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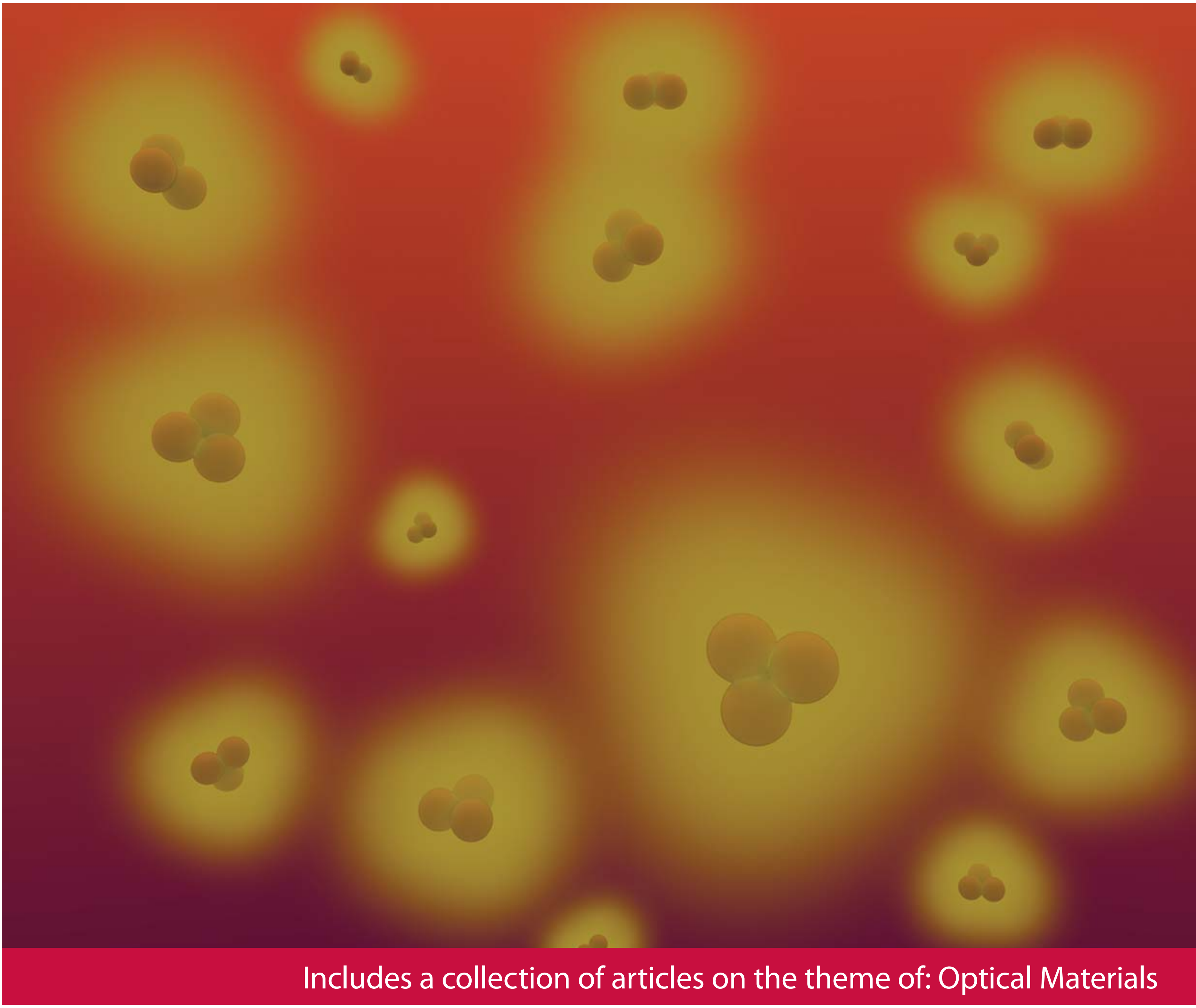

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MINIREVIEW

Fluorescent silver nanoclusters

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Silver nanoclusters are a class of fluorophores with attractive features, including brightness, photostability and subnanometer size. In this review we overview the different scaffolds that are used as stabilizer for silver nanoclusters (*e.g.* polymers, dendrimers, DNA oligomers, cryogenic noble gas matrixes, inorganic glasses, zeolites and nanoparticles), and we briefly discuss the recent advances.

Introduction

The electronic and optical properties of metals depend greatly on their size, in particular in the nanometre range (Fig. 1). Bulk metals are electrically conducting and good optical reflectors, as caused by the sea of freely moving delocalized electrons in the conduction band. On the other hand, metal nanoparticles display intense colors due to surface plasmon resonance, a feature attributed to the collective oscillation of conduction electrons upon interaction with light. When the size of metals is further reduced to around 1 nm or less, down to a few atoms, the band structure becomes discontinuous and is broken down into discrete energy levels, somewhat similar as the energy levels of molecules. Therefore these metal nanoclusters are said to have molecule-like properties and do not anymore exhibit plasmonic properties. They represent the missing link between metal atoms and nanoparticles. Nonetheless, metal nanoclusters can still interact with light *via* electronic transitions between energy levels, resulting in intense light absorption and emission.[1–5]

Without stabilization, metal nanoclusters would strongly interact with each other and aggregate irreversibly as to reduce their surface energy. A proper stabilizing scaffold is thus indispensable. A scaffold that allowed detailed spectroscopic study of few-atom metal nanoclusters already in the 1970s was the cryogenic noble gas matrix. Major pioneering contributions on matrix-isolated metal nanoclusters were done by the group of Ozin[6] and the group of Ertl.[7] Also zeolites as scaffolds for nanoclusters have been studied for decades, originally in part for their catalytic potential,[8] and recently for optical encoding of data.[9]

Organic scaffolds for fluorescent silver nanoclusters are relatively new, first reported by the group of Dickson in 2002.[10] These organic scaffolds hold big potential, as the interaction between the ligands and nanoclusters can be adjusted to finely

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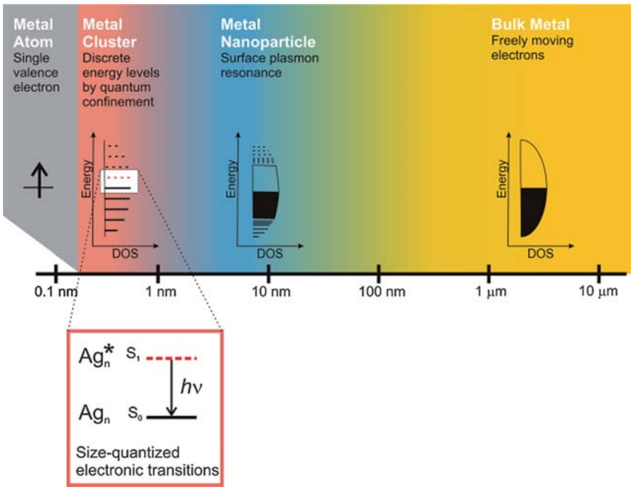


Fig. 1 The effect of size on metals. Whereas bulk metal and metal nanoparticles have a continuous band of energy levels, the limited number of atoms in metal nanoclusters results in discrete energy levels, allowing interaction with light by electronic transitions between energy levels. Metal nanoclusters bridge the gap between single atoms and nanoparticles. Reprinted from ref. 3 with permission of Springer Science + Business Media.

tune the spectroscopic properties. For example, by playing with the nucleotide sequence of DNA oligomers it is possible to synthesize silver nanoclusters that emit from the blue to near-infrared region.[11] These silver nanoclusters can become applicable as fluorescent biolabels, because of their biocompatibility, brightness and photostability.[12] Furthermore, the nanoclusters can act as sensitive probes, as their optical response is highly dependent on the interaction with the organic scaffold. It is demonstrated that the nanoclusters can sense small molecules (*e.g.* cysteine),[13] solvents[14] and even the single nucleotide mutation causing sickle cell anemia.[15]

The most common fluorophores in fluorescence applications are organic dyes and semiconductor quantum dots, and both have their own strengths and weaknesses. For example, organic dyes usually suffer from a relatively poor photostability, in contrast to the semiconductor quantum dots. Nevertheless, the semiconductor quantum dots are relatively large (10–20 nm), which makes them cumbersome as a fluorescent label attached to molecules or few-nanometre-sized proteins, and they often contain toxic elements, which may compromise their use for *in vivo* applications. Silver nanoclusters have an appealing set of features that complements the properties of organic dyes and semiconductor quantum dots. They are reported to have excellent photostability, have subnanometre size, are non-toxic and thus suit well as a fluorescent probe for biological applications.[12,21,22]

1. Silver nanoclusters in organic scaffolds

The formation and stabilization of silver nanoclusters in solution have been accomplished in various ways (Fig. 2). The proper choice of parameters for the reaction, including the temperature, the reducing method, the stabilizers and the initial ratio of silver : stabilizer, plays a crucial role for the successful synthesis of silver nanoclusters. Small variations in the synthetic procedure may lead to the formation of large non-fluorescent plasmonic silver nanoparticles. Despite the many reports of the last few years about the synthesis of silver nanoclusters, it remains difficult to design general rules for the synthesis of silver nanoclusters, as similar reagents and reaction conditions may lead to silver nanoparticles instead of nanoclusters.

The activation of the reaction to produce silver nanoclusters can be performed following several routes. The silver ions from dissolved silver salts can be reduced, either by a chemical reductant (*e.g.* sodium borohydride, sodium hypophosphite), by light (photoreduction with visible or ultraviolet light) or by γ-rays (by radiolysis of water). The chemical reduction and the photoreduction are the most commonly used methods and will be discussed here. The radiolytic method was used to produce silver nanoclusters in solution, but luminescence was not reported.[23,24]

It is well known that the specific properties of silver nanoclusters, such as the stability and fluorescence quantum yield, depend largely on the scaffold used during reduction. The largest fluorescence quantum yield reported for silver nanoclusters prepared in organic scaffolds is achieved using DNA (quantum yield of 64%),[25] while polyacrylates provide stable solutions over years.[14] The sensitivity of silver nanoclusters to the local environment can be exploited for sensing purposes, though it represents a difficulty when designing the synthesis, the proper recipe for the production of silver nanoclusters with a certain scaffold might not be useful when using a different scaffold.

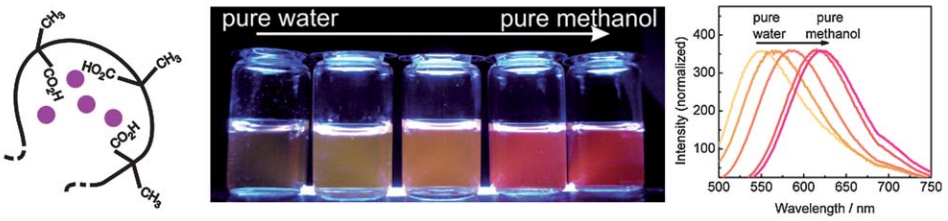
For gold nanoclusters, the ligands play a major role in enhancing the fluorescence of the nanoclusters in two different ways, by charge transfer from the ligands to the gold core through direct bonding or by direct donation of delocalized electrons of electron-rich atoms or groups of the ligands to the gold core.[26] In the case of silver, the role of ligands or scaffolds on the fluorescence properties are not yet understood.

In this section we will summarize the most relevant procedures and results achieved in the last decade organized by the scaffold/stabilizer used during synthesis. A suitable scaffold for the synthesis of silver nanoclusters typically allows multiple and strong interactions with silver ions by complexation or by ionic bonds. Special care is needed to avoid aggregation, since all the suitable scaffolds can also be used for the nucleation and growth of large non-fluorescent silver nanoparticles, for example in acrylates,[27,28] dendrimers[29] or DNA.[30] To limit the size to few-atom nanoclusters, proper scaffolds thus must be combined with suitable concentrations and with appropriate reduction methods.

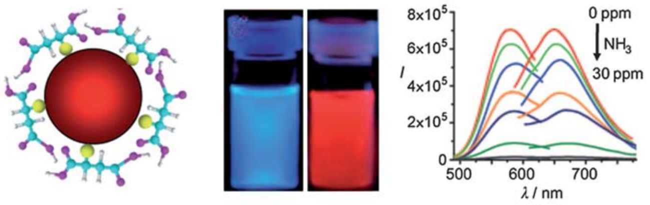
1a. Dendrimers, polymers and short molecules

Dickson and coworkers reported the first stable aqueous solution of fluorescent silver nanoclusters in 2002.[10] An OH-terminated dendrimer was used as scaffold. The dendrimer, a second-generation OH-terminated poly(amidoamine) with a large excess of OH-groups with respect to silver ions (OH : Ag ~ 1 : 0.2) hosts a small amount of silver ions during photoreduction with blue light and prevents further growth of the formed nanoclusters. The nanoclusters in the OH-terminated dendrimer exhibit distinct fluorescence peaks ranging from 533 to 648 nm.

(a) Stabilized with polymers



(b) Stabilized with short molecules



(c) Stabilized with DNA oligonucleotides

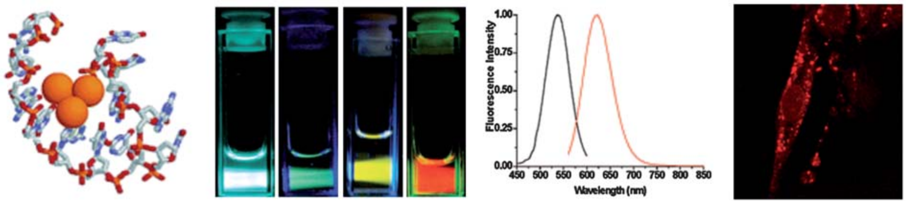


Fig. 2 Fluorescent silver nanoclusters, their organic scaffolds, properties and applications. (a) Schematic drawing of silver nanoclusters protected by carboxyl groups of poly(methacrylic acid). Photograph under UV-light of samples in water/methanol mixtures, from pure water on the left to pure methanol on the right. Emission spectra of the samples imaged. Reproduced from ref. 14, 16 with permission of Wiley-VCH Verlag GmbH & Co. KgaA. (b) Silver nanoclusters prepared by interfacial etching from silver nanoparticles and stabilized with small molecules (*i.e.* mercaptosuccinic acid). Picture of samples under UV-light. Fluorescence quenching by addition of NH_3. Reprinted from ref. 17 with permission of Royal Society of Chemistry and from ref. 18 with permission of Wiley-VCH Verlag GmbH & Co. KgaA. (c) Representation of silver nanoclusters encapsulated in DNA oligonucleotides. Photographs under UV-light of samples with different oligonucleotides and hence different emitters. Emission spectra of the last sample, showing red emitters. Confocal fluorescence microscopic image of live cells incubated with (anti-heparin sulfate)-(DNA oligonucleotides)-(silver nanoclusters). Reprinted from ref. 11, 19 with permission of American Chemical Society and from ref. 20 with permission of John Wiley & Sons.

The authors mentioned that the nanoclusters could be suitable labels to study biological systems.[10]

Kumacheva *et al.* used poly(*N*-isopropylacrylamide-acrylic acid-2-hydroxyethyl acrylate) microgel particles as a scaffold to form fluorescent silver nanoclusters. The microgel particles contain not only OH-groups but also COOH-groups. However, the authors claim that only the acrylates are critical for the formation of nanoclusters. Additionally, the scaffold exhibits stimuli-responsive volume changes under variation of external factors such as temperature and pH, facilitating the application of nanoclusters. The nanoclusters were prepared with UV-irradiation in molar ratio COOH : Ag of 1 : 1.[31] Acrylates were already used for the synthesis of small silver nanoclusters by γ-irradiation but no fluorescence was reported.[24]

Claiming that a high density of COOH-groups benefits the formation of fluorescent silver nanoclusters compared with short linear polymers, a star-like polymer with 36 arms, polyglycerol-*block*-poly(acrylic acid), was later used to prepare silver nanoclusters under similar conditions.[32] Nevertheless, also linear polyacrylates such as poly(methacrylic acid) can act as an excellent scaffold for the preparation of silver nanoclusters in water solution, by photoreduction with visible light,[14] UV-light[33] or sonochemically[34] (Fig. 2a). Molar ratios COOH : Ag can be very flexible, 1 : 0.15–1 : 3, when using visible light as reported by Ras *et al.*[14] Poly(methacrylic acid) stabilized nanoclusters have an excellent stability (no visible changes for 3-year-old samples),[14] a high quantum yield (~18.6%)[33] and can be transferred to other scaffolds[14,35,36] or solvents[16] and can sense the local environment.[14] The sonochemical process was as well successfully used with scaffolds as poly(acrylic acid) and poly(acrylic acid-*co*-maleic acid).[34] Silver nanoclusters formed in acrylates present in general a single emission band centered in the region from about 550 nm to 650 nm. Silver nanoclusters prepared in poly(methacrylic acid) exhibit electrochemiluminescence, also called electrogenerated chemiluminescence.[14]

In addition to polymers, also small molecules containing both carboxylic groups or thiols have been used to stabilize silver nanoclusters in solution. In water solution and by reduction with sodium borohydride, scaffolds as captopril and glutathione[37] or dihydrolipoic acid[38] lead to silver nanoclusters with weak fluorescence. When using dihydrolipoic acid the molar ratio thiol : Ag was 20 : 1 and the Stokes shift was very large (>200 nm). These nanoclusters can be used for sensing purposes since the fluorescence is quenched by particle aggregation mediated through the Hg(II). With mercaptosuccinic acid as scaffold, Ag_7 and Ag_8 nanoclusters were produced by etching of large silver nanoparticles at the interface of several aqueous/organic biphasic systems. In this latter case, the fluorescence quantum yield could be increased up to 9% with decreasing temperature[18] (Fig. 2b). The nanoclusters present two emission bands at 650 and 440 nm corresponding to Ag_8 and Ag_7 respectively. Also Ag_9 silver nanoclusters could be prepared in the absence of solvent, using a solid-state route.[39] First silver nitrate powder was mixed and ground with mercaptosuccinic acid powder. Subsequently, sodium borohydride powder was added followed by grinding. The clusters formed in the solid state and could be purified by polyacrylamide gel electrophoresis.

The sensitivity of silver nanoclusters to their chemical environment reveals in absorption[40] as well as in fluorescence properties, for example when prepared in poly(methacrylic acid) clusters are sensitive to solvents,[14,16,41] to cysteine[13] and to Cu(II) ions,[42] whereas the ones formed in thiols can sense Hg(II) ions.[38]

1b. DNA, proteins and peptides as scaffolds for silver nanoclusters

DNA oligonucleotides are able to bind silver ions, preferentially the heterocyclic bases and the single-stranded DNA over the phosphates and the double-stranded DNA. For the formation of silver nanoclusters various oligonucleotide conformations have been used, such as linear, or with the shape of a hairpin or a loop.[43,44] Linear oligonucleotides, mainly consisting of 12 bases, were found to be excellent scaffolds for the formation of emissive silver nanoclusters by sodium borohydride reduction of solutions with molar ratio bases : Ag 2 : 1.[11,19,45,46]

Time-dependent measurements and spectra recorded at various excitation wavelengths reveal the presence of distinct emitters. It has been proven as well that silver nanoclusters are very sensitive to the local environment, such as the various DNA bases, the discrete base sequence or the length of the oligonucleotide leading to particular emission properties ranging from visible to near-IR (Fig. 2c).[11,12,44,47,48] We describe shortly the effects of these parameters.

Regarding the selection of the bases, ^1H NMR spectra and theoretical studies[49] indicate that silver has a higher affinity for cytosine bases compared to other bases, and mass spectra[44] show comparable affinities of silver for cytosine and guanine, encouraging researchers to use oligonucleotides with high content of cytosine bases. Silver nanoclusters templated in linear 12-mer cytosine oligonucleotide produce four emitters with fluorescence quantum yield up to 17%.[46] Substitution of cytosines by thymines results in a smaller number of emitters. Interestingly chemical conversion between emitters can occur by oxidation or reduction.[46,48] Fully reduced nanoclusters with red emission could react with O_2 to form oxidized nanoclusters with blue/green emission. The reverse reaction, a chemical reduction of the blue/green emitters, is possible using $NaBH_4$ and results in red-emissive nanoclusters.[46,48] The base sequence tuning results in distinct emission properties, that is different emission wavelengths through the visible spectrum till the near-IR and different fluorescence quantum yields, the highest determined being 37%.[11] With longer oligonucleotides, also distinct emitters can be produced and fluorescence quantum yields of 64% can be achieved.[25]

The high fluorescence quantum yield and the photostability make silver nanoclusters good candidates as fluorophores for biological labeling. The application, however, requires that the nanoclusters can be functionalized to enable their attachment to biological molecules, like proteins. A good example of such functionalization of silver nanoclusters is demonstrated by Dickson *et al.* using a thiolated 24-mer oligocytosine.[20] *Via* maleimide coupling, the DNA strands could be linked to proteins (*e.g.* avidin). Staining of biotinylated fixed cells with the conjugate avidin–DNA–silver nanoclusters produces fluorescent spots in the cells possibly resulting from endocytosis.[20] Silver nanoclusters can also be prepared first in the DNA oligonucleotides which are later complexed with a transfection reagent (lipofectamine) to facilitate internalization of the silver nanoclusters.[50] Alternatively, one can prepare the silver nanoclusters in poly(acrylic acid) and then transfer them to a DNA oligonucleotide linked to antibodies.[35] A significant advantage of silver nanoclusters as fluorescent marker is that their small size likely causes minimal perturbance to the protein to which they are attached. Furthermore, silver nanoclusters exhibit no significant toxicity according to tests using HeLa cells.[50]

The specific detection of DNA targets is possible with a DNA detection probe that "lights up" upon target binding. For instance, red fluorescence of DNA–Ag nanoclusters can be enhanced 500-fold when placed in proximity to guanine-rich DNA sequences.[51] Thus, guanine proximity can trigger reversible transformation of silver nanoclusters between a dark species and a bright red-emitting species. Other sensing applications of silver nanoclusters in DNA are the recognition of single nucleotide mutations in DNA[15] or the detection of copper ions[52] and mercury ions by fluorescence quenching.[53]

Regarding investigations to fluorescently stain proteins with silver nanoclusters, there are several possibilities due to the affinity of silver for proteins. One is to produce silver nanoclusters by photoactivation using lower concentration of silver ions than compared to the traditional staining procedure in fixed cells with silver particles. The silver nanoclusters here show a broad emission band between 500 and 700 nm.[54] In a different approach, an enzyme, bovine pancreatic α-chymotrypsin (CHT), was used as template during chemical reduction of silver ions (molar ratio CHT : Ag of 1 : 10) with sodium borohydride. The emission of silver nanoclusters stable in solution occurs at 680 nm.[55] Another possibility consists of preparing the silver nanoclusters by photoreduction in the presence of the fluorophore thioflavin T (ThT) (molar ratio ThT : Ag of 1 : 1). The intense emission at 450 nm, 50-fold stronger than the fluorescence of pure ThT, is explained as a combination of the emission of silver nanoclusters templated by ThT and a metal-enhanced fluorescence of ThT. Amyloid proteins stained with fluorescent ThT-silver nanoclusters present 100-fold stronger luminescence than reported for silver nanoclusters in dendrimers.[56]

Synthetic oligopeptides of 9–18 amino acids can template the synthesis of silver nanoclusters prepared by reduction of silver ions with sodium borohydride.[54,57] The nanoclusters produce weak fluorescence (fluorescence quantum yield of 3%) at 630 nm when excited at 400 nm. Several oligopeptides with different compositions were used as templates producing silver nanoclusters with distinct optical properties. However, the relation between the template structure and optical properties is not yet clear. These nanoclusters can be evenly loaded in living cells whereas in fixed cells strong staining in the nuclear region occurs.[54,57] A dipeptide-based supramolecular hydrogel can serve also as template to synthesize silver nanoclusters by using sunlight. Each gelator peptide has two free COOH groups that can complex with silver ions. The nanoclusters remain attached to the larger gel fibres of average diameter 50 nm which form a three-dimensional nanofibrillar network.[58]

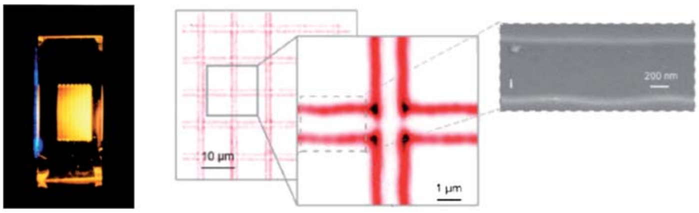
2. Silver nanoclusters in inorganic scaffolds

Inorganic scaffolds were the first type of stabilizers available for silver nanoclusters. Under suitable conditions they can produce nanoclusters of a well-defined number of atoms, and they can cover a wide emission wavelength range from UV to near-IR.

Matrix-isolation is a method to produce few-atom ligand-free metal nanoclusters, and since the early work by Ozin in the 1970s, it has provided lots of insight in the photophysical properties of oligomeric silver and was used to verify the outcome of computational studies.[6,59,60] In matrix-isolation, the silver atoms are thermally evaporated and transported by a flow of noble gas (*e.g.* Ar) followed by their deposition on a cooled transparent substrate, like quartz. The cooling of the gas at the cold walls leads to nucleation and growth of the nanoclusters. Parameters that allow control of the cluster size distribution include the gas-to-metal ratio and the temperature of the substrate. At temperatures approaching 50 K, silver atoms are able to diffuse throughout the Ar matrix to form nanoclusters, whereas at lower temperatures clustering is limited to the surface of the growing matrix layer.[63] Alternatively, the bulk diffusion and subsequent cluster formation can also be induced by light.[6] The group of Ertl reported that the aggregation of silver atoms to nanoclusters is accompanied with emission of light (=chemiluminescence).[7] Although cryogenic matrix-isolation is a good tool to study metal nanoclusters, it is not suitable for applications in ambient conditions.

Inorganic glasses provide a solid matrix that stabilizes silver nanoclusters by immobilization, and thus preventing their tendency to aggregate to large nanoparticles (Fig. 3a). In general the process to make the silver nanoclusters in a glass consists of two steps. First a Ag^+-doped glass is prepared, either by melting $AgNO_3$ together with the other raw materials, or by immersion of a glass slide in a molten salt bath containing the silver salt, so that Ag^+ replaces Na^+ in an ion exchange reaction, or by ion implantation. In the second step the Ag^+-doped glass is activated to form the fluorescent nanoclusters. Activation is achieved by laser irradiation,[61,62] synchrotron irradiation,[64] thermal annealing or controlled quenching of the melt.[65] The nanoclusters are reported to be very stable against photobleaching, have short lifetimes and have a high quantum yield up to 20%. The glass matrix is transparent and therefore these fluorescent glasses could find applications as optical components in the form of thin films and fibers. Furthermore, direct laser writing on the Ag^+-doped glass enables fabrication of 3D fluorescent structures at the nanometre scale opening up applications in optical data storage and nanophotonics (Fig. 3b).[62,66] The recorded images do not photobleach and are claimed to have temperature, ageing, and humidity tolerance, making silver cluster glasses suitable as a perennial storage medium over many centuries.[66]

(a) Stabilized in inorganic glasses



(b) Stabilized in zeolites

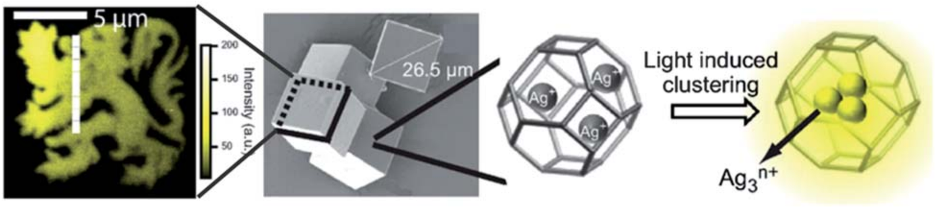


Fig. 3 Silver nanoclusters in inorganic scaffolds. (a) Fluorescent silver nanoclusters prepared in a soda-alumino-phosphate glass doped with silver ions upon exposure to near-ultraviolet femtosecond laser pulses. Reprinted from ref. 61 with permission of American Institute of Physics. 3D patterning at the nanoscale of fluorescent nanostructures in a silver-containing glass. Reprinted from ref. 62 with permission of American Chemical Society. (b) Two-photon activation of a silver-exchanged zeolite A enables writing of submicron scale fluorescent patterns. Reprinted from ref. 9 with permission of Wiley-VCH Verlag GmbH & Co. KgaA.

Zeolites are excellently suited to produce silver nanoclusters of well-defined sizes, because of the confinement offered by the zeolite cages (Fig. 3b).[8,67,68] Zeolites are crystalline, highly porous materials with a negatively charged aluminosilicate framework. Their pores are in the Ångström size range and contain exchangeable cations to preserve charge neutrality. Silver nanoclusters are prepared by activation of a zeolite loaded with silver cations. Multiple activation pathways have been demonstrated, including heat treatment,[69,70] (single photon) UV light excitation[68] and two-photon excitation.[9] During the activation, the silver ions combine to form the nanoclusters that can grow only to oligomeric size due to the limited cage dimensions. The two-photon excitation using near-infrared laser enabled the formation of silver nanoclusters with submicron resolution in the 3D space of a giant zeolite crystal (Fig. 3b).[9] The silver nanoclusters hosted in zeolite crystals display excellent photostability upon UV illumination. Applications have been suggested as wavelength converters for fluorescent lamps and biocompatible labels.

3. Silver nanoclusters supported by silver nanoparticles or silver oxide

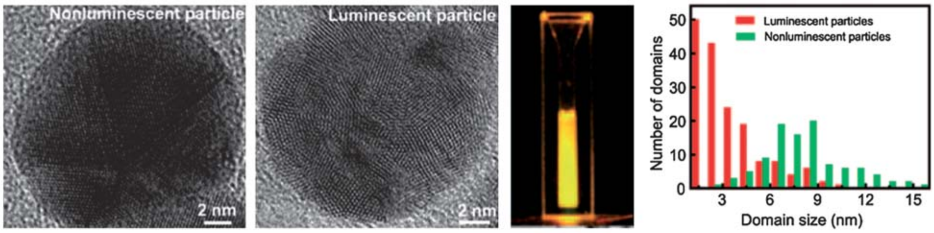
As explained in the Introduction, silver nanoparticles larger than 2 nm exhibit plasmon resonance effects and typically do not present fluorescence. However, there are recent reports about luminescent silver nanoparticles. The emission of light from large silver particles is likely produced from surface-bound nanoclusters or subnanodomains within silver nanoparticles.

Polycrystalline silver nanoparticles with diameter of 18 nm synthesized by thermal reduction of silver ions in glycine matrix are stable in aqueous solution and exhibit bright luminescence (Fig. 4a).[71] The authors showed that the nanoparticles with single-crystalline or twined structures with domain sizes averaging 8 nm are not luminescent, whereas the polycrystalline nanoparticles with numerous small domains in the range of 1–2 nm exhibit luminescence. The luminesence is claimed to originate from these small domains, with sizes comparable to the electron Fermi wavelength (approx. 0.5 nm). The small domains present in the nanoparticles result in discrete energy states that allow optical transitions.

Fluorescent silver nanoparticles with diameters of about 3.7 nm can be produced by photogenerated ketyl radicals that reduce Ag^+ from silver trifluoroacetate in the presence of cyclohexylamine as stabilizer.[73] The nanoparticles emit at 528 nm when prepared in toluene and at 580 nm in tetrahydrofuran. The fluorescence quantum yield is 11%. In this case, the authors claim that the luminescence arises from particle-supported small metal nanoclusters (predominantly Ag_2).[73]

Microgels of poly(*N*-isopropylacrylamide-*co*-acrylic acid-*co*-acrylamide) can be used to template the formation of fluorescent silver nanoparticles.[74] The polyacrylamide fragments serve as stabilizing ligands. Poly(acrylic acid) fragments induce a pH-sensitive volume phase transition of the gel by shrinking during protonation, resulting in an enhancement of the photoluminescence intensity. Whereas the pNIPAM component is used to induce temperature-responsive volume phase transition to control the size particle, it is suggested that the small

(a) Stabilized within multidomain silver particles



(b) Stabilized on silver oxide surfaces

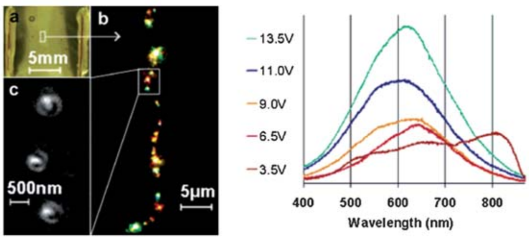


Fig. 4 a) TEM images of a non-luminescent and a luminescent silver nanoparticle. An aqueous solution of luminescent nanoparticles under 532 nm laser excitation. Domain size distributions obtained from luminescent (red) and non-luminescent (green) nanoparticles. Reprinted from ref. 71 with permission of American Chemical Society. (b) Silver nanocluster electroluminescence: discolored Ag_2O region between copper electrodes on a glass substrate in vacuum with 9 V DC potentials applied across the film; multicolored electroluminescence from single Ag_n (n = 2–8) molecules within the electrically discolored region; dipole emission patterns characteristic of single-molecule behavior. Electroluminescence spectra at various applied voltages. Reprinted with permission of American Chemical Society from ref. 72.

silver nanoclusters can coexist with the large silver nanoparticles.[74]

A PEG-based non-ionic block copolymer, poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide), templates the synthesis of luminescent silver particles, with a mean diameter of 7.6 nm. The fluorescence can be observed even in the absence of the block copolymer but the fluorescence intensity increases with the polymer concentration. It is claimed that the emission is caused by silver nanoclusters chemo-adsorbed on the nanoparticle surface.[75]

The cases described in this paragraph deal with fluorescent silver nanoparticles, and although the authors did not mention nanoclusters, we believe the fluorescence may be caused by silver nanoclusters. For instance, silver nanoparticles with diameters of 10 nm can be coated by poly(4-vinylphenylboronic acid-*co*-2-(dimethylamino)ethyl acrylate) to obtain a fluorescent nanocomposite gel capable of sensing glucose.[76] The gel exhibits volume phase transitions induced by glucose, thereby changing the local refractive index of the medium surrounding the silver particles and producing changes in their fluorescent properties.[76] Another example of fluorescent metal nanoparticles are Ag–Au bimetallic cores coated by a PEG-based hydrogel prepared with 2-(2-methoxyethoxy)ethyl methacrylate as monomer and poly(ethylene glycol) dimethacrylate as crosslinker. The reversible thermo-responsive volume phase transition of the PEG-based gel can modify the environment of the metal nanoparticles and hence their fluorescence intensity, facilitating application for sensing environmental temperature increases, offering a thermo-triggered drug release and fluorescently imaging of cancer cells.[77]

Fluorescent silver nanoclusters can also form on surfaces of silver halides[78] or silver oxides.[79] The reaction using silver halides represents in fact the first step in photography, in which exposure of silver halide crystals to illumination results in creation of electrons and holes that reduce silver ions to surface-bound silver nanoclusters, thereby forming the latent image. These silver nanoclusters are fluorescent at low temperatures.[78] The silver nanoclusters on silver oxide are produced by illumination of silver oxide particles, leading to a multicolored room-temperature fluorescence.[79] Emission appeared from many localized sites on the silver oxide particle, each of which exhibited distinctive emission patterns characteristic of single silver nanoclusters. An interesting characteristic is that the emission color of each localized site changes over time (green, red and yellow). This has been attributed to the silver nanocluster size and charge constantly being modified (Ag_2^+, Ag_2, Ag_3^+,…) under illumination and chemical reaction. The silver nanoclusters on silver oxide also emit light upon a DC potential, *i.e.* they are electroluminescent (Fig. 4b).[72] Optoelectronic devices have been constructed from them, including LEDs and logic AND and XOR gates.[72]

Conclusions

In this review we have summarized the advances in the study of silver nanoclusters from synthesis and properties to applications. The silver nanoclusters feature light absorption and luminescent properties originating from electronic transitions between energy levels. The wide emission range tunable from UV to the near-IR region, the quantum confinement effects, the outstanding photostability, the high fluorescence efficiency, the large Stokes shift, electroluminescence, chemiluminescence and electrogenerated chemiluminescence, the stimuli-responsive fluorescence, the good biocompatibility and the particularly small size make these fluorophores to become versatile tools promising for applications in single-molecule studies, fluorescence imaging, fluorescence sensing, data storage devices and labeling.

A suitable scaffold is essential for the nanoclusters to remain stable. For several decades, solid inorganic scaffolds were used, such as cryogenic noble gases, inorganic glasses and zeolites, and they had greatly expanded our knowledge on the metal nanoclusters, and lead to applications including catalysis and 3D micropatterning. The solution-based synthesis of fluorescent silver nanoclusters, as initiated by the group of Dickson, can be considered as a major breakthrough that has opened a new avenue for silver nanocluster research to fluorescence application areas where traditionally organic dyes and semiconductor quantum dots are the dominant fluorophore. It allowed to produce silver nanoclusters in scaffolds of molecular dimensions, so that they become suitable as fluorescent marker, *e.g.* to label proteins. Because the silver nanoclusters have a set of properties complementary to those of organic dyes and semiconductor quantum dots, we believe that their future looks bright.

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