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A Review on Mycosynthesis, Mechanism, and Characterization of Silver and Gold Nanoparticles

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Abstract The study on green synthesis methods and on its potential applications for the mankind are in trend over 10 or more decades in the field of bioscience. A significant number of works have been done on the synthesis of nanoparticles by a microorganism which clearly shows the reliability of this method. Microbes can be used for synthesis process at much lower cost and in an environment-friendly approach. Among microbes, fungi can be mass grown in vitro, and they demonstrate an easy downstream processing for nanoparticles. Biosynthesized nanoparticles are the by-product of their resistance mechanism of the metal concerned, in different sizes and shapes. Also, presently, there are various methods of characterizing the metal nanoparticles. In this review, we will analyze aspects of mycosynthesis of silver and gold nanoparticles and their different characterization techniques. We are intended to give a clear picture of the importance and downside of this method.

Keywords Mycosynthesis · Silver nanoparticles (AgNPs) · Gold nanoparticles (AuNPs) · Mechanism · Downstream process

1 Introduction

Nanostructured particles ranging from 1 to 1000 nm in size are well-known for creating wonders in the field of nanobiotechnology in recent years. Bioscience studies have revealed that natural mechanisms in microorganisms like bioaccumulation, biomineralization, precipitation, and biosorption against toxic compounds lead to synthesis and design of these particles. The simple detoxifying process in microbes is solely responsible for the synthesis of nanoparticles and termed as nanobiosynthesis. This, according to Siddiqi and Husen [1], is the most benign and inexpensive route so far. Whereas, chemical and physical syntheses are always considered hazardous and costly that make them disadvantageous. Furthermore, drawbacks of using chemicals in synthesis processes include their interference in biomedical functions. The toxic chemicals get absorbed on surfaces of the particles resulting in undesirable effect during their use in medical applications. Also, it is comparatively easier to control the shapes and sizes of the particles for certain pharmaceutical and therapeutic purposes as desired. Though plants as well can be extensively used in preparing nanoparticles, the most preferred route is the microbial synthesis since it is undoubtedly eco-friendly, cost-effective, manipulative, and compatible with any kind of biomedical research [2].

The microbial processes utilize extracts from different species of bacteria, fungi, actinomycetes, algae, etc. for nanoparticle synthesis [3]. However, fungi are the most ideal among all microorganisms given that it allows a higher level of productivity due to its ability to secrete higher amount of proteins [4]. Also, the presence of mycelia provides them with a larger surface area, and they are economically reasonable as well as easy to grow. Besides, they show considerably simple downstream processing during synthesis. In case of many fungi, it is known to be NADH-dependent reductase enzyme that is responsible for the reduction of metals, whereas, in others, it is various types of proteins, organic acids, polysaccharides, glucose, etc. [5–8].

The present trend in bio-nanoscience involves the synthesis of especially metal nanoparticles because of their varied range

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of applications in the field of biology, chemistry, and physics. Among different metals that have been studied for their importance, silver and gold showed maximum use in biological applications. They are incredibly considered as antimicrobials, antioxidants, catalysts, anticancerous, and so on [1, 9–15]. Silver and gold NPs are therefore studied vastly over a period of time which led different research groups to biosynthesize and explore them tremendously. NPs are produced using extracts from several fungal species such as *Penicillium citrinum* [16], *Aspergillus niger* and *Aspergillus parasiticus* [17], *Aspergillus flavus* [18], *Aspergillus concius*, *Penicillium janthinellum* and *Phomosis* sp. [19], *Pleurotus ostreatus* [20], *Aspergillus terreus* [21], *Bryophilous rhizoctonia* [22], *Fusarium oxysporum* [23], and *Fusarium semitectum* [24]. Similarly, AuNPs are also synthesized by many such fungi namely, *Aspergillus fumigates* [25], *Geobacillus* sp. [26], and *Aspergillus fischeri* [13]. Besides fungi, yeasts are known best for their easy handling methods, with simple nutrient in the laboratory and therefore, are always chosen over bacteria for the nanoparticle synthesis. These eukaryotic systems, for example, *Candida glabrata* and *Schizosaccharomyces pombe*, are often used and had been reported in several kinds of literature [27]. Au-Ag alloy synthesized by yeast is also applied as various important aspects of these methods prove to produce a bulky number of metal nanoparticulate when compared to bacteria-mediated synthesis processes.

Once the metal particles are synthesized either randomly or as per desired properties, the next step is to characterize them. The distinctiveness of nanoparticles can be determined by special instruments working on different principles such as UV–visible spectrophotometer (UV–Vis spectra), X-ray diffractometer (XRD), dynamic light scattering (DLS), scanning and transmission electron microscopes (SEM and TEM), energy dispersive spectroscopy (EDS), and Fourier transform infrared (FT-IR) spectroscopy. These instruments are used to analyze the particles based on their size, shape, surface plasmon resonance (SPR), the charge on them, side groups present on them, and metallic characteristics [28, 29].

Hence, the review intends to give the readers an idea about the typical methods and means of biosynthesis of silver and gold nanoparticles carried out using different fungal species in the recent past. Also, we elaborate on the characterization methods and their principles which help recognize these particulates in the laboratory. This study on characterization of silver and gold nanoparticles will prove to be important for researchers to proceed in learning various mechanisms behind production processes and to plan any further experiments on the basis of synthesis routes. Characterization techniques help in an appropriate understanding of size, shape, side groups, and particle distribution or dispersity pattern of bionanoparticles which is significant in vital biomedical investigations involving silver and gold.

2 Green Synthesis and Its Importance

Green syntheses as the name suggests involve through and through the use of environmentally friendly and non-toxic means in synthesizing nanoparticles. Ganesan et al. [30] elaborated that unlike chemical and physical processes, these methods employ plants or microorganisms which are inexpensive, easily available, simple to grow, and safe to handle. Microbes have been vastly explored being easy to be cultured on Petri plates from different sources like soil, air, water, food and food crops, and plants on economical media. Use of high temperature, pressure, harsh reducing agents, organic solvents, and capping agents like sodium borohydride, chloroform, and toluene are common in the case of chemical and physical synthesis processes [16]. Such systems causing a hazard to the surroundings are undesirable for the environment. Also, in relevance to biomedical aspects, such harmful chemical might show side effects and interfere with the actual treatment practices. In context to human health, minimum risk is desired; thus, functions of metal nanoparticles in medical diagnostic imaging, pharmaceutical products, disease diagnostics, medical implants, wound dressing, and medical treatment protocols such as antimicrobials, antioxidants, antiinflammatory agents, anticancerous agents, and biological catalysts, are highly crucial. It was confirmed that biologically synthesized nanoparticles avoid such high possibilities of toxic problems [31]. Also, besides being eco-friendly and biocompatible, this path is a better way to achieve monodispersity and well-defined dimensions of the particles. Proteins or enzymes secreted by microorganisms or plants lead to the high productivity of comparatively superior quality nanoparticles [32]. The finer features help us create characteristic materials for specific applications, and they show the definite mode of action, which contributes to the advancement of various unknown research areas.

Many review articles have been published to describe different ways of biosynthesizing nanoparticles, especially microorganisms. Literature demonstrates how diverse group of metal nanoparticles as well as their alloys are biologically blended by bacteria, fungi, actinomycetes, and yeasts [1, 27, 29, 33].

2.1 Mechanism of Synthesis

The mechanism behind green synthesis of nanoparticles has been studied very little so far. Different types of microorganisms show different techniques; however, the reduction reaction is the main principle behind all kinds of mechanisms (Fig. 1). Intracellular and extracellular routes are both seen taking place due to cellular peptides and polysaccharides which lead to enzymatic oxidation, reduction, sorption, and chelation. Intermembranous transport and consequent nucleation and growth of the nanoparticles lead to extracellular formations of

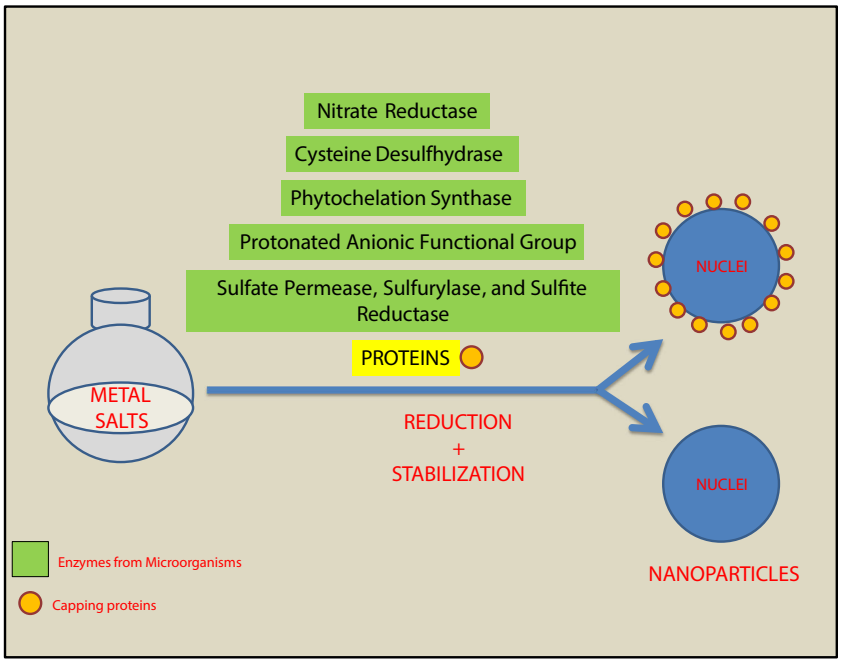

Fig. 1 Mechanistic aspects of nanoparticle formation and stabilization

nanoparticles [32]. They also reported production of AgNPs using protonated anionic functional groups present on the cell wall of lactic acid bacteria. In the case of fungus, *F. oxysporum*, it is clearly explained how NADPH-dependent nitrate reductase enzyme reduces silver ions by transferring an electron to it with the help of NADP as co-factor. Also, it was explained that AgNPs are reduced by quinine derivatives of naphthoquinones and anthraquinones [34]. A similar process of reduction of gold ions (Au^{3+}) to Au^{0} is demonstrated using *Stenotrophomonas maltophilia*, a gram-negative bacterium [35]. Zinc sulfide (ZnS) nanoparticles were synthesized intracellularly by bacteria, *Rhodobacter sphaeroides*, by secreted enzymes like sulfate permease, sulfurylase, and sulfite reductase [36], again, cadmium sulfide (CdS) nanoparticles are produced extracellularly using a protein, cysteine desulfhydrase, secreted by *Rhodopseudomonas palustris* [37]. Extracellular process of synthesis of AgNPs is extensively elucidated using *Verticillium* sp. and *F. oxysporum*, where the silver ions are found to be get trapped on the fungal cell wall and reduced to silver nuclei by reductase enzyme present on the cell surface, ending in accumulation of these silver ions on the generated nuclei [38, 39]. In the case of yeast, an oxidoreductase mechanism is witnessed. Phytochelation synthase was secreted for reducing Cd^{+}, whereas, *Saccharomyces cerevisiae* produced cadmium telluride quantum dots (CdTe QDs) extracellularly with help of protein ligands [40, 41]. The extracellular formation of silver and gold nanoparticles with help of secreted melanin produced by important yeast named *Yarrowia lipolytica* [42]. We thus conclude, various naturally produced enzymes and chemicals like nitrate reductase enzyme, quinine derivatives of naphthoquinones and anthraquinones, sulfate permease, sulfurylase, and sulfite reductase, cysteine desulfhydrase, phytochelation synthase, and mycobased melanin can be extracted from different organisms by autolysis method or using lysis-promoting agents so as to carry out synthesis of a varied range of bio-nanoparticles in an easier way.

Basically, the intracellular method involves trapping, bioreduction, and capping of the nuclei produced. Intracellular methods occupy certain ion transportation and electrostatic interaction between microbial cells and metal ions leading to the formation of nanoparticles, whereas the extracellular method comprises of secretion of enzymes, bioreduction, and capping of particles. A most common enzyme isolated so far is nitrate reductase which might be responsible for majority of AgNP production processes [43]. However, Singh [44] explained extracellular method is preferable for the synthesis of nanoparticles, i.e., because downstreaming and purification are easier when compared to the intracellular process which consecutively makes intracellular processes over all time-consuming and biosynthesis is unlikely to be costly. The extracellular method of synthesis can be wholly cheap and convenient against intracellular method which stands out to be the main purpose for biosynthesis (Fig. 2).

3 Mycosynthesis of Nanoparticles

Once the microbes are isolated, they can be screened for the production of nanoparticles and further purified for various applications. Among all microorganisms, bacteria and fungi were most often used until now. Researchers isolated *Pseudomonas stutzeri* from silver mines in 1999 which is known to have synthesized AgNPs for the first time in history,

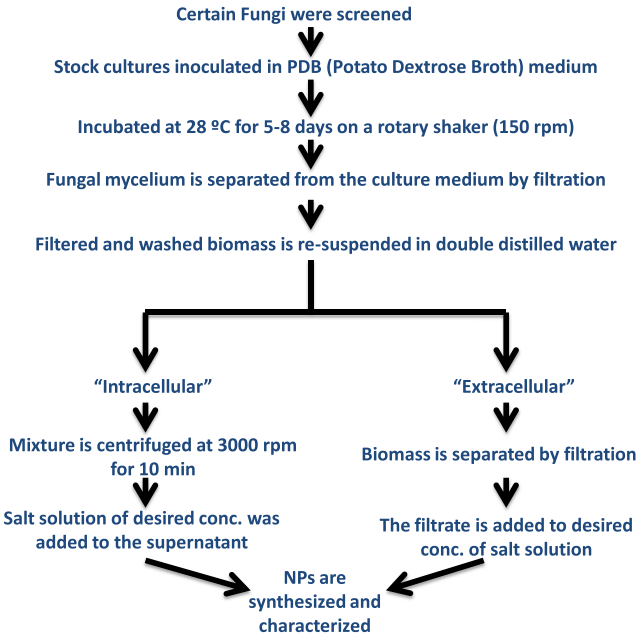

Fig. 2 Schematic representation of mycosynthesis of nanoparticles under extracellular and intracellular routes

and there have been many bacteria recognized to produce various metal nanoparticles [45]. Some of the bacterial species identified as efficient producers of nanoparticles are *Magnetospirillum magneticum*, *Desulfovibrio desulfuricans* NCIMB 8307 [46], *Pseudomonas aeruginosa* [47], *Plectonema boryanum* UTEX 485 [48–50], *Rhodopseudomonas capsulate* [51], *Escherichia coli* [52], *Bacillus licheniformis*, *Lactobacillus* sp. [53–55], and *Bacillus cereus* [56].

However, if we consider fungi, it showed equal potentials in synthesizing nanoparticles and thus are studied as elaborately as bacterial synthesis processes. Fungi were found to be more convenient since their biomasses can be easily developed and optimized according to our need, biomasses are simpler to handle, cheaper to grow, i.e., they are economically viable, their mycelial mats provide larger surface area for greater wall-binding capacity, produce comparatively higher quantity of enzymes responsible for diverse processes, help in comparatively larger scale of production of nanoparticles, have higher bio-accumulation capacity of nanoparticles due to their elevated tolerance level, and their synthesis can be either intra/extracellular but downstreaming is simpler in this case [5, 38, 57–60]. Fungal species include different *Aspergillus* spp., *Fusarium* spp., *Verticillum* spp., and *Trichoderma* spp., and endophytic fungi [4, 38, 39, 58, 61–65] are known to produce silver, gold, as well as a variety of other metal nanoparticles. It was revealed that metals being toxic to the bacterial or fungal cells are reduced by certain reductase enzymes secreted by them, like α-NADPH-dependent reductases, nitrate-dependent reductases, or the metal ion bonds to the cell surface causing biosorption [66]. Both ways, microbes strive to remove the unnecessary particles from its surrounding. Fungi are more preferred organisms for such process when carried out in larger scales. A fungi secrete diverse group of enzymes, proteins, or peptides as reducing agents like naphthoquinones, anthraquinones, and nitrate reductase which break down metal ions into metal nanoparticles. These are specific to particular metals and act on them easily [39, 67, 68]. Also, some metal induces high oxidative stress in fungi and thus secretes proteins to inhibit any harmful actions of the metal. Due to the use of such enzyme and protein secretions, the size and shape of the nanoparticles can be simply controlled. These enzymatic processes are gaining a lot of interest in recent science even though the mechanism behind most of the processes is yet to be revealed. As mentioned earlier, mycosynthesis can be either extracellular or intracellular type. Extracellular synthesis involves secretion of responsible fungal enzymes into the media due to the presence of silver salts that creates a stressed environment for the organism. Furthermore, the metal is reduced into nanoparticles by these enzymes. Whereas, the negatively charged cell wall of fungi interrelates with positively charged metal ions due to enzymes present on cell wall membrane, in the case of the intracellular route [69].

Yeast, being members of kingdom fungi, are slightly different from the typical fungus since they are unicelled. As their cellular organization is similar to fungi, they are also considered for synthesizing silver and gold nanoparticles. As reported earlier, several yeast species successfully produced AuNPs, for example, a cold-adapted yeast, *Y. lipolytica* NCYC 78 [70], a tropical marine yeast *Y. lipolytica* NCIM 3589 [71], *S. cerevisiae* AP22, and CCFY-100 [72]. On the other hand, silver nanoparticles were synthesized by the silver-tolerant strain MKY3, dermatophytic species *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Kluyveromyces marxianus*, and *Candida utili* [73–76].

Ninganagouda et al. [77] demonstrated fungal synthesis of metal nanoparticles in three simple steps: Firstly, the organism of interest in mass cultured in respected media, most commonly potato dextrose broth (PDB). Secondly, well-grown mycelia mats are filtered from the chemically composed media, washed, and re-suspended in double-distilled water. This step is carried out to avoid any interference of the chemicals present in the media of the process. Thirdly, the suspension obtained is formulated with different concentrations of metal salts and incubated. Nevertheless, intracellular production process necessitates the use of the mycelia mat instead of the filtrate since the responsible enzymes are established within the cell walls [31, 38, 45]. Particles formed are polydisperse due to the presence of different types of enzymes with assorted potency as secreted by the organisms employed.

3.1 Silver Nanoparticles

Silver, as a metal, has always been significant in human health and diseases due to its natural powerful antimicrobial properties which help in wound healing, skin care, water purifier, etc. They easily interact with the microbial membranes and destroy cells which avoid or treat infectious diseases [78–80]. Certain microbes are becoming silver-resistant, and therefore, silver ions have failed in healing ailments in spite of having a number of beneficial qualities. Silver ions have very low toxic threshold, and they are not preferred as safe drugs in many therapies [81]. These shortcomings of silver metal led to nanosilver synthesis. Fungal synthesis of AgNPs has conquered large area in the field of nanobiotechnology where many scientists had and even now are exploring mycosynthesis of silver particles. Different fungal endophytes and pathogens were selected for synthesizing AgNPs by various researchers. Some of the important species such as, *Fusarium graminearum*, *Fusarium solani*, *F. oxysporum*, *Fusarium culmorum*, *Fusarium scirpi*, *Fusarium tricinctum*, *A. flavus*, *Aspergillus* sp., *A. terreus*, *Penicillium* sp., *Fusarium moniliforme*, *A. concius*, *P. janthinellum*, *Phomosis* sp. and many others proved to have fair ability to produce silver nanobiomolecules [19, 21, 82, 83]. Certain fungi isolated from various environmental sources were also evident for the synthesis of these colloidal particles. Scientists and different groups carried out formulations to illustrate fabrication of silver by fungi such as *A. flavus* [18], *P. citrinum* [84], *Rhizoctonia* sp. [22], *F. oxysporum* [85], and *F. semitectum* (KSU-4) [24]. Other fungi like *Trichoderma viridae* [86], *Coriolus versicolor* [8], *Penicillium fellutanum* [87], and *Trichoderma asperellum* [65] were also investigated for the synthesis of silver particles as well (Table 1).

The mechanism behind silver nanobiosynthesis process which fundamentally involves reduction instigated by electron transfer from the NADH by NADH-dependent reductase as electron carrier has partially been studied by several scientists [107]. They concluded α-NADPH-dependent nitrate reductase, phytochelatin, or hydrogenase enzyme is chiefly responsible for AgNPs in several instances. Silver salt like silver nitrate is vastly used for bioreduction methods of AgNPs, and they are formed due to reducing agents mentioned above, and stabilizing agents are produced by fungi. According to assays performed, the cell wall containing carboxylate groups is negatively charged which interacts to the positively charged silver metal and fabricates nanosilver [108]. Fungal species, i.e., *Phaenerochaete chrysosporium* indicated secretion of reducing sugar which was also known to facilitate AgNP synthesis [109].

3.2 Gold Nanoparticles

Among metal nanoparticles, AuNPs have gained a lot of significance due to its inert nature which makes it undoubtedly non-toxic for human cells. Gold is scarcely investigated so far in comparison to silver for the synthesis of their relevant nanoparticles. Though gold as a metal is expensive, various research groups have extensively considered it for the synthesis of nanoparticles and applying them in biomedical applications [102, 110, 111]. Gold has proved to be the most biocompatible, stable, and eco-friendly nanomolecule. They have shown great functions in biosensors, cancer diagnostics, and therapy, in needle-free drug delivery and as antimicrobials [112]. According to previous reports, fungi have been extensively used in mycosynthesis in gold particle production. AuNPs in the size range of 10 to 200 nm were fabricated by diverse scientific groups by various fungal species like *A. fumigates* [25], *Cylindrocladium floridanum* [113], *Sclerotium rolfsii* [101], *Epicoccum nigrum* [102], *F. solani* [103], *A. terreus* IF0 [104], *Hormoconis resinae* [98], *P. chrysosporium* [90], *Penicillium rugulosum* [114], *C. versicolor* [8], *Penicillium brevicompactum* [115], *A. niger* [105], *Candida albicans* [116], *Nocardia farcinica* [117], *Rhizopus oryzae* [118], and *Penicillium chrysogenum* [119]. Fungi are known to be more efficient in producing the smaller size of AuNPs than bacteria. However, less than 50 fungal species are so far screened for the AuNP synthesis, and they are listed in Table 1.

Mechanism of AuNP synthesis is barely considered as part of nanobiotechnology research as yet, but it is explained in simple ways about how the enzymes and proteins work in synthesis process [90]. They clarified the routes where, enzymes like Laccase helps in the extracellular synthesis and protein side groups like amino groups, sulfhydryl groups, and carboxylic groups act in forming loci on the cell surface leading to crystal growth in case of the intracellular pathway. It is also stated that AuNP synthesis takes place again due to the same reason as that of AgNP synthesis, i.e., toxic stress in the microbial environment.

Explained earlier in this article, yeast is important in synthesizing silver and gold nanoparticles, but they are least studied in the field of nanotechnology. Different yeasts studied so far are cited in Table 2.

These nanoparticles being some of the best materials presently for application on biomedical devices, we richly pursue study on the mechanism of making its synthesis process effortless. We can also introduce different parameters in the synthesis process to get better yield, specific shapes, and sizes of the particles pertaining to different applications.

4 Downstreaming and Purification of Nanoparticles

Fungi are always preferred in today's nanobiotechnology research for synthesizing nanoparticles because of their easy and simple downstreaming process. In the case of bacterial production, the separation of enzymes secreted requires a lot of equipment like centrifugation, sonicator, and chemical agents such as methanol, whereas fungal filtrate can be effortlessly

Table 1 List of fungal species synthesizing silver and gold nanoparticles

Species	Ag/Au NP	Size (nm)	Morphology	Intracellular/ extracellular	Reference
Alternaria sp.	Ag	20–60	Polydisperse spherical	–	Gajbhiya et al. [88]
F. oxysporum	Ag	3–25	Spherical	Extracellular	Gaikwad et al. [82]
Penicillium sp.	Ag	3.71–65.92	Cubic nanoclusters	Intracellular	Chandrappa et al. [83]
Phoma sp. 3.2883	Ag	71.06–74.46	Spherical	Intracellular	Chen et al. [89]
Verticillium	Ag	25 ± 12	Spherical	Intracellular	Mukherjee et al. [38]
Trichoderma asperellum	Ag	13–18	Spherical	–	Mukherjee et al. [65]
Phaenerochaete chrysosporium	Ag	15–200	Spherical	Extracellular	Sanghi et al. [90]
F. solani	Ag	16.23	Spherical	Extracellular	Ingle et al. [91]
Aspergillus clavatus	Ag	10–25	Polydisperse spherical or hexagonal	Extracellular	Verma et al. [92]
A. fumigates	Ag	5–25	Spherical	Extracellular	Bhainsa and D'souza [61]
A. fumigates	Ag	50	Spherical	Extracellular	Navazi et al. [93]
Helminthosporum solani	Au	15–20	Mostly spheres, and number of rods, triangles, pentagons, pyramids, and stars	Extracellular	Kumar et al. [94]
A. terreus	Ag	1–20	Spherical	Extracellular	Li et al. [95]
F. acuminatum	Ag	5–40	Spherical	Extracellular	Ingle et al. [96]
F. semitectum	Ag	10–60	Spherical	Extracellular	Basavaraja et al. [97]
Hormoconis resinae	Au	3–20	Spherical	Extracellular	Mishra et al. [98]
Neurospora crassa	Ag	60	Spherical	Intracellular	Castro-Longoria et al. [59]
Penicillium brevicompactum	Ag	23–105	Spherical	Extracellular	Shaligram et al. [99]
Penicillium fellutanum	Ag	5–25		Extracellular	Kathiresan et al. [87]
Coriolus versicolor	Capped Ag	–	Spherical	Extracellular	Sanghi and Verma [8]
Fusarium oxysporum	Ag	5–13	Spherical	Extracellular	Husseiny et al. [47]
Trichoderma viridae	Ag	5–40	Spherical/occasionally rod-shaped	Extracellular	Fayaz et al. [100]
Penicillium citrinum	Ag	109	Spherical	Extracellular	Honary et al. [84]
Rhizoctonia sp.	Ag	25–50	Plate-like	Extracellular	Raudabaugh et al. [22]
Cylindrocladium floridanum	Au	19.05	Spherical	–	Badrinarayanan and Sakthivel [25]
Sclerotium rolfsii	Au	25	Triangles, hexagonals, decahedrals, and rods, and isotrophic sphericals	Extracellular	Kannan et al. [101]
Epicoccum nigrum	Au	5–50	Spherical/rod-shaped	Extracellular	Sheikloo et al. [102]
Fusarium solani	Au	20–50	Spherical	Extracellular	Gopinath and Arumugam [103]
Aspergillus terreus IFo	Au	10–19	Spherical/rod	Extracellular	Priyadarshini et al. [104]
Phanerochaete chrysosporium	Au	10–100	Spherical	Extracellular	Sanghi et al. [90]
Aspergillus niger	Au	10–30	Various shapes	Extracellular	Soni and Prakash [105]
Coriolos versicolor	Au	5–30	Spherical or elliptical	Extracellular	Sanghi and Verma [106]

segregated from the mycelia mats by simple filtration technique. This helps in saving time, lesser use of intricate instruments, and thus making it an overall manageable procedure [10, 57, 125]. The downstream process in fungal synthesis method involves the separation of biomass from the filtrate which eventually can be used in the formulation with metal salts. Once the particles are formed, the colloidal solution containing these particles is freeze-dried and purified for

characterization. Freeze-drying is carried out by lyophilizers, and the powder obtained is purified by different approaches which involve washing multiple times with Millipore water, heating at high temperature (within a melting point of metal concerned), and treating with agents (hydrogen peroxide, phosphoric acid) to remove organic materials.

The metal nanoparticles synthesized by fungal route have no particular method of purifying them so far, yet some researchers have carried out different techniques for this purpose. They have demonstrated varied ways of purifying nanoparticles to get rid of the impurities that might be present from the media used or the biomass involved or due to the organic molecules that cause aggregation in them. Silver nanoparticle formulations were spun at 10,000 rpm for 10 min twice to purify and washed multiple times to remove impurities by centrifuging at 14,000 rpm for 30 min by a group of researchers, whereas, another group passed the formulation through microfilters so as to obtain the same size range of particles and then separated them by discontinuous sucrose density gradient which is done using ultracentrifugation technique [126, 127]. To purify monodispersed solution of stable gold particles, Al-Kazazz et al. [128] used dialysis membrane instead of employing an organic solvent like other researchers, since dialysis process does not require harmful chemicals. Again, removing unwanted impurities in form of supernatant from the concentrated pellets by centrifuging samples at high speed (2000, 5000, 10,000, 15,000 rpm for 20 min) is another common technique used [129], where large aggregates are removed first and gradually the pellets are rewashed to get finer and pure particles. These methods consequently help collect pure nanoparticles which can be undoubtedly further exploited in biomedical functions.

5 Characterization of Bio-Nanoparticles

Nanoparticle formation has to be confirmed by certain approaches so as to be able to employ them in their respective applications in future. Most preliminary mean to follow particle formation in a formulated solution is by visual observation of the color change. It can be further affirmed by detecting specific peaks given by nanoparticles in the visible regions from UV–vis spectrum of formulations using a spectrophotometer within the range 250 to 800 nm [130, 131]. X-Ray diffraction (XRD) analysis data is another important evidence of nanoparticle formation. Morphology, size, composition, and the distribution of nanoparticles can be studied by transmission electron microscopic (TEM) analysis, scanning electron microscope (SEM), energy dispersive spectroscopy (EDAX), dynamic light scattering (DLS), etc. The biomolecules influencing the synthesis and stabilization of nanoparticles are traced by FT-IR spectrum [1, 28, 132].

Broadly, AgNP production in the test solution has shown a color change from pale yellow to reddish brown or dark

Table 2 List of yeast species synthesizing silver and gold nanoparticles

Species	Ag/Au nanoparticle	Size (nm)	Morphology	Intracellular/extracellular	Reference
P. jadinii	Au	Few to 100	Triangles, hexagons, spheres, and rods	Intracellular/extracellular	Gericke and Pinches [120]
MKY3	Ag	2–5 nm	Hexagonal	Extracellular	Kowshik et al. [74]
Pichia capsulata	Ag	–	–	Extracellular	Subramanian et al. [121]
Hansenula anomala	Au	2–70	–	–	Kumar et al. [94]
Saccharomyces cerevisiae	Au	20–100	Spherical	Extracellular	Lim et al. [122]
	Ag	5–20	–	Extracellular	
Candida guilliermondii	Au	50–70	Spherical	Extracellular	Mishra et al. [123]
	Ag	10–20	Spherical	Extracellular	
Yarrowia lipolytica	Au	15–20	Nanoparticles and nanoplates	Extracellular and intracellular	Pimprikar et al. [124]
Saccharomyces cerevisiae, AP22 and CCFY-100	Au	15–20 at 1 h	Spherical	Extracellular	Sen et al. [72]
Candida utilis	Ag	20–80	Spherical	Extracellular	Waghmare et al. [73]
Trichophyton rubrum,	Ag	< 50	Spherical	Extracellular	Moazeni et al. [75]
Trichophyton mentagrophytes	Ag	50–100	Spherical	Extracellular	
Microsporum canis		50–70	Spherical	Extracellular	
Kluyveromyces marxianus	Ag	3–12	Spherical	Extracellular	Ashour [76]
Candida utilis	Ag	6–20	Spherical	Extracellular	

brown depending on the concentration of the metal salt used. As reported in several articles, brown solution confirmed the presence of AgNPs due to surface plasmon resonance (SPR) of the particles, and the color intensity increases with the increase in a number of nanoparticles in solution [19, 21, 77, 133]. Even when the biomass of the fungus is directly used for the purpose of synthesis, the color of the biomass turns brownish from colorless [18]. In contrast, control, that either consists of cell filtrate or metal salt solution alone, does not show any change in color.

Varied shades of red like ruby red, burgundy red, or a light or reddish purple color are commonly formed with different concentrations of chloroauric acid ($HAuCl_4$) due to the presence of AuNPs and their surface plasmon vibrations [25, 27, 134, 135]. Statements from scientists clearly depict the initial pale yellow color of the formulation changes with the change in gold salt concentrations, where, as the concentration increased the color was intensified starting from 0.5 mM with pinkish red color to 1.5 mM with a dark purple color. However, with a higher concentration of gold, the particles start agglomerating and interfere in further characterization studies [136].

Further characterization methods include two types of techniques: firstly, microscopy-based ones like SEM, TEM, and AFM which give an idea about the physical look of the particles and secondly, spectroscopy-based such as UV–vis, XRD, FT-IR, and DLS that help to determine their composition, structure, and properties.

5.1 UV–Visible Spectroscopy

This works on the principle of measuring the SPR frequency of the particles in the solution. Mukherjee et al. [65]described that the intensity of the peak obtained is related to a number of nanoparticles present, where, breadth and height of the peak are directly proportional to the concentration of particles. The number of particles in the test solution determines the height, while dispersity represents the broadness of UV spectrum peak. Also, polydispersity decides the number of peaks in UV spectrum analysis and the size of the particles shows certain shifts (blue and red shift) in the absorption spectra, i.e., peak is obtained at the position more towards higher wavelength if a particle size is big and vice versa [82, 137]. The wavelength region of surface plasmon absorption peak, the number of absorption peaks, and spectrum broadening are predominantly related to the shape of the nanoparticle; therefore, the UV–visible spectroscopy helps immensely in determining the size of particles as well.

As depicted in Figs. 3 and 4, the occurrence of the plasmon band in this instrument for AgNPs as well as AuNPs is measured at the resolution of 1 nm within the wavelength range of 250 to 800 nm [24, 136]. A colloidal solution of AgNP shows an intense peak at 400–450 nm according to the majority of studies carried out [19, 65, 77, 85, 138]. Gold particles illustrate an absorbance at 500–550 nm as reported in various works [95, 103, 132, 139].

5.2 X-Ray Diffraction (XRD)

X-Ray diffraction is the method to study simple or complicated crystalline structures, size, and diffraction patterns of any particles. The powdered sample of nanoparticles after freeze-drying is characterized by passing beams of X-ray which on collision with atoms gets scattered and interferes with each other. This process is recorded, and certain peaks are obtained in form of a graph which depicts the structure and size of the particular nanoparticle. This analysis by Shah et al. [31] confirms the crystalline structure according to the standard crystallographic database. The diffraction intensities are recorded in the range of 8 to 80° 2θ angles.

Silver in powdered nanoform represents face center cubic (fcc) crystalline structure following the comparison of the peaks found in XRD graphs with the Joint Committee on Powder Diffraction Standards (JCPDS) File No. 04-0783 database. Mainly, four peaks are observed at planes (111), (200), (220), and (311) at 2θ angles calculated by Bragg's law [21, 133, 137, 140]. Gold nanocrystals also show similar fcc structure, and peaks are the same planes as silver [105, 134].

5.3 Fourier Transform Infrared Spectroscopy (FT-IR)

This particular study confirms any biomolecular compounds capping the nanoparticles and in turn makes them more stable in nature. This analytical technique measures infrared versus wavelength of light which helps to determine functional groups on mycosynthesized nanoparticles (Figs. 3 and 4). Powdered samples are recorded with a resolution 4 cm^{-1} in the range of 400–4000 cm^{-1} [24]. The commonly observed surface residues in the case of the fungal mediated AgNPs are amino acids and peptides which act as capping agents which prevent agglomeration. Previous articles confirm the presence of carboxylate ions (–COO), alkenes (C═C), alkanes (C–C), amides (C–N), alcohols (–OH), phenols, primary and secondary amines (–NH), and carbonyl (C═O) groups which extend stability to the particle fabricated [23, 65]. These groups originate depending on distinct fungal extract used for the synthesis process. Again, AuNPs are also found to be capped with similar groups like primary, secondary, or aromatic or aliphatic amines, hydroxyl, methylene, as well as carbonyl, amide I, and amide II [141].

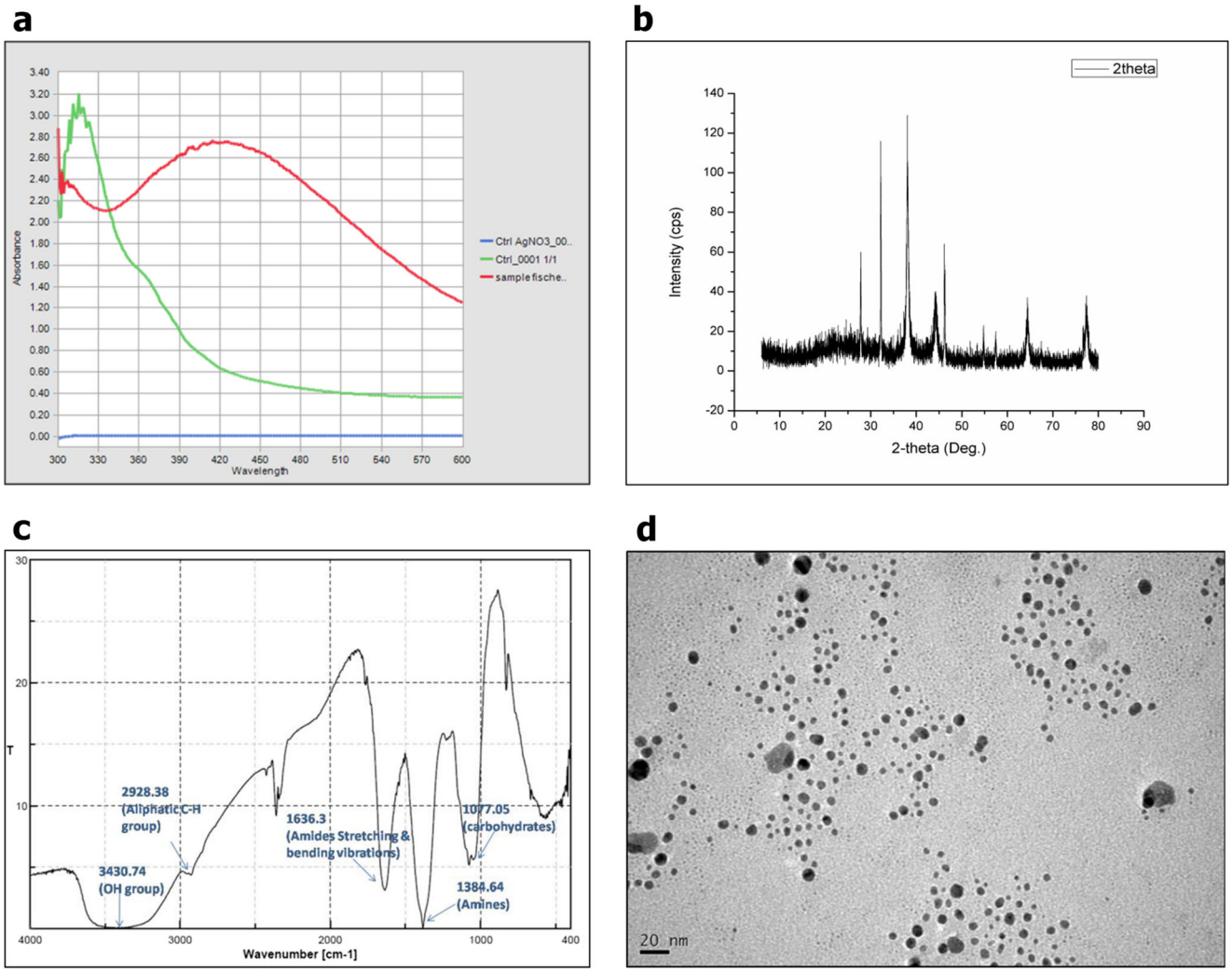

Fig. 3 Illustration of various characterization methods of silver nanoparticles (AgNPs)

5.4 Scanning and Transmission Electron Microscopy (SEM and TEM)

SEM or scanning electron microscope is an instrument which involves scanning of surface of the sample and backscattering of the ray is recorded. Since metal nanoparticles are highly electrically conductive, it is easy to scan them through SEM. Similarly, transmission electron microscope (TEM) helps in capturing images of nanoparticle samples based on the interaction of the electron beam in the high vacuum-conditioned chamber. SEM can be carried out by directly placing samples on the black surface that prevents unwanted scattering of the incident beam. In the case of TEM, the sample is prepared on carbon-coated copper grids using a single drop of the colloidal solution.

These microscopy techniques basically assist in finding out the size and shape of particles. So far, researchers detected various shapes like spherical, diamond, rod-like, and cubic with a wide range of sizes starting from as small as 1 to 100 nm in diameter [20, 83, 86, 105, 134, 142, 143] as demonstrated in the characterization figures. Thus, we can conclude the dominating shape and size of the particles in a formulated solution and standardize procedures to obtain the shape and size of our own interest.

5.5 Energy Dispersive Spectroscopy (EDS)

The method also known as energy dispersive X-ray (EDX) analysis is applied to find out the composition of nanoparticle sample [1]. The test powder is measured at an accelerating voltage of 10 kV, and optical absorption band peaks are formed at typical positions for specific elemental metal due to SPR. Metallic silver nanocrystallites show an absorption band at ~2 to 3 keV along with C and O signatures in some instances [21, 77, 144], while gold showed a band at 2 keV characteristic of AuNPs. There is a presence of carbon, oxygen, and nitrogen atoms due to the extract used in synthesis [145, 146].

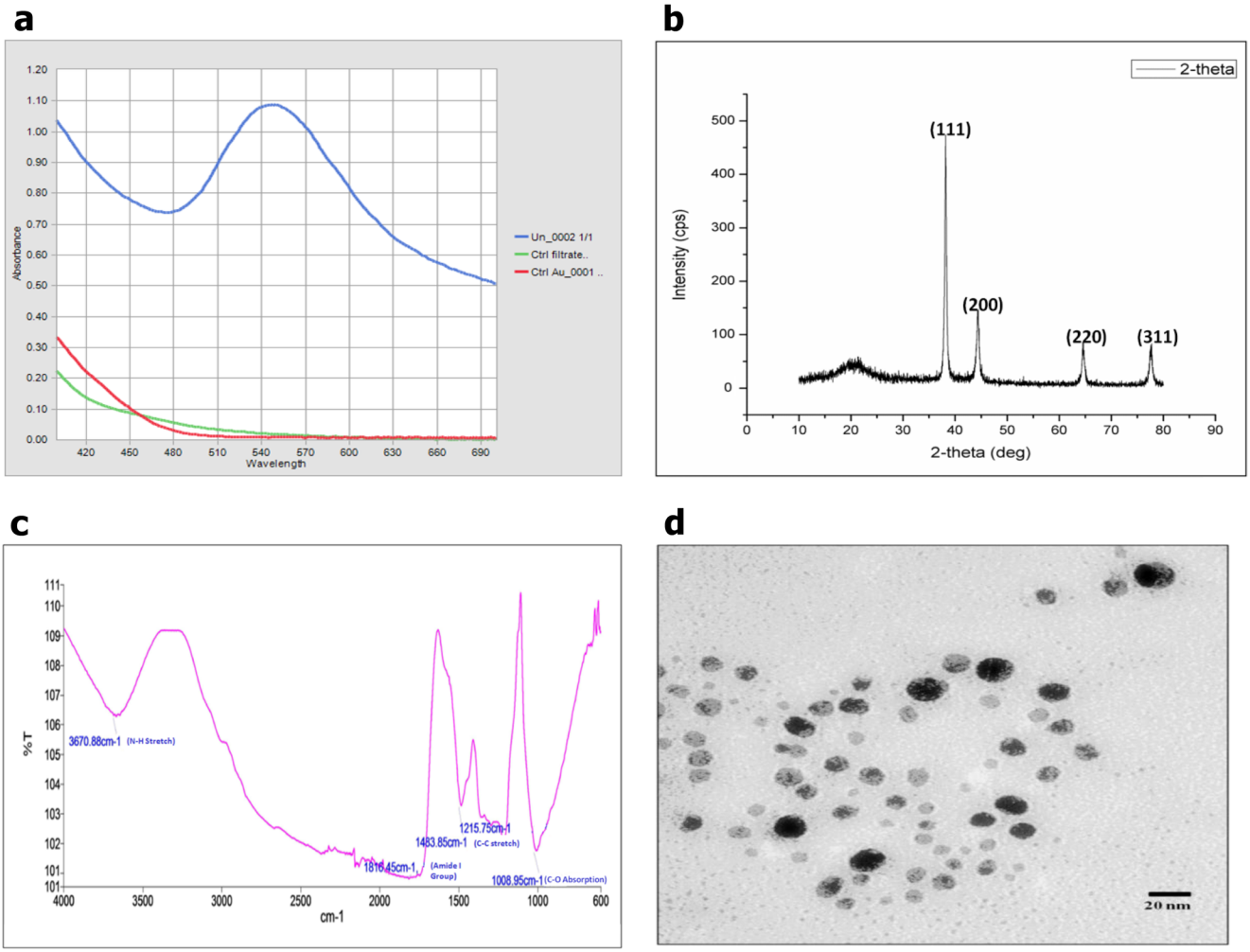

Fig. 4 Graphs depicting characterization methods of gold nanoparticles (AuNPs)

5.6 Dynamic Light Scattering (DLS)

The size distribution and surface charge of the particles can be checked by this technique. This works on the principle of interaction of particles with light. The amount of light scattered in the colloidal solution due to the presence of particles can be related to their diameter. According to Akbari et al. [147], this technique can be used to measure particles in the range of 1–500 nm but, it is difficult to record their sizes when agglomerated. The pure filtered nanoparticle dried samples are dissolved in a solvent, and the mean size, as well as charge on them, is analyzed by means of the instrument at 25 °C with a scattering angle of 90° [141]. Moreover, the time-dependent study of the nanoparticle formation can also be carried out applying DLS. Zeta potential is measured by the peak number and peak area which fundamentally involve the charge on the particles. Negative charge provides evidence of particle size being smaller than 100 nm and vice versa. The smaller the particles, the more stable they are [23, 132]. Sometimes, the size determined by DLS, XRD, and TEM differs depending on the capping agent present on the nanoparticles, where the size calculated by DLS is bigger compared to other measurements [65].

6 Drawbacks and Future Prospects

Mycosynthesis of nanoparticles being useful in several ways has gained attention in present nanotechnology research, and it is very much employed in different synthesizing metal nanoparticles. However, every process has its own shortcomings from a scientific point of view. Similarly, problems behind fungal synthesis methods are studied here and specified. To start with, pathogenic microorganisms especially extracts from fungal species used might contain disease-causing spores which if spread may lead to safety risk sometimes and acts as a hazard to the ecosystem [3]. Secondly, mycosynthesis of silver and gold nanoparticles is yet to be studied elaborately; therefore, researchers do not possess the

ability to control nanoparticle formation, their shape, size, and size distribution faultlessly. Also, very less knowledge has been acquired on the stability and aggregation of mycosynthesized particles. Next, biggest disadvantage of fungal synthesis is polydispersity in the formulation due to unknown reasons. Alongside, the particle size is also found to be bigger in some cases which might interfere in future application aspects [147, 148]. Lastly, since fungus is a eukaryotic organism, it is difficult to deal with its genes in order to identify responsible enzymes in this route. In short, the mechanism of reaction of formation and secretion of the enzymes in this process is hardly understood which causes a major drawback in further recognition of mycosynthesis [69, 148]. Apart from the above-mentioned problems, particles formed by fungal route are essential to be purified; however, downstreaming is difficult as it requires equipment like centrifugation, hot air oven, and lyophilizer, and it comparatively is time-consuming.

As we have discerned, the fact that mycosynthetic process of producing AgNP and AuNP is much safer, and economically, it should be considered to be studied in further details. Their immense use in pharmaceutics and other important aspects of medical sciences like delivery of the drug, cancer therapy, gene treatment, DNA analysis, antibacterial factors, biosensors, increasing response rates, separation science, and MRI is leading us to broadly deal with their morphological characteristic. Also, different methods for purifying mycosynthesised particles have also been explored recently which can be undoubtedly applied in the biomedical field. Mycosynthesis process being inexpensive and eco-friendly is analytically exploited by scientists, and numbers of fungi are being screened. Consequently, discoveries in this field are entitled to develop in future as silver and gold nanomaterials are important as antibiotics, antibiofilm agents, catalysts, anticancerous agents, antiproliferative agents, etc.

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