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Review Article

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# Fluorescent Giant Clusters with Myriad Applications

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## Abstract

The synergistic evolution of highly emissive giant clusters thiolated $Au^{I}_{core}$-$(Ag_{2,3})_{shell}$ has been proven to be of immense importance due to their myriad applications. The long-term emission stability (excitation energy 400 nm, emission maximum 560 nm and quantum yield 5%) of $Ag_{2,3}$ clusters has been found due to the drift of electron density from silver to gold. Usually, clusters, the missing link between nanoparticles (NPs) and atoms, are < 2 nm in size with less stability. However, thiolated $Au^{I}_{core}$-$(Ag_{2,3})_{shell}$ giant clusters have a diameter of ~ 600 nm. They have been so stable that they have been obtained in various solvents (along with water) and even in solids for months. Such clusters have been biocompatible (MTT assay) with remarkable antibacterial properties. Antibacterial cotton and paper were designed by impregnating the clusters. In addition, selective detection of toxic pollutants (with nanogram level limit of detection) and cancer cells have also been observed. Depending on the synthetic protocol (e.g., photo-chemical, green-chemical, modified hydrothermal, etc.), the variation of properties and applications have been noted.

## Keywords

Synergism, Sensing, Gold, Silver, Antibacterial activity, Bioimaging, Cell viability

## Introduction

Nanotechnology has sparked a great deal of public interest due to the need for and applications of NPs in various human undertakings, including industry, agriculture, commerce, medicine, and public health [1]. Exposure to NPs in the environment is inevitable given that they are already prevalent in our daily lives [2]. A NP is a group of molecules or atoms that are linked together, having a diameter or dimension in at least one direction between 1 and 100 nm. A metal particle with a diameter of ≤ 2 nm is formally referred to as a "nanocluster" (NCs) [3]. Due to their extraordinarily tiny size, profusion of unsaturated active sites, and distinctive electronic structure that differs from that of conventional NPs, NCs have demonstrated significant catalytic activity and distinctive selectivity in several catalytic events. More significantly, they serve as model catalysts to show the relationship between atomic-scale structure and catalytic performance because of their clear structure and monodispersity [4]. Noble metal NCs like gold (Au) and silver (Ag), which generally have diameters under 2 nm, have drawn interest because of their special ability to fill the "missing link" between atomic and NPs behavior. Ferromagnetism, chirality, quantum behavior, single-molecule optoelectronics, photoluminescence (PL), bioassay, and sensing are only a few examples of recent research, concentrating on their quantum electronic features. Like semiconductor quantum dots with tight confinement of the quantum-size range, the particle size of NCs is less than the exciton Bohr radius

(CdSe quantum dots are 4 - 5 nm in size) [5].

One of nature's most amazing phenomena, PL, is what gives numerous natural things relating to their unique color. The two primary types of PL—fluorescence and phosphorescence—are electrical transitions from singlet and triplet excited states to the singlet ground state, respectively [6, 7]. Because of their extreme simplicity, flexibility, sensitivity, multi-analyte detection, and non-invasive measurement, the utilization of biosensors—a vital spectrum technology—for fluorescence analysis has unquestionably become more promising [8].

For life to exist on earth, water is a very important supply. Overpopulation, human activities, rapid industrialization, unskilled exploitation of natural water resources, and unplanned urbanization have all had a negative impact on the water quality [9, 10]. The collection of metals and metalloids having an atomic density of more than 4000 kg/m$^3$ is referred to as heavy metal. Even in extremely low quantities, heavy metals are poisonous by nature and may seriously harm both humans and animals [11, 12].

The quality of patients' healthcare has been greatly enhanced by advances in biomedical imaging. With increased precision in illness identification and surgical operations, image-guided therapy has decreased the significant risk of human mistakes [13, 14].

In this review article, we focused on the synergism of Au and Ag to obtain emissive giant clusters with salient features, stability, the origin of fluorescence, and applications such as antibacterial (modification of cotton paper**)** bioimaging, heavy metal sensing with their mechanisms, and cell viability.

## NCs

An emerging type of atomically precise nanomaterials, metal NCs—also referred to as ultra-small metal NPs—fill the space between single atoms and plasmonic nanomaterials. Intriguing physical-chemical characteristics of these materials include luminescence, chirality, magnetism, catalysis, and electrochemistry, which may be explained in terms of their quantum size effect and discrete electronic states [15, 16].

One of these nanomaterials' most exciting and fascinating properties is PL. Such luminescent NCs have considerable potential for use in cell labelling, bioimaging, chemical sensing phototherapy, and drug administration since they often demonstrate greater photo-stability and biocompatibility [16].

Many novel and intriguing properties of NPs that are not present in bulk materials have been uncovered. Due to their potential uses in optics, sensing, drug administration, and catalysis, metal NPs have been the focus of much investigation. In particular, the field of NPs research has been substantially expanded by the discovery of metal NCs below 2 nm (such as Pt, Au, Ag, and Cu). A new kind of luminous nanomaterial that has received a lot of interest recently is metal nanoclusters, which are described as clusters of a few to a few hundred atoms. As they get closer to the Fermi-wavelength of electrons, these nano-systems start to resemble molecules, giving them the ability to fill a crucial gap between solitary metal atoms and plasmonic metal NPs [17, 18].

## Synergism of Au and Ag

A synergistic impact is one where the combined effect of two or more species is greater than the sum of the effects of the individual species. NC science has documented a synergism between Au and Ag, resulting in improved fluorescence, greater photochemical stability, and useful applications. The electronic component, nuclearity, size effect, and other factors result in aggregation-induced fluorescence, autofluorescence, core-shell contact, doping or alloying, oxidation-induced interaction, and Ag effect, among other phenomena [19].

Fluorescence synergism between Au and Ag has been proven into a highly promising phenomenon. Recently, several researchers have entered this field because of its many exciting findings. Ag and Au-based bimetallic particles are easily synthesized in a variety of size regimes and compositions, because they have comparable lattice constants. As a result, mixed crystals may be generated throughout practically the entire concentration range of Au and Ag (Figure 1) [20-22].

## Giant Cluster

Giant cluster creation in solutions, in nature, and in business is becoming more and more important. However, the development of massive clusters in solutions of tiny, comparatively soluble, but nonamphiphilic compounds has received very little study [23].

The size of the cluster is proposed to be 2 nm; however, giant clusters are much larger in size in comparison to the usual NC. Ganguly et al. [19] synthesized a Au(I)$_{core}$ Ag(0)$_{shell}$ NCs (TAuAgGC), protected by oxidized glutathione, which was highly fluorescent in nature ($\lambda_{em}$ 560 nm, $\lambda_{ex}$ 400 nm and the quantum yield is 4%). From the transmission electron microscope image, it was evident that the diameter of the particle was approximately 600 nm. Here Au core acted as a scaffold on which fluorescing Ag clusters were decorated resulting in a highly fluorescent giant cluster. However, Ag or

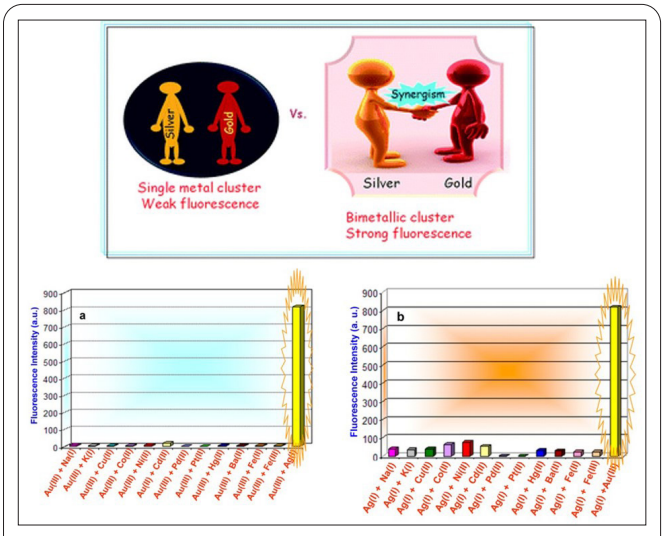



**Figure 1:** Synergism of Au and Ag to generate fluorescent giant cluster with versatile uses [19, 25].

Au alone in the same experiment condition could not show fluorescence. Au was pivotal to design the Ag cluster, resulting in the highly emissive giant cluster. However, the TAuAgGC, as discussed above was exclusively fluorescent. Such property helped to be employed in myriad applications [24, 25].

Gehin et al. [26] synthesized giant core-shell nano spherical clusters, containing 32 Co or 32 Ni atoms with 6 p-tert-Butyl Thiacalix [4]arene units. Core-shell Co13@TM20 clusters (TM = Mn, Fe, Co, and Ni) were reported by Jing et al. [27]. The Co13@Mn20 cluster was discovered to have a massive total magnetic moment (113 B), which is interesting.

## Origin of Fluorescence

The TAuAgGA were strongly fluorescent. The X-ray photoelectron spectroscopy revealed that the core Au was in +1 oxidation state, while shell Ag was present in zero-valent state. The fluorescent giant clusters had long-term stability due to an intriguing synergism between the Au(I) core and Ag(0) shell. The travel of the electron density from the shell (Ag) towards the core (Au) was attributed to the stability [25].

It has been confirmed by analysis of the TAuAgGA of the MALDI mass spectrum that $Ag_2$ and $Ag_3$ clusters were formed. Liquid chromatography-mass spectral analysis was also done to validate the production of $Ag_2/Ag_3$ clusters because MALDI is a hard ionization technique. It is significant to note in this context that many groups have provided explanations for the luminous properties of Ag clusters composed of 2 - 8 atoms [28].

## Synthesis of Giant Cluster

For the first time, Ganguly et al. [25] described a method for producing monodispersed spherical TAuAgGC from the corresponding precursor compounds (silver nitrate, chloroauric acid, and glutathione solution) (Figure 2). Exposure of UV (ultraviolet) light, sunlight as well as the hydrothermal method was employed to obtain such fluorescent giant clusters.

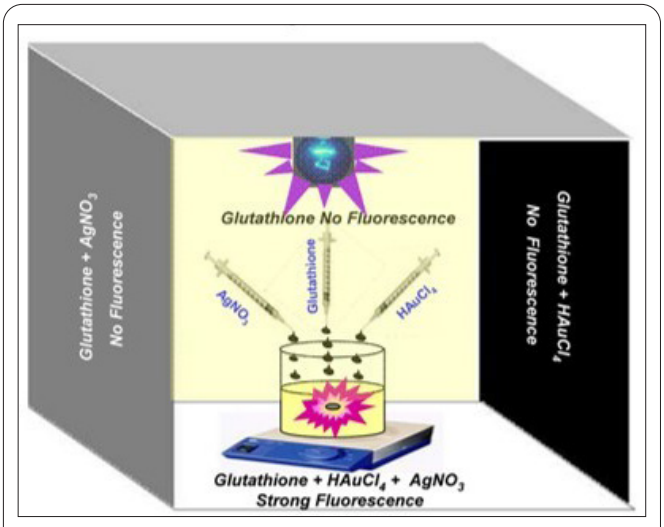



**Figure 2:** TAuAgGC displayed the synthetic protocol of fluorescent TAuAgGC in the presence of sunlight, glutathione, and Au-Ag [25].

Depending on the synthetic protocol the emission was slightly different. Bright sunlight exposure for 12 h resulted in giant clusters with $\lambda_{em}$ of 564 nm, while modified hydrothermal for 70 min with 100 W bulb produced giant clusters having 635 nm of $\lambda_{em}$. It is noteworthy that ten hours of UV light exposure synthesized giant clusters with $\lambda_{em}$ 565 nm [29].

## Stability of Cluster Giant Cluster

TAuAgGC was highly stable without any change in fluorescence for up to 3 months. Due to its extreme durability, TAuAgGC could be converted into a solid powder, dispersible in several water-miscible solvent systems without significantly changing the emission maximum. Sodium dioctyl sulfosuccinate might also be used to create very stable fluorescent clusters in water-immiscible solvents. Moreover, the giant clusters were utilized to produce fluorescent film, cotton, and paper substrates (with effective antibacterial activity). The particles in TAuAgGC contained positively charged surfaces (+11.9 mV) [25].

## Sensing

### Amino acid sensing

The ability of the body to recognize and respond to variations in dietary levels, notably those of glucose, lipids, and amino acids, is essential for survival. Changes in nutrition sensing, therefore, have a close connection to human diseases, particularly metabolic disorders. The primary components that support life are the three macronutrients glucose, lipids, and amino acids [29-31].

#### Cysteine sensing

Cysteine (Cys) serves crucial functions in biological processes such as metabolism, protein synthesis, and detoxification because of its involvement in reversible redox reactions. Additionally, it plays a significant role in several pharmaceutical compositions, such as antibiotics and solutions for skin problems. Numerous problems, including decreased hematopoiesis, leucocyte loss, psoriasis, etc. are brought on by a lack of Cys. Changes in Cys levels are thought to increase the chance of developing Alzheimer's and Parkinson's disorders [32]. Due to Cys' great fundamental role in biological systems, selective detection of Cys is currently a hot topic to study [33-35].

Massive TAuAgGC with strong fluorescence showed drastic quenching in the presence of Cys in comparison to other amino acids (Arginine, tyrosine, glutamic acid, aspartic acid, 4-hydroxy proline, isoleucine, histidine, lysine, leucine methionine, phenylalanine, threonine, tryptophan, asparagine, serine, threonine, isoleucine, and valine). The limit of detection of Cys was down to 5 x $10^{-8}$ M. The quenching was also investigated in other non-aqueous miscible solvents (acetone, ethanol, isopropanol, acetonitrile methanol). For all the cases, quenching was highly significant in the presence of Cys. For the case of water-immiscible solvents, TAuAgGC was vacuum dried to obtain solid fluorescent material, redispersed in water-immersible solvents. Some solvents displayed milky white suspension after adding Cys. On the other hand, some water-miscible solvents remained transparent with quenched

fluorescence.

### Mechanism

Cys selectively quenched the fluorescence of TAuAgGC. Cys, with a molecular weight of 121 Da is substantially smaller than tripeptide glutathione with a molecular weight of 307 Da. As a result, the giant clusters broke and subsequent fluorescence quenching was observed when the tiny thiolated ligand (Cys) replaced the glutathione, previously present in the protective layer of the TAuAgGC. Thus, the fluorescence of TAuAgGC was destroyed. However, Cys could not be identified in the presence of glutathione from infrared and UV, because glutathione is a tripeptide with Cys, Glu, and Gly skeleton [35].

## Solvent sensing

Organic solvents are valuable because of their unique capacity to dissolve a variety of useful materials, including fats, waxes, and resins, while maintaining chemical stability [36].

As carbon-based solvents, organic solvents are classified by their volatility, boiling point, molecular weight, and color as their main characteristics. Due to the significant risks that come with organic solvents and the fact that they are utilized for so many different things, we should consider their toxicity more. Nearly every solvent poses a health risk if consumed or breathed more than the permitted amount, and most of them irritate the skin when they meet it [36, 37].

Carbon disulfide and N-hexane, which cause peripheral sensorimotor polyneuropathy; toluene, which can cause severe brain damage, hearing loss, and an embryopathy known as fetal solvent syndrome; and styrene, which results in a nonspecific encephalopathy in people; are examples of solvents with distinct and distinctive neurotoxic features [37].

### Dimethyl sulfoxide sensing

Dimethyl sulfoxide (DMSO) is a popular organic solvent, that is also a natural substance. Additionally, micromolar quantities of DMSO have been found in drinks and a variety of foods, including tea, coffee, and wine. Due to its unpleasant odor, dimethyl sulfide, a reductive product of DMSO, is unfavorable for beverages or foods [38-43].

An analytical technique called electrochemical enzymatic biosensing has been discovered for aqueous DMSO samples. The manufacture of the selective gas sensor for DMSO was described by Pirsa and Alizadeh [44].

DMSO was the only common water-miscible solvent, that quenched the fluorescence selectively of TAuAgGC. The limit of detection of DMSO was 178 ppm. TAuAgGC detected contamination of DMSO not only in water, but also in various non-aqueous miscible solvents such as methanol, acetonitrile isopropanol, ethanol, acetone, etc. Only $Pb^{2+}$ restored the fluorescence (TAuAgGC + DMSO) system. Other metals could not restore the fluorescence TAuAgGC + DMSO system (Figure 3) [45].

### Mechanism

According to transmission electron microscope image, the big fluorescent spherical particles in the TAuAgGC were indeed Au core-Ag shells. The big particles were broken into a tiny sphere by the addition of DMSO, which significantly reduced the fluorescence in TAuAgGC [45].

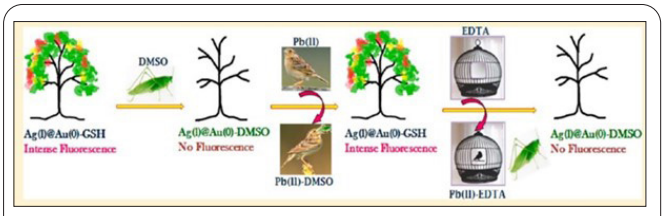



**Figure 3:** The schematic representation of the detection of $Pb^{2+}$ and DMSO with TAuAgGC [45].

In DMSO, the connection between the sulfur and oxygen atoms is a polarised double bond-dative bond intermediate. The electrostatic portion of the S-O interaction, which has a strong dipolar component, results in a negative charge centered on oxygen. According to the zeta potential value, the very negatively charged oxygen in DMSO facilitates the simple replacement of glutathione from the positively charged large clusters [45].

From a steric standpoint, replacing big glutathione with tiny DMSO was also advantageous. Giant clusters' positive charge was decreased by oxygen in DMSO, which resulted in a significant decrease in fluorescence [45].

## Heavy metal sensing

One of the main causes of our environment's rapid degradation, which regrettably overuses natural resources, is heavy metal pollution. $Pb^{2+}$, $Cr^{3+}$, $Hg^{2+}$, $As^{3+}$, and $Cu^{2+}$ are the main heavy metals that have recently been discovered to be environmentally hazardous. As a result, several efforts have been undertaken to identify and measure heavy metals routinely and analytically. However, modern technologies are constantly needed to detect heavy metals in a sensitive and user-friendly manner. For this reason, nanotechnology was developed. These nanotechnologies improved the performance of analytical tools and were discovered to be very sensitive, selective, and capable of operating quickly. Nano-based sensors are useful for on-the-spot detection or on-field detection with high linear resolution and are simple to integrate into the system [46-49].

Toxic metal ions have seriously affected the biological system and the living environment as one of the most harmful hazardous pollutants. It is essential to detect dangerous metal ions accurately and quickly, necessitating the creation of inexpensive, simple-to-use sensors with high sensitivity. Porous nanomaterials, noble metal NPs, and semiconductors have specifically been used in the building of sensors, together with analytical techniques including, colorimetry, electrochemistry, mass spectrometry, fluorescence, and surface-enhanced Raman scattering [48].

### $Pb^{2+}$ sensing

One, among the most dangerous heavy metal cations, is $Pb^{2+}$. Children who consume even extremely modest levels of $Pb^{2+}$ have a number of health issues, including, anaemia,

sluggish nerve conduction velocity, and memory loss. Children should not be exposed to blood levels of lead that exceed 100 mg/L (0.48 mM), according to the Centres for Disease Control. Selective and quantitative detection of $Pb^{2+}$ in water at neutral pH is a growing field of research [50, 51].

The fluorescence of TAuAgGC was quenched selectively with DMSO and $Pb^{2+}$ selectively restored the quenched fluorescence. $Ba^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{2+}$ $Cu^{2+}$, $Fe^{2+}$, $Hg^{2+}$, $K^{+}$, $Mg^{2+}$, $Mn^{2+}$, $Na^{2+}$, $Ni^{2+}$, and $Zn^{2+}$ did not any enhancement of fluorescence. Pb-induced enhanced fluorescence was again quenched with the addition of $Na_2EDTA$. The limit of detection of $Pb^{2+}$ was found to be $14 \times 10^{-8}$ M [44].

The fluorescence of TAuAgGC was quenched selectively in the presence of Cys and this quenched fluorescence was restored by the $Pb^{2+}$. No other metal ion was able to restore the lost fluorescence in such a dramatic manner. The detection limit of Cys and $Pb^{2+}$ was down to $5 \times 10^{-8}$ M and $15 \times 10^{-8}$ M, respectively. Thus, TAuAgGC was the justified platform for lead sensing.

### Mechanism

$Pb^{2+}$ removed the capping of DMSO due to the interaction of the sulfur of DMSO and $Pb^{2+}$ making TAuAgGC free. Thus, enhancement of fluorescence was observed. X-ray photoelectron spectroscopy revealed that Pb was in a +2-oxidation state after the treatment with the (TAuAgGC + DMSO) system. A slight increase in binding energies indicated that $Pb^{2+}$ also attracted the electron density of Ag(0) like Au(I), causing stability and an increment of fluorescence intensity [44].

Cys selectively quenched the fluorescence of TAuAgGC because cysteine replaced glutathione in the TAuAgGC. But $Pb^{2+}$ restored the fluorescence of the (TAuAgGC + Cys system). Compared to glutathione, $Pb^{2+}$ has a higher affinity for Cys.

Cys is highly affinized to $Pb^{2+}$. The $Pb^{2+}$-Cys complex has a stability constant of 1.75 104 l $mol^{-1}$. Thus, TAuAgGC + Cys became an efficient sensor for $Pb^{2+}$ sensing [35].

### $Hg^{2+}$ sensing

Human health is seriously threatened by mercury poisoning, and mercury emissions worldwide are increasing at an alarming rate. Significant sources of mercury emissions include the burning of fossil fuels and solid waste. Mercury has a long atmospheric half-life, which contributes to the widespread poisoning of land and water. The problem worsens when bacteria convert ionic and elemental mercury into methylmercury, a potent neurotoxin that enters the food chain. Serious sensory, motor, and cognitive are among the symptoms of mercury poisoning in humans that are the most severe [52, 53].

Fluorescent TAuAgGC was synthesized with sun exposure, and TAuAgGC displayed quenching with $Hg^{2+}$. Fluorescent TAuAgGC offers a highly sensitive and selective sensing platform for the detection of $Hg^{2+}$ down to 6 nM [28].

### Mechanism

The fluorescence was efficiently and selectively quenched when $Hg^{2+}$ ions were added to the fluorescent TAuAgGC repeatedly, and its emission peak gradually shifted from green to red. The red shift and quenching of fluorescence have been attributed to the $Hg^{2+}$ induced aggregation and metallophilic $5d^{10}(Hg^{2+})$-4d10($Ag^{+}$) interaction [28].

### Anion sensing

An important and growing class of molecules used in anion sensing are metal-based receptors. Anion sensor design has long been a research priority because it allows for the on-site, real-time detection and measurement of both healthy and harmful anions. In general, interactions between an ion and an ion receptor, such as electrostatic interactions, and ion-pair complexation, hydrogen bonds have been used to sense anions [54, 55].

### Sulphide anion sensing

A hazardous component known as sulphide ($S^{2-}$) anion is widely used in industrial settings including tanneries, oil refineries, food processing facilities, and paper/pulp production facilities. $S^{2-}$ ions commonly function as a reactant. $S^{2-}$ ion is also produced as a by-product of several industrial and manufacturing processes. $S^{2-}$ is extremely hazardous to living things. In an aqueous media, it also functions to create hydrogen sulfide and remove dissolved oxygen. Due to exposure in several occupational situations, $S^{2-}$ induces toxicity in humans that is remarkable. A constant increase in $S^{2-}$ in the human body invites a variety of physiological discomforts and metabolic challenges. Once more, protonation makes it even more poisonous. With too much $S^{2-}$, concrete deteriorates, and metal surfaces corrode in addition to health issues [56-60].

$S^{2-}$ ion quenched the fluorescence of TAuAgGC. The color of TAuAgGC was pale yellow but after the addition of $S^{2-}$ ion, the yellow color of TAuAgGC changed into orange. Thus, TAuAgGC was a fluorometric and colorimetric sensor for sensing of $S^{2-}$ ions. The limit of detection for $S^{2-}$ via the fluorometric mechanism was 10 nM respectively [29].

$S^{2-}$ ions cover the surface of TAuAgGC, producing an orange color, and eliminate glutathione from the fluorescent TAuAgGC due to its smaller size and high diffusing capacity. The reduction of Au(I) to Au(0), which resulted in the aggregation of Ag clusters and subsequently the destruction of the fluorescence, was caused by $S^{2-}$ having a stronger reducing ability than glutathione [19, 29].

## Antibacterial Activity

Noble metals are recognized for their well-documented antibacterial effects, which are connected to particle form. Due to their bacteriostatic and bactericidal qualities, noble metal particles are frequently utilized for antimicrobial surfaces on textiles and polymers fibers. Additionally, they are used to make surgical masks, equipment, catheters, and scaffolds for tissue engineering. Additionally, the development of effluent treatment membranes, water purification systems, and antimicrobial coatings all depend increasingly on the

antibacterial properties of noble metals [61, 62].

Fluorescent TAuAgGC made by Ganguly et al. were impregnated in cotton and paper to create luminous cotton and paper with antibacterial activity. Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains responded to the antibacterial properties of cotton wool and paper substrate. The luminous cotton wool and paper substrate were surrounded by transparent zones, as the disc diffusion test method showed. The number of Gram-positive and Gram-negative bacteria decreased on the cotton wool/paper treated with fluorescent particles. According to studies, the luminous cotton wool increased the survival of Gram-positive bacteria by 98.6% and decreased the vitality of Gram-negative bacteria by 99.91% after 3 h of incubation (Figure 4) [62].

## Cell Imaging

At the start of the 20th century, the businesses Carl Zeiss and Carl Reichert developed the first fluorescence microscopes. Fluorescence imaging has evolved into an important detection technology in biological and medical research because of its specific characteristics of non-invasiveness, high sensitivity, and real-time responsiveness. Many fluorescent materials, including metal nanoclusters, quantum dots, and carbon dots, have been established recently for bioimaging. Small organic molecules have also received a lot of interest in addition to these materials because of their good biocompatibility, controllable emission wavelengths, and flexible functionalization techniques [63, 64].

Live-cell imaging is an essential analytical technique in labs studying biological subjects like cell biology, neurology, pharmacology, and developmental biology. High light intensity and prolonged exposure durations, which are frequently required for imaging fixed cells and tissues (where photobleaching is a major concern), must be avoided while imaging active cells. In live-cell microscopy, there is often a trade-off between maintaining healthy cells and getting clear pictures. Therefore, to avoid high light intensity and prolonged exposure time, spatial and temporal resolutions are typically limited in an experiment. Living cells are imaged using several contrast-enhanced optical microscopy methods. The bulk of the study uses one of the several types of fluorescence microscopy, which is usually combined with transmitted light [63-65].

The literature is replete with findings on cell imaging using Au-Ag NCs. Due to their molecule-like optical characteristics, simplicity of production, and biocompatibility, fluorescent Au, and Ag NCs are attractive sensing materials [64].

In around 70 min, Ganguly et al. produced a highly fluorescent TAuAgGC via modified hydrothermolysis. This is an illustration of aggregation-induced fluorescence, which only manifested when the ratio of Ag to Au in the glutathione matrix is equal. The highly fluorescent solution with a pale-yellow tinge has shown potential as a competitor for bioimaging [60].

The viability of the synthesized TAuAgGC solution as a cell imaging agent was confirmed by optical microscopy research for live human fibroblast (foreskin origin) cells (passage-4) cells (Figure 5). It was seen that the clusters penetrated the cells and spread throughout the cytoplasm. It was clear that the distribution of the cluster was mostly in the cytoplasm. Therefore, the likelihood that the clusters will become mutagenic because of the oxidative stress in primary cells was drastically reduced. For several hours, the cells were seen to be unbleached. The emission bands of the Ag clusters and the nuclear stain DAPI did not significantly overlap in the cells' unique, healthy shapes and sizes. While performing comparable optical microscopy, a considerable shift in results

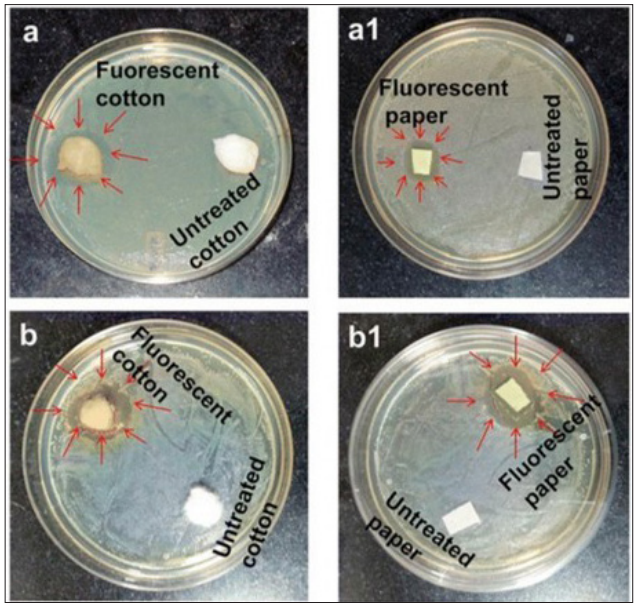



**Figure 4:** TAuAgGC-modified cotton and paper displayed antibacterial activity for Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) [62].

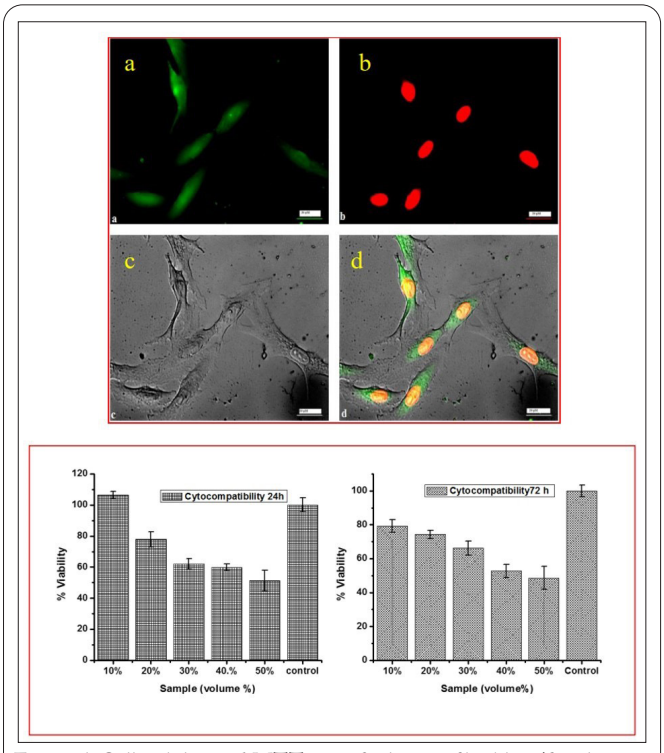



**Figure 5:** Cell viability and MTT assay for human fibroblast (foreskin origin) cells using TAuAgGC [60].

was noted [66].

## Cell Viability

All types of cell culture depend critically on the determination of cell viability. In other cases, such as toxicity tests, it serves as the experiment's primary goal. A more realistic image of, for example, anabolic activity can be obtained by using cell viability to connect cell behavior with cell number. There are several ways to determine how viable a cell is, from the most basic trypan blue dye exclusion assay to the more sophisticated study of individual cells utilizing Raman microscopy. The assay used will depend on several factors, including price, speed, and the complexity of the equipment needed [66].

The MTT test is still one of the most useful and well-liked viability assays that rely on living cells converting substrate into chromogenic product. The MTT test uses mitochondrial reductase to change the water-soluble yellow dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into an insoluble purple formazan. The concentration of the formazan is then evaluated by using optical density at 570 nm after it has been solubilized. The product is a very sensitive assay and linear up to 106 cells each well. Like the Alamar blue test, one can identify cell stress upon exposure to a harmful substance without experiencing direct cell death since modest changes in metabolic activity can result in significant changes in MTT [67, 68].

The MTT assay findings of TAuAgGC from the typical cell culture showed that the cells are considerably cytocompatibility (Figure 5). The material was proven to have more effectiveness viability than the control in a short-term trial (24 h), demonstrating the bioactive characteristic of the TAuAgGC. However, as the concentration was raised, the viability gradually decreased, with an $IC_{50}$ value of around 50%, which was five times higher than the concentration needed for an imaging investigation. In 72-h research, the vitality of the cells decreased to 79% at the appropriate concentration of the fluorescent particle. Additionally, it is shown that the $IC_{50}$ value increases to a 40% concentration. Consequently, the synthesized TAuAgGC was biocompatible [60].

## Conclusion and Future Perspective

This review article was designed for the first time regarding fluorescent giant clusters. The synthetic protocol, stability, origin of fluorescence, synergism, and applications (sensing, bioimaging, antibacterial activity, and cell viability) were illustrated vividly by employing fluorescence giant clusters. Au(I) remained at the core, while Ag(0) in the form of $Ag_2$ and $Ag_3$ were ascribed for the long-term strong fluorescence of giant clusters. This paper will hopefully be an important account of cluster chemistry, especially for the choice of capping agent. Here Au also capped Ag along with organic stabilizing agents. The versatile applications of giant clusters are highly promising and may be an asset for researchers, venturing into the field of environmental nanoscience to cope up with real environmental challenges.

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## Conflict of Interest

There is no conflict of interest.

### References

1. Sim S, Kui N, Wong NK. 2021. Nanotechnology and its use in imaging and drug delivery (Review). *Biomed Rep* 14(5): 42. https://doi.org/10.3892/br.2021.1418
2. Ray PC, Yu H, Fu PP. 2009. Toxicity and environmental risks of nanomaterials: challenges and future needs. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 27: 1-35. https://doi.org/10.1080/10590500802708267
3. Zhang P, Wyman I, Hu J, Lin S, Zhong Z, et al. 2017. Silver nanowires: synthesis technologies, growth mechanism and multifunctional applications. *Mater Sci Eng B* 223: 1-23. https://doi.org/10.1016/j.mseb.2017.05.002
4. Du Y, Sheng H, Astruc D, Manzhou Z. 2020. Atomically precise noble metal nanoclusters as efficient catalysts: a bridge between structure and properties. *Chem Rev* 120: 526-622. https://doi.org/10.1021/acs.chemrev.8b00726
5. Cheng-An J, Lin CA, Chih-Hsien L, Jyun-Tai H. 2009. Synthesis of fluorescent metallic nanoclusters toward biomedical application: recent progress and present challenges. *J Med Biol Eng* 29: 276-283.
6. Chatterjee DP, Pakhira M, Nandi AK. 2020. Fluorescence in "non-fluorescent" polymers. *ACS Omega* 5(48): 30747-30766. https://doi.org/10.1021/acsomega.0c04700
7. Zhang H, Zheng X, Xie N, He Z, Liu, et al. 2017. Why do simple molecules with "isolated" phenyl rings emit visible light? *J Am Chem Soc* 139(45): 16264-16272. https://doi.org/10.1021/jacs.7b08592
8. Jeong Y, Kook YM, Lee K, Koh WG. 2018. Metal enhanced fluorescence (MEF) for biosensors: general approaches and a review of recent developments. *Biosens Bioelectron* 111: 102-116. https://doi.org/10.1016/j.bios.2018.04.007
9. Patil RR. 2014. Urbanization as a determinant of health: a socioepidemiological perspective. *Soc Work Public Health* 29(4): 335-341. https://doi.org/10.1080/19371918.2013.821360
10. Zhong X, Li Z, Shi R, Yan L, Zhu Y, et al. 2022. Schiff base-modified nanomaterials for ion detection: a review. *ACS Appl Nano Mater* 5: 13998-14020. https://doi.org/10.1021/acsanm.2c03477
11. Kim Y, Johnson RC, Hupp JT. 2001. Gold nanoparticle-based sensing of "spectroscopically silent" heavy metal ions. *Nano Lett* 4: 165-167. https://doi.org/10.1021/nl0100116
12. Alissa EM, Ferns GA. 2011. Heavy metal poisoning and cardiovascular disease. *J Toxicol* 2011: 870125. https://doi.org/10.1155/2011/870125
13. Vardhan KH, Kumar PS, Panda RC. 2019. A review on heavy metal pollution, toxicity and remedial measures: current trends and future perspectives. *J Mol Liq* 290: 111197. https://doi.org/10.1016/j.molliq.2019.111197
14. Lahoti HS, Jogdand SD, Lahoti H. 2022. Bioimaging: evolution, significance, and deficit. *Cureus* 14(9): e28923. https://doi.org/10.7759/cureus.28923
15. Kang X, Zhu M. 2019. Tailoring the photoluminescence of atomically precise nanoclusters. *Chem Soc Rev* 48: 2422-2457. https://doi.org/10.1039/c8cs00800k

16. Tao Y, Li M, Ren J, Qu X. 2015. Metal nanoclusters: novel probes for diagnostic and therapeutic applications. *Chem Soc Rev* 44: 8636-8663. https://doi.org/10.1039/c5cs00607d

17. Looij SMV, Hebels ER, Viola M, Hembury M, Oliveira S, et al. 2022. Gold nanoclusters: imaging, therapy, and theranostic roles in biomedical applications. *Bioconjug Chem* 33: 4-23. https://doi.org/10.1021/acs.bioconjchem.1c00475

18. Yau SH, Varnavski O, Goodson III. 2013. An ultrafast look at Au nanoclusters. *Acc Chem Res* 7: 1506-1516. https://doi.org/10.1021/ar300280w

19. Ganguly M, Jana J, Pal A, Pal T. 2016. Synergism of gold and silver invites enhanced fluorescence for practical application. *RSC Adv* 6**:** 17683-17703. https://doi.org/10.1039/c5ra26430h

20. Link S, Wang ZL, El-Sayed MA. 1999. Alloy formation of gold–silver nanoparticles and the dependence of the plasmon absorption on their composition. *J Phys Chem B* 103: 3529-3533. https://doi.org/10.1021/jp990387w

21. Mahl D, Diendorf J, Ristig S, Greulich C, Li ZA, et al. 2012. Silver, gold, and alloyed silver–gold nanoparticles: characterization and comparative cell-biologic action. *J Nanopart Res* 14: 1153. https://doi.org/10.1007/s11051-012-1153-5

22. Tiedemann D, Taylor U, Rehbock C, Jakobi, S. Klein, et al. 2014. Reprotoxicity of gold, silver, and gold–silver alloy nanoparticles on mammalian gametes. *Analyst* 139: 931-942. https://doi.org/10.1039/c3an01463k

23. Nasser D, Ferreiro-Rangel C, Sweatman B, 2022. Molecular dynamics investigation of giant clustering in small-molecule solutions: the case of aqueous PEHA. *J Phys Chem B* 126: 8882-8891. https://doi.org/10.1021/acs.jpcb.2c04489

24. Ganguly M, Jana J, Mondal C, Pal A, Pal T. 2014. Green synthesis of highly fluorescent Au(I)@$Ag_2$/$Ag_3$-thiolate core-shell particles for selective detection of cysteine and Pb(II). *Phys Chem Chem Phys* 16: 18185. https://doi.org/10.1039/c4cp01782j

25. Ganguly M, Pal A, Negishi Y, Pal T. 2013. Synthesis of highly fluorescent silver clusters on gold(I) surface. *Langmuir* 29: 2033-2043. https://doi.org/10.1021/la304835p

26. Gehin A, Ferlay S, Harrowfield JM, Fenske D, Kyritsakas N, et al. 2012. Giant core–shell nanospherical clusters composed of 32 Co or 32 Ni atoms held by 6 p-tert-Butylthiacalix[4]arene units. *Inorg Chem* 51: 5481-5486. https://doi.org/10.1021/ic300550v

27. Jing Q, Cao HB, Ge GX, Wang YX, Yan HX, et al. 2011. Giant magnetic moment of the core-shell $Co_{13}$@$Mn_{20}$ clusters: first-principles calculations. *J Comput Chem* 32: 2474-2478. https://doi.org/10.1002/jcc.21831

28. Ganguly M, Mondal C, Pal J, Pal A, Negishic Y, et al. 2014. Fluorescent Au(I)@$Ag_2$/$Ag_3$ giant cluster for selective sensing of mercury(II) ion. *Dalton Trans* 43: 11557-11565. https://doi.org/10.1039/c4dt01158a

29. Hu X, Guo F. 2021. Amino acid sensing in metabolic homeostasis and health. *Endocr Rev* 42: 56-76. https://doi.org/10.1210/endrev/bnaa026

30. Dato S, Hoxha E, Crocco P, Iannone F, Passarino G, et al. 2019. Amino acids and amino acid sensing: implication for aging and diseases. *Biogerontology* 20: 17-31. https://doi.org/10.1007/s10522-018-9770-8

31. Wood ZA, Schroder E, Harris JR, Poole LB. 2003. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 28: 32-40. https://doi.org/10.1016/s0968-0004(02)00003-8

32. Joy T, Rao MS, Madhyastha S. 2018. N-Acetyl cysteine supplement minimize tau expression and neuronal loss in animal model of Alzheimer's disease. *Brain Sci* 8: 185. https://doi.org/10.3390/brainsci8100185

33. Carmel R, Jacobsen DW. 2001. Homocysteine in Health and Disease. Cambridge University Press.

34. Pérez-Ruiz T, Martínez-Lozano C, Tomás V, Casajús R. 1995. Flow injection fluorimetric determination of thiourea. *Talanta* 42(3): 391-394. https://doi.org/10.1016/0039-9140(95)01421-7

35. Dişbudak A, Bektaş S, Patır S, Genç O, Denizli A. 2002. Cysteine-metal affinity chromatography: determination of heavy metal adsorption properties. *Sep Purif Technol* 26: 273-281. https://doi.org/10.1016/S1383-5866(01)00181-2

36. Joshi DR, Adhikari N. 2019. An overview on common organic solvents and their toxicity. *Int J Pharm Res* 28: 1-18. https://doi.org/10.9734/jpri/2019/v28i330203

37. Costa LG, Aschner M. 2014. Organic Solvents. In Aminoff MJ, Daroff RB (eds) Encyclopedia Neurological Sciences (Second Edition). Academic Press, pp 694-695.

38. Mora SJ, Lee P, Shooter D, Eschenbruch R. 1993. The analysis and importance of dimethylsulfoxide in wine. *Am J Enol Vitic* 44: 327-332. https://doi.org/10.5344/ajev.1993.44.3.327

39. Pearson TW, Dawson HJ, Lackey HB. 1981. Naturally occurring levels of dimethyl sulfoxide in selected fruits, vegetables, grains, and beverage. *J Agric Food Chem* 29: 1089-1091. https://doi.org/10.1021/jf00107a049

40. Simo R, Malin G, Liss PS. 1998. Refinement of the borohydride reduction method for trace analysis of dissolved and particulate dimethyl sulfoxide in marine water samples. *Anal Chem* 70: 4864-4867. https://doi.org/10.1021/ac980345o

41. Ui K, Abo M, Okubo A, Yamazaki S. 2004. An improved method for the analysis of dimethyl sulfoxide in water samples. *Anal Sci* 20: 223-225. https://doi.org/10.2116/analsci.20.223

42. Abo M, Ogasawara Y, Tanaka Y, Okubo A, Yamazaki S, et al. 2003: Dimethyl sulfoxide sensor using dimethyl sulfoxide reductase from *Rhodobacter sphaeroides*. *Biosens Bioelectron* 18: 735-739. https://doi.org/10.1016/S0956-5663(03)00043-5

43. Yonehara H, Fujii SI, Sato K, Abo M, Yoshimura. 2004. Construction of a dimethyl sulfoxide sensor based on dimethyl sulfoxide reductase immobilized on a au film electrode. *Anal Sci* 23: 55-58. https://doi.org/10.2116/analsci.23.55

44. Pirsa S, Alizadeh N. 2012. A selective DMSO gas sensor based on nanostructured conducting polypyrrole doped with sulfonate anion. *Sens Actuators B Chem* 68: 303-309. https://doi.org/10.1016/j.snb.2012.04.027

45. Ganguly M, Mondal C, Jana J, Pal A, Pal T. 2014. Photoproduced fluorescent Au(I)@($Ag_2$/$Ag_3$)-thiolate giant cluster: an intriguing sensing platform for DMSO and Pb(II). *Langmuir* 30: 348-357. https://doi.org/10.1021/la403848z

46. Hawkes SJ. 1997. What is a "heavy metal"? *J Chem Educ* 74: 1374. https://doi.org/10.1021/ed074p1374

47. Kaur J, Sengupta P, Mukhopadhyay S. 2022. Critical review of bioadsorption on modified cellulose and removal of divalent heavy metals (Cd, Pb, and Cu). *Ind Eng Chem Res* 61:1921-1954. https://doi.org/10.1021/acs.iecr.1c04583

48. Shrivastava P, Jain VK, Nagpal S. 2022. Nanoparticle intervention for heavy metal detection: a review. *Environ Nanotechnol Monit Manage* 17: 100667. https://doi.org/10.1016/j.enmm.2022.100667

49. Xu X, Yang S, Wang Y, Qian K. 2000. Nanomaterial-based sensors and strategies for heavy metal ion detection. *Green Anal Chem* 2: 100020. https://doi.org/10.1016/j.greeac.2022.100020

50. Lin-Fu JS. 1992. Modern History of Lead Poisoning: A Century of Discovery and Rediscovery. In Needleman HL (ed) Human Lead Exposure. CRC Press, pp 23-43.

51. How to Prevent Lead Poisoning in Children. [https://www.cdc.gov/nceh/lead/docs/how-to-prevent-lead-poisoning-in-children.html] [Accessed December 27, 2023]

52. Boening DW. 2000. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere* 40: 1335-1351. https://doi.org/10.1016/s0045-6535(99)00283-0

53. Harris HH, Pickering IJ, George GN. 2003. The chemical form of mercury in fish. *Science* 301: 1203-1203. https://doi.org/10.1126/science.1085941

54. Beer PD, Bayly SR. 2005. Anion Sensing by Metal-based Receptors. In Stibor I (ed) Anion Sensing. Topics in Current Chemistry. Springer, Berlin, pp 125-162.
55. Lee KY, Kim DW, Heo J, Kim JS, Yang J, et al. 2006. Novel colorimetric sensing of anion with gold nanoparticles-embedded plasticised polymer membrane. *Bull Korean Chem Soc* 27(12): 2081. https://doi.org/10.5012/bkcs.2006.27.12.2081
56. Gore AH, Vatre SB, Anbhule PV, Han SH, Patila SR, et al. 2013. Direct detection of sulfide ions [$S^{2-}$] in aqueous media based on fluorescence quenching of functionalized CdS QDs at trace levels: analytical applications to environmental analysis. *Analyst* 138: 1329-1333. https://doi.org/10.1039/c3an36825d
57. Yang XF, Wang LP, Xu HM, Zhao ML. 2009. A fluorescein-based fluorogenic and chromogenic chemodosimeter for the sensitive detection of sulfide anion in aqueous solution. *Anal Chim Acta* 631: 91-95. https://doi.org/10.1016/j.aca.2008.10.037
58. Wang ZX, Zheng CL, Ding SN. 2014. Label-free detection of sulfide ions based on fluorescence quenching of unmodified core–shell Au@Ag nanoclusters. *RSC Adv* 4: 9825-9829. https://doi.org/10.1039/c3ra45019h
59. Choi MM. 1998. Fluorimetric optode membrane for sulfide detection. *Analyst* 123**:** 1631-1634. https://doi.org/10.1039/a801702f
60. Sun H, Lu D, Xian M, Dong C, Shuang S. 2016. A lysozyme-stabilized silver nanocluster fluorescent probe for the detection of sulfide ions. *Anal Methods* 8(22): 4328-4333. https://doi.org/10.1039/c6ay00300a
61. Lu Z, Rong K, Ju Li, Yang H, Chen R. 2013. Size-dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria. *J Mater Sci Mater Med* 24: 1465-1471. https://doi.org/10.1007/s10856-013-4894-5
62. Ogunsona EO, Muthuraj R, Ojogbo E, Valerio O, Mekonnen TH. 2020. Engineered nanomaterials for antimicrobial applications: a review. *Appl Mater Today* 18: 100473. https://doi.org/10.1016/j.apmt.2019.100473
63. Wang S, Zhou K, Lyu X, Li H, Qiu X, et al. 2023. The bioimaging story of AIEgens. *Chem Biomed Imaging* 1(6): 509-521. https://doi.org/10.1021/cbmi.3c00056
64. Ellen CJ. 2013. Overview of live-cell imaging: requirements and methods used. *Anat Rec* 296(1): 1-8. https://doi.org/10.1002/ar.22554
65. Shiang YC, Huang CC, Chen WY, Chen PC, Chang HT. 2012. Fluorescent gold and silver nanoclusters for the analysis of biopolymers and cell imaging. *J Mater Chem* 22**:** 12972-12982. https://doi.org/10.1039/c2jm30563a
66. Stoddart MJ. 2011. Cell Viability Assays: Introduction. In Stoddart MJ (ed) Mammalian Cell Viability. Methods in Molecular Biology. Humana Press, pp 1-6.
67. Kumar P, Nagarajan A, Uchil PD. 2018. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc* 2018(6): 1-4. https://doi.org/10.1101/pdb.prot095505
68. Ghasemi M, Turnbull T, Sebastian S, Ivan K. 2021. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *Int J Mol Sci* 22: 12827. https://doi.org/10.3390/ijms222312827