Smart oligopeptide gels: *in situ* formation and stabilization of gold and silver nanoparticles within supramolecular organogel networks†

Sudipta Ray, Apurba Kumar Das and Arindam Banerjee*

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Tripeptide with redox active chemical entities based smart organogels have been used for *in situ* formation and stabilization of gold and silver nanoparticles within the supramolecular gel networks and the gold nanoparticles are aligned in arrays along the gel nanofibers of peptide 1–toluene gels.

Supramolecular organogels have numerous applications including structure-directing agents for synthesis of nanoporous materials, templates for assembling nanoparticles, electro-optical display materials, media for the growth of large organic, inorganic and macromolecular crystals of high optical quality, and others.[1] Tuning the synthesis of nanoscale materials is one of the most significant challenges faced by modern chemistry. Supramolecular organogels consisting of multiple entangled fibrillar networks have been exploited to direct the shape and nanostructures of the inorganic materials.[2] Various inorganic nanostructures can be obtained using supramolecular organogels as templates. One such example is the helical nanofibers prepared by Shinkai[3] and coworkers using supramolecular organogels as a structure-directing agent. Similarly the groups of Hanabusa[4] and Stupp[5] have developed transition metal nanotubes and CdS ribbons respectively using supramolecular gels as templates. Supramolecular hydrogels have been employed as a template for synthesizing inorganic nanotubes.[6] Recently, Hanabusa and his coworkers reported the preparation of helical silica nanostructures using amino acid based gelators as the structure-directing agent.[7] The formation of 'pearl necklace' architecture of CdS with inorganic nanoparticles having a diameter of about 30 nm has been reported by Lu[8] and his coworkers using organogels as a template. However, there are only a few reports of the use of supramolecular gels for the construction of gold and silver nanostructures.[9–12] Liu *et al.* have shown the formation of silver nanohelices using a racemic gelator as a template.[9] Kimura and his coworkers have described self-organization of gold nanoparticles into a network structure using thiol-terminated gelators.[10] They heated a gelator with octanethiol stabilized gold nanoparticles and on cooling further gelation caused gold nanoparticles to assemble into fibrous aggregates of the gel network. Recently, Smith and his coworkers have reported gold nanoparticle synthesis by UV irradiation of a supramolecular organogel containing $HAuCl_4$ and tetraoctylammonium bromide.[11] Very recently, Vemula and John have used urea-containing hydro/organogelators to prepare and stabilize gold nanoparticles by *in situ* reduction.[12] However, none of the above reports regarding the *in situ* synthesis of gold and silver nanostructures using supramolecular gels as a template include short peptide molecules as gelators. In this report, we present *in situ* synthesis and stabilization of different shaped gold and silver nanoparticles within the gel-phase network using redox active tyrosine containing new oligopeptide based supramolecular organogels.

One interesting issue is the type of distribution, stability and the shape of the gold nanoparticles (GNPs) and silver nanoparticles (SNPs) within the gel phase. These nanoparticles can be distributed either all over the gel or they can be aligned in a particular array along the gel micro/nanostructures. It is interesting to fabricate the aligned arrays of GNPs and SNPs using gel fibers as templates for possible optical device uses. For these reasons, we have synthesized a series of terminally protected tyrosine containing new oligopeptides **1–3**[13] (Fig. 1) which self-assemble to form gels in various organic solvents. There are several examples of peptide based hydrogelators/organogelators,[14] but none of these low molecular weight gelators have been exploited for the *in situ* synthesis of gold and silver nanoparticles within the gel-phase network. In gel forming peptides the presence of tyrosine residue(s) can be used to reduce Ag^{+}/Au^{+3} within the gel phase into Ag^{0}/Au^{0} nanoparticles. This can promote the *in situ* formation and stabilization of GNPs and SNPs within the gel network structures.

The minimum gelation concentrations (MGC) and the results of gelation tests for these peptide gelators are listed in ESI Table 1.† Peptides **1** and **2** produce gels in various organic solvents like benzene, 1,2-dichlorobenzene, toluene, *p*-xylene, *m*-xylene, nitrobenzene and tetralin. Peptide **1** forms a gel in dimethyl sulfoxide whereas peptide **2** forms a gel in chloroform. Peptide **3** can gelate solvents like 1,2-dichlorobenzene, nitrobenzene and methanol–water (1 : 1) solvent. Gel melting temperatures (T_{gel}) of these

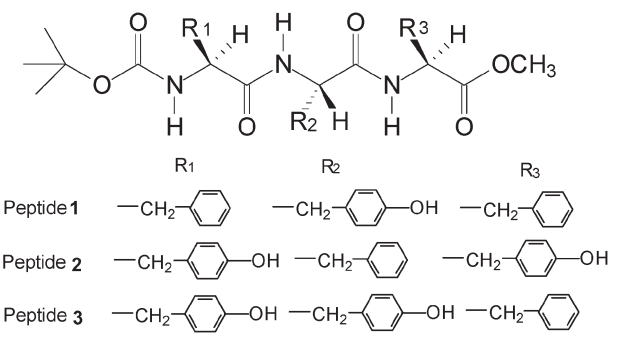

Fig. 1 Schematic representation of tripeptides **1**, **2** and **3** showing their chemical structures.

Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India. E-mail: bcab@mahendra.iacs.res.in; Fax: +91-33-2473-2805

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peptide gelators were analyzed by the inverted test tube method. The morphology of these gelator peptides were characterized by transmission electron microscopy (TEM) and field emission scanning electron microscopy (FE-SEM). The SEM images of the xerogels obtained from peptide **1** in toluene show entangled nanofibrillar networks with an average diameter of 180 nm (ESI Fig. S7a†). The TEM images of the xerogels prepared from gels of peptide **2** in toluene and of peptide **3** in methanol–water (1 : 1) (1% w/v) reveal a 3D network of nanofibrillar structure (ESI Fig. S7b and Fig. S7c†).

To study the self-assembling behavior of these gel-forming peptides, FT-IR and temperature dependent ^{1}H NMR experiments were done. Peptide **2** forms a gel in $CHCl_3$. So, temperature dependent ^{1}H NMR chemical shifts of peptide **2** gel in $CDCl_3$ (1% w/v) have been recorded from the temperature 25 °C (gel state) to 75 °C (solution state) (ESI Fig. S8†). Significant changes in chemical shifts have been observed for all amide NHs. These results indicate that all amide NHs are involved in intermolecular hydrogen bonding to form the gel state. The FT-IR spectrum of a toluene gel formed by the peptide **2** shows absorption bands at 3298 and 1649 cm^{-1} which can be assigned as intermolecularly hydrogen bonded N–H and C=O stretching vibrations respectively (ESI Fig. S14†).

Previous reports have demonstrated that gold and silver nanoparticles have been synthesized by tyrosine, alkylated tyrosine and tyrosine based peptides.[15] Incorporating the redox active tyrosine residue into the gel forming tripeptides, we want to explore the *in situ* synthesis of gold and silver nanostructures within the gel-phase network.

The following method has been used for the synthesis of gold nanoparticles in peptides **1**– and **2**–toluene gels. Toluene (5 mL) and tricaprylylmethylammonium bromide (70 μL) were mixed in a beaker and the mixture was continued to stir for half an hour. An aqueous yellow colored solution of $HAuCl_4$ (20 mg in 2 mL) was added to it for transferring the chloroaurate to the toluene layer. The toluene layer was then separated. Gelator peptide **1** (10 mg) or **2** (10 mg) was added in 800 μL of toluene chloroaurate solution and it was heated above 100 °C to produce a homogeneous solution. Upon cooling a stable gel has been formed and the yellow coloration was not changed for several days. Triethylamine (5 μL) was added into the toluene gel and it was heated in an oil bath above 100 °C to produce a clear solution. Then the yellow color was rapidly changed to a colorless solution and it was turned into a violet solution within a few minutes indicating the formation of gold nanoparticles *via* the oxidation of tyrosine residues of peptide **1** or **2**. Upon slow cooling, after 1 h a gold nanoparticle embedded violet colored gel was formed. The presence of a surface plasmon band around 548 nm suggests the existence of gold nanoparticles (ESI Fig. S9a†). Peptide **3** forms a stable gel in methanol–water (1 : 1) within the pH range from 7 to 11. The gelator peptide **3** (20 mg) was added in 400 μL of methanol–water (1 : 1) solution at pH 10 and it was heated until the appearance of a clear solution. Upon cooling below room temperature the complete volume of the respective solvent was immobilized and a stable gel was formed. 1 mg of $HAuCl_4$ was added into the gel and the mixture was heated above 50 °C to dissolve. Upon cooling, the yellow color of the solution gradually changed to a colorless solution [Au(III) to Au(I)] and then it turned into bluish violet color, indicating the formation of gold nanoparticles [Au(0)] *via* oxidation of tyrosine residues of peptide **3**. After 1 h a gold nanoparticle embedded bluish violet colored gel was formed. A surface plasmon band around 551 nm was observed in the UV-vis spectrum indicating the formation of gold nanoparticles[11] (ESI Fig. S9b†). The following method was used for the *in situ* preparation of silver nanoparticles in tyrosine containing peptide **3** gels. 1 mg of $AgNO_3$ was added into the methanol–water gel of peptide **3** and the mixture was heated above 50 °C until the appearance of a clear solution. Upon slow cooling the color of the transparent solution changed from light yellow to strong yellow within 1 h indicating the formation of silver nanoparticles *via* oxidation of tyrosine residues of the tripeptide **3** and then the silver nanoparticle embedded gel was formed within a few minutes. A surface plasmon band around 363 nm suggests the existence of silver nanoparticles (ESI Fig. S9c†).

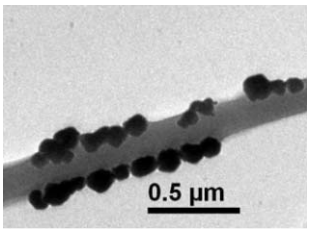

Fig. 2 TEM image indicating the definite alignment of gold nanoparticles along a gel nanofiber obtained from the peptide **1**–toluene gel.

Transmission electron microscopy was used to characterize the gold and silver nanoparticles embedded gels. A small piece of the gel of a particular peptide in its respective solvent was placed on a carbon-coated copper grid (300 mesh) and it was allowed to dry under reduced pressure at room temperature for two days. Fig. 2 shows that gold nanoparticles (with an average diameter of 50–80 nm) were aligned in a definite array along the gel nanofiber obtained from peptide **1**–toluene gel. Gold nanoparticles (with an average diameter of 15–20 nm) in the toluene gel-phase network of peptide **2** were visualized by TEM (Fig. 3). Fig. 4a clearly illustrates the presence of gold nanoparticles within the gel network structure of peptide **3**. Fig. 4b shows the TEM image of individual gold nanoparticles with different morphologies including spherical and hexagonal with various particle sizes ranging from 15 nm to 40 nm. Previous reports include the synthesis of hexagonal shaped gold nanoparticles of various sizes using different chemical and biological methods.[16] However, none of these methods include the

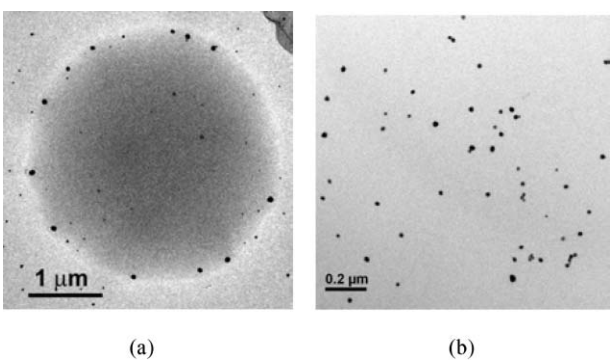

Fig. 3 (a) TEM image of gold nanoparticles formed within the gel network structure of peptide **2**–toluene gel. (b) TEM picture of the *in situ* formed gold nanoparticles at a higher magnification.

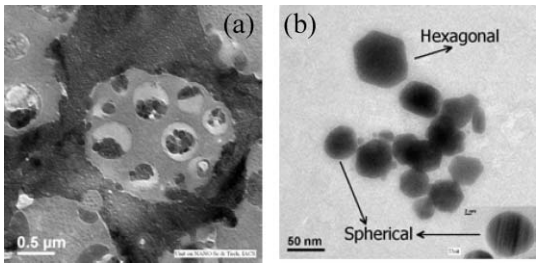

Fig. 4 (a) TEM image of gold nanoparticles within the gel network structure of peptide **3**–methanol–water (1 : 1) gel and (b) magnified TEM image of gold nanoparticles showing hexagonal and spherical morphology.

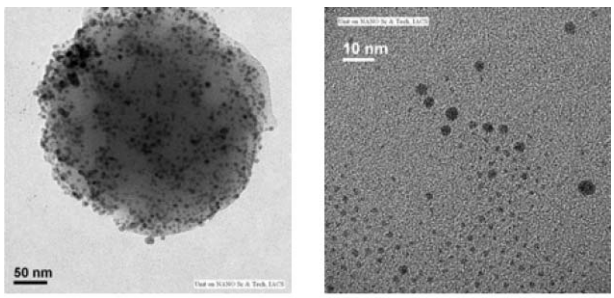

Fig. 5 (a) TEM image of silver nanoparticles embedded in a spherical sponge-like gel network structure of peptide **3**–methanol–water (1 : 1) gel and (b) TEM image of these silver nanoparticles at higher magnification.

formation of hexagonal gold nanoparticles within the gel phase. Fig. 5 shows the TEM images of silver nanoparticles (with an average diameter of 2–10 nm) formed within the methanol–water gel of peptide **3**. A characteristic EDX profile for silver nanoparticles is given in ESI Fig. S12c.† From X-ray diffraction studies of the gel and gel–metal nanoparticle composite, it was observed that gold/silver nanoparticle embedded peptide gel network structures are similar to the respective peptide gel network structure without the presence of the gold/silver nanoparticles (ESI Fig. S13†). In order to understand the role of the tyrosine residue in the reduction of Au(III), we have synthesized and studied another tripeptide Boc–Ala-Phe-Ala–OMe (AFA) without any tyrosine residue. AFA forms effective organogels in toluene, but it failed to reduce the $HAuCl_4$ under similar conditions to produce the gold nanoparticles within the gel network. This result convincingly demonstrates that the tyrosine residue has a definite role for the preparation of gold/silver nanoparticles by *in situ* reduction.

We have successfully demonstrated the *in situ* preparation of gold and silver nanoparticles into a gel network structure using tyrosine containing oligopeptide based organogelators. The tyrosine residue(s) of gelator peptides have been successfully utilized to reduce Au^{+3}/Ag^{+} into colloid Au^{0}/Ag^{0} nanoparticles and after the reduction, the gelator peptides retain their gelation properties intact. Hence, these nascent metal nanoparticles are trapped and stabilized within the supramolecular gel-phase network. This is an wonderful demonstration of the exploitation of tyrosine containing gel-forming oligopeptides for the *in situ* preparation of gold and silver nanoparticles and their concomitant stabilization within the supramolecular assemblies. Another remarkable feature is that the alignment of *in situ* prepared gold nanoparticles along the nanostructured gel fibers of peptide **1**-toluene gels. Such materials may be important for the future development of nanostructured advanced materials from the conjugates of tyrosine containing oligopeptide based smart gels and Au/Ag nanoparticles, which may open up applications in the promising field of supramolecular devices. Gels containing useful chemical entities like tyrosine make themselves suitable for the applications, such as *in situ* formation of gold and silver nanoparticles with different shapes and sizes without any reducing and stabilizing agents and this can be utilized to make smart gels containing useful functionalities for the *in situ* preparation and stabilization of metal nanoparticles within the gel network structure in future.

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