JB.187.15.5195-5202.2005

Tahir K, Nazir S, Li B, Khan AU, Khan ZUH, Gong PY, Khan SU, Ahmad A (2015) *Nerium oleander* leaves extract mediated synthesis of gold nanoparticles and its antioxidant activity. Mater Lett 156:198–201. https://doi.org/10.1016/j.matlet.2015.05.062

Tamuly C, Hazarika M, Borah SC, Das MR, Boruah MP (2013a) In situ biosynthesis of Ag, Au and bimetallic nanoparticles using *Piper pedicellatum* C.DC: green chemistry approach. Colloids Surf B Biointerfaces 1:627–634. https://doi.org/10.1016/j.colsurfb.2012.09.007

Tamuly C, Hazarika M, Bordoloi M (2013b) Biosynthesis of Au nanoparticles by *Gymnocladus assamicus* and its catalytic activity. Mater Lett 108:276–279. https://doi.org/10.1016/j.matlet.2013.07.020

Tao Y, Ju E, Ren J, Qu X (2015) Bifunctionalized mesoporous silica-supported gold nanoparticles: intrinsic oxidase and peroxidase catalytic activities for antibacterial applications. Adv Mater 27:1097–1104. https://doi.org/10.1002/adma.201405105

Teal AR, Wymer PEO (1991) Enzymes and their role in Biotechnology. The Biochemical Society, London. https://wellcomelibrary.org/item/b1966235x

Terkeltaub R (2009) Gout: novel therapies for treatment of gout and hyperuricemia. Arthritis Res Ther 11:236. https://doi.org/10.1186/ar2738

Thadathil N, Velappan SP (2014) Recent developments in chitosanase research and its biotechnological applications: a review. Food Chem 150:392–399. https://doi.org/10.1016/j.foodchem.2013.10.083

Tkachenko AG, Xie H, Liu Y, Coleman D, Ryan J, Glomm WR, Shipton MK, Franzen S, Feldheim DL (2004) Cellular trajectories of peptide-modified gold particle complexes: comparison of nuclear localization signals and peptide transduction domains. Bioconjugate Chem 15:482–490. https://doi.org/10.1021/bc034189q

Turkevich J, Stevenson PC, Hillier J (1951) Nucleation and growth process in the synthesis of colloidal gold. Discuss Faraday Soc 11:55–75. https://doi.org/10.1039/DF9511100055

Ul N, Jalil K, Shahid M, Rauf A, Muhammad N, Khan A, Shah MR, Khan MA (2015) Green synthesis and biological activities of gold nanoparticles functionalized with Salix alba. Arabian J Chem: https://doi.org/10.1016/j.arabjc.2015.06.025

Underkofler LA, Barton RR, Rennert SS (1957) Production of microbial enzymes and their applications. Appl Microbiol, 6:212–221. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1057391/

Vahabi K, Mansoori GA, Karimi S (2011) Biosynthesis of silver nanoparticles by fungus *Trichoderma Reesei* (a route for large-scale production of AgNPs). Insciences J 1:65–79. https://doi.org/10.5640/insc.010165

Vakili B, Nezafat N, Negahdaripour M, Yari M, Zare B, Ghasemi Y (2017) Staphylokinase enzyme: an overview of structure, function and engineered forms. Curr Pharm Biotechnol 18:1026–1037. https://doi.org/10.2174/1389201019666180209121323

Velasco-Lozano S, López-Gallego F, Mateos-Díaz JC, Favela-Torres E (2016) Cross-linked enzyme aggregates (CLEA) in enzyme improvement – a review. Biocatalysis 1:66–177. https://doi.org/10.1515/boca-2015-0012

Vellard M (2003) The enzyme as drug: application of enzymes as pharmaceuticals. Curr Opin Biotechnol 14:444–450. https://doi.org/10.1016/S0958-1669(03)00092-2

Venkatpurwar VP, Pokharkar VB (2010) Biosynthesis of gold nanoparticles using therapeutic enzyme: in-vitro and in-vivo efficacy study. J Biomed Nanotech 6:667–674. https://doi.org/10.1166/jbn.2010.1163

Verma ML (2017a) Fungus-mediated bioleaching of metallic nanoparticles from agro-industrial by-products. In: Prasad R (ed) Fungal Nanotechnology. Fungal Biology. Springer, Cham. https://doi.org/10.1007/978-3-319-68424-6_5

Verma ML (2017b) Critical evaluation of toxicity tests in context to engineered nanomaterials: An introductory overview. In: Kumar V, Dasgupta N, Ranjan S (eds) Nanotoxicology. CRC Press, Boca Raton. https://doi.org/10.1201Fb21545-1

Verma ML (2017c) Enzymatic nanobiosensors in the agricultural and food industry. In: Ranjan S, Dasgupta N, Lichtfouse E (eds) Nanoscience in Food and Agriculture 4. Sustainable Agriculture Reviews, vol 24. Springer, Cham. https://doi.org/10.1007/978-3-319-53112-0_7

Verma ML (2017d) Nanobiotechnology advances in enzymatic biosensors for the agri-food industry. Environ Chem Lett 15:555–560. https://doi.org/10.1007/s10311-017-0640-4

Verma ML, Barrow CJ (2015) Recent advances in feedstocks and enzyme-immobilised technology for effective transesterification of lipids into biodiesel. In: Kalia V (ed) Microbial Factories. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2598-0_6

Verma ML, Kanwar SS (2010) Purification and characterization of a low molecular mass alkaliphilic lipase of *Bacillus cereus* MTCC 8372. Acta Microbiol Immunol Hung 57:187–201. https://doi.org/10.1556/AMicr.57.2010.3.4

Verma ML, Kanwar SS (2012) Harnessing the potential of thermophiles: The variants of extremophiles. Dyn Biochem Process Biotechnol Mol Biol 6:28–39. http://www.globalsciencebooks.info/Online/GSBOnline/images/2012/DBPBMB_6%28SI1%29/DBPBMB_6%28SI1%2928-39o.pdf

Verma ML, Azmi W, Kanwar SS (2009) Synthesis of ethyl acetate employing celite-immobilized lipase of *Bacillus cereus* MTCC 8372. Acta Microbiol Immunol Hung 56:229–242. https://doi.org/10.1556/AMicr.56.2009.3.3

Verma ML, Azmi W, Kanwar SS (2011) Enzymatic synthesis of isopropyl acetate catalysed by immobilized *Bacillus cereus* lipase in organic medium. Enzyme Res 2011:919386, 7 pages. https://doi.org/10.4061/2011/919386

Verma ML, Barrow CJ, Kennedy JF, Puri M (2012) Immobilization of β-galactosidase from *Kluyveromyces lactis* on functionalized silicon dioxide nanoparticles: Characterization and lactose hydrolysis. Int J Biol Macromol 50:432–437. https://doi.org/10.1016/j.ijbiomac.2011.12.029

Verma ML, Rajkhowa R, Barrow CJ, Wang X, Puri M (2013a) Exploring novel ultrafine Eri silk bioscaffold for enzyme stabilisation in cellobiose hydrolysis. Bioresour Technol 145:302–306. https://doi.org/10.1016/j.biortech.2013.01.065

Verma ML, Naebe M, Barrow CJ, Puri M (2013b) Enzyme immobilisation on amino-functionalised multi-walled carbon nanotubes: Structural and biocatalytic characterisation. PLoS One 8:e73642. https://doi.org/10.1371/journal.pone.0073642

Verma ML, Chaudhary R, Tsuzuki T, Barrow CJ, Puri M (2013c) Immobilization of β-glucosidase on a magnetic nanoparticle improves thermostability: Application in cellobiose hydrolysis. Bioresour Technol 135:2–6. https://doi.org/10.1016/j.biortech.2013.01.047

Verma ML, Barrow CJ, Puri M (2013d) Nanobiotechnology as a novel paradigm for enzyme immobilization and stabilisation with potential applications in biofuel production. Appl Microbiol Biotechnol 97:23–39. https://doi.org/10.1007/s00253-012-4535-9

Verma ML, Puri M, Barrow CJ (2016) Recent trends in nanomaterials immobilised enzymes for biofuel production. Critical Rev Biotechnol **36**:108–119. https://doi.org/10.3109/07388551.2014.928811

Vinod VTP, Saravanan P, Sreedhar B, Keerthi Devi D, Sashidhar RB (2011) A facile synthesis and characterization of Ag, Au and Pt nanoparticles using a natural hydrocolloid gum kondagogu (*Cochlospermum gossypium*). Colloids Surf B Biointerfaces 83:291–298. https://doi.org/10.1016/j.colsurfb.2010.11.035

Wu W, Huang J, Wu L, Sun D, Lin L et al (2013) Two-step size- and shape-separation of biosynthesized gold nanoparticles. Sep Purif Technol 106:117–122. https://doi.org/10.1016/j.seppur.2013.01.005

Xie J, Lee JY, Wang DI, Ting YP (2007) Identification of active biomolecules in the high-yield synthesis of single-crystalline gold nanoplates in algal solutions. Small 3:672–682. https://doi.org/10.1002/smll.200600612

Xin Y, Yin M, Zhao L, Meng F, Luo L (2017) Recent progress on nanoparticle-based drug delivery systems for cancer therapy. Cancer Biol Med 14:228–241. https://doi.org/10.20892/j.issn.2095-3941.2017.0052

Yang N, WeiHong L, Hao L (2014) Biosynthesis of Au nanoparticles using agricultural waste mango peel extract and its in vitro cytotoxic effect on two normal cells. Mater Lett 134:67–70. https://doi.org/10.1016/j.matlet.2014.07.025

Yari M, Ghoshoon MB, Vakili B, Ghasemi Y (2017) Therapeutic enzymes: applications and approaches to pharmacological improvement. Curr J Pharma Biotechnol 18:531–540. https://doi.org/10.2174/1389201018666170808150742

Yeh CS, Cheng FY, Huang CC (2012) Bioconjugation of noble metal nanoparticles and their applications to biolabeling and bioimaging. In: Lai-Kwan C, Chang HT, from Bioimaging to Biosensors: Noble Metal Nanoparticles in Biodetection https://doi.org/10.1201Fb13162-2

Yu JJ, Park KB, Kim SG, Oh SH (2013) Expression, purification, and biochemical properties of arginase from *Bacillus subtilis* 168. J Microbiol 51:222–228. https://doi.org/10.1007/s12275-013-2669-9

Yu X, Jiao Y, Chai Q (2016) Applications of gold nanoparticles in biosensors. Nano LIFE 6:11. https://doi.org/10.1142/S1793984416420010

Zaidi KU, Ali AS, Ali SA, Naaz I (2014) Microbial tyrosinases: promising enzymes for pharmaceutical, food bioprocessing, and environmental industry. Biochem Res Int 2014:854687, 16 pages. https://doi.org/10.1155/2014/54687

Zaitsev S, Spitzer D, Murciano JC (2010) Sustained thrombo prophylaxis mediated by an rbc-targeted pro-urokinase zymogen activated at the site of clot formation. Blood 115:5241–5248. https://doi.org/10.1182/blood-2010-01-261610

Zhang H, Sang Q, Zhang W (2012) Statistical optimization of chitosanase production by *Aspergillus* sp. QD-2 in submerged fermentation. Ann of Microbiol 62:193–201. https://doi.org/10.1007/s13213-011-0246-1

Zhang M, Cheng F, Gan F (2015) Electrochemical nitrite nanosensor based on Au nanoparticles/graphene nanocomposites. Int J Electrochem Sci 10:5905–5913. https://doi.org/10.1515/chempap-2015-0099

Zhao H, Wang Z, Jiao X, Zhang L, Lv Y (2012) Uricase-based highly sensitive and selective spectrophotometric determination of uric acid using BSA-stabilized Au nanoclusters as an artificial enzyme. Spectroscopy Lett 45:511–519. https://doi.org/10.1080/00387010.2011.649440