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### Short-Peptide-Based Hydrogel: A Template for the In Situ Synthesis of Fluorescent Silver Nanoclusters by Using Sunlight

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Dedicated to Professor Animesh Chakravorty on the occasion of his 75th birthday

Abstract: N-terminally Fmoc-protected dipeptide, Fmoc-Val-Asp-OH, forms a transparent, stable hydrogel with a minimum gelation concentration of 0.2% w/v. The gelation property of the hydrogel was investigated by using methods such as transmission electron microscopy, field-emission scanning electron microscopy, atomic force microscopy and Fourier transform infrared spectroscopy. The silver-ion-encapsulating hydrogel can efficiently and spontaneously produce fluorescent silver nanoclusters under sunlight at physiological pH (7.46) by using a green chemistry approach. Interesting-

### Introduction

Noble metal nanoclusters have attracted considerable attention due to their marvellous optical and chemical properties and interesting applications in single-molecular spectroscopy, catalysis, biological/chemical sensing and biological imaging.<sup>[1]</sup> Few-atom gold/silver clusters are even smaller than the corresponding plasmon-supporting metal nanoparticles. Interestingly, they show molecule-like electronic transitions within the conduction band, which results in fluorescence properties.<sup>[2]</sup> These fluorescent nanoclusters are attractive candidates for biolabelling and bioimaging due to their bright fluorescence, ultrafine size, very low fluorescence intermittency and nontoxicity. There is a growing

ly, in the absence of any conventional reducing agent but in the presence of sunlight, silver ions were reduced by the carboxylate group of a gelator peptide that contains an aspartic acid residue. These clusters were investigated by using UV/Vis spectroscopy, photoluminescence spectroscopy, high-resolution transmission electron microscopy (HR-TEM), atomic force microscopy (AFM) and X-ray diffraction (XRD)

**Keywords:** fluorescence • gels • green chemistry • nanoparticles • peptides

studies. Mass spectrometric analysis shows the presence of a few atoms in nanoclusters containing only Ag<sub>2</sub>. The reported fluorescent Ag nanoclusters show excellent optical properties, including a very narrow emission profile and large Stokes shift (>100 nm). The reported fluorescent Ag nanoclusters within hydrogel are very stable even after 6 months storage in the dark at 4°C. The as-prepared hydrogel–nanocluster conjugate could have applications in antibacterial preparations, bioimaging and other purposes.

need for the development of environmentally friendly methods for the synthesis of nanomaterials targeted for biomedical applications. An eco-friendly reducing and capping agent, an environmentally suitable solvent system and room-temperature synthesis are essential criteria for an absolutely green nano-structured synthesis.<sup>[3]</sup>

Of the noble metals, fluorescent gold nanoclusters have been extensively studied because of their chemical stability and facile synthetic procedure.<sup>[1d,f,2c,4]</sup> However, relatively few studies have been focused on the silver clusters because these clusters are generally not very stable in water and have a natural tendency to aggregate to form larger nanoparticles. There are several methods to prepare fluorescent silver nanoclusters, which include radiolytic,<sup>[5]</sup> chemical reduction<sup>[6-10]</sup> and photochemical (using UV light) approaches.<sup>[2a,11-15]</sup> Dickson and co-workers made a pioneering contribution to the preparation of silver clusters by using different methods.<sup>[1a,b,6]</sup> There are many reports for the synthesis of fluorescent silver clusters by using DNA as a scaffold in aqueous solution.<sup>[7,8]</sup> The role of sequence-dependent DNA templates in the formation of fluorescent Ag nanoclusters has also been reported recently.<sup>[8]</sup> Jin and co-workers recent-

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ly reported Ag<sub>7</sub> clusters by using 2,3-mercaptosuccinic acid as a ligand.<sup>[10]</sup> Formation of silver nanoclusters have also been reported within silver-exchanged zeolites formed by heat treatment<sup>[16]</sup> and within glass by using femtosecond laser irradiation.<sup>[17]</sup> Kumacheva and co-workers reported the successful photogeneration of fluorescent Ag nanoclusters in poly(N-isopropylacrylamide-acrylic acid-2-hydroxyethyl acrylate) copolymer-based hydrogel microspheres by UV irradiation.<sup>[12]</sup> Recently, the formation of fluorescent Ag nanoparticles in p(NIPAM-AA-AAm) microgels by using NaBH<sub>4</sub> was reported.<sup>[9c]</sup> However, environmentally harmful UV irradiation/gamma irradiation or toxic sodium borohydride has been used as a reducing agent in all above-mentioned cases. To the best of our knowledge, there is only one report on an absolutely green synthesis of fluorescent gold nanoclusters by using a commercially available protein, bovine serum albumin (BSA), at physiological temperature.<sup>[18]</sup> Herein we report the green synthesis of fluorescent Ag nanoclusters within a dipeptide-based supramolecular hydrogel by using sunlight irradiation (for a few minutes) at physiological pH and room temperature.

### **Results and Discussion**

**Preparation and characterisation of the dipeptide-based hydrogel**: The N-terminally protected dipeptide Fmoc-Val-Asp-OH forms a stable, transparent hydrogel in the pH range 1–12 (Figure 1, inset). The minimum gelation concen-



tration is 0.2 % w/v at pH 7.46, that is, a single peptide molecule can gelate almost 12611 water molecules. This indicates that the gelator peptide is very efficient at forming hydrogels. The gelator peptide was first dissolved in a minimum volume of dimethyl sulfoxide with gentle heating, and then cooled to room temperature. Upon addition of the required amount of water to the solution, a transparent thermoreversible hydrogel was immediately produced. The temperature at which the gel-to-sol transition occurs is called the gelmelting temperature ( $T_{gel}$ ) and it increases as the concentration of the gelator peptide increases, until the plateau region is reached (Figure 1). This suggests that the formation of the supramolecular gel network is essentially complete at the higher concentration region.

The morphology of the supramolecular structure formed by the hydrogel was investigated by using field-emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM) and atomic force microscopy



Figure 1. The change in  $T_{gel}$  with respect to the gelator concentration. Inset: A photograph of the hydrogel at 0.2 % w/v at physiological pH.

(AFM). The TEM image (Figure 2a) of a slightly diluted solution of this peptidic hydrogel in water shows an entangled three-dimensional network structure of fibrous aggregates. The width of the gel fibres is within the range of 35 to



Figure 2. a) TEM and b) FE-SEM images of the hydrogel.

42 nm and each fibre is a few micrometers in length. Individual junctions between these fibres were also observed, which are probably responsible for stabilising the supramolecular hydrogel network. The FE-SEM image (Figure 2b) of the freeze-dried hydrogel shows a dense intertwined fibrous aggregate. The width of these fibres remains 30 to 45 nm. It has been found from this study that the width of these gel fibres is much higher than that of the molecular dimension of the gelator peptide. This clearly indicates that several molecular chains are self assembled to form the supramolecular aggregated fibrilar structure. The AFM image (Figure 3a) of the hydrogel also shows a uniform three-dimensional nanofibrillar network structure with an average diameter of 50 nm in the gel state. The uniform nature of the aggregates observed in these morphological studies suggests that this peptide molecule self-assembles in a hierarchical fashion to form a one-dimensional supramolecular polymer of intertwined fibres to give a larger network of superstructures in a gel. An FT-IR study (Figure 3b) of freeze-dried hydrogel was carried out to understand the intermolecular interaction between the gelator peptides in the gel state. The presence of bands at  $\tilde{\nu} = 3294$ , 1647 and

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Figure 3. a) AFM image of hydrogel (scale bar: 1  $\mu m)$  and b) FT-IR spectrum of the dried gel.

1537 cm<sup>-1</sup> can be assigned as the hydrogen-bonded N–H stretching (amide A), C=O stretching (amide I) and N–H bending (amide II) bands, respectively.<sup>[19]</sup> The amide I and amide II bands suggest the presence of an intermolecularly hydrogen-bonded structure in the gel state. Another peak at  $\tilde{\nu}$ =1719 cm<sup>-1</sup> appeared; this is characteristic of the C=O stretching band of carboxylic acid.<sup>[19]</sup>

Synthesis of fluorescent silver nanoclusters within the hydrogel: Preparation of nanoparticles in the gel phase is a fascinating research area. Gels in the swollen stage provide a large free space between the three-dimensional cross-linked networks that acts as a nanoreactor for the nucleation and growth of nanoparticles. Stability, longevity and controlled growth of nanoparticles are easily achieved within the gel phase. There are several examples of the formation of metal nanoparticles in gel-phase materials. The entrapment of presynthesised metal nanoparticles in gels is relatively common.<sup>[20]</sup> However, examples of the in situ synthesis of metal/semiconductor nanoparticles in gels by using the corresponding gel as a reaction medium are limited.<sup>[21-24]</sup> We previously reported the in situ synthesis of Au/Ag nanoparticles in an oligopeptide-based supramolecular organogel by using the redox-active tyrosine moiety of the gelator peptide.<sup>[22]</sup> Other reports include the reduction of Ag+ in an organogel by using DMF as a gelling solvent and hydroquinone as a reducing agent.<sup>[23]</sup> However, the three-dimensional network provided by hydrogels is an important template for

producing nanoparticles because hydrogels are generally nontoxic, biocompatible materials. A controlled deposition of silver nanoparticles into the polymer microgel has been achieved by Kumacheva and co-workers by using UV light or in presence of an external reducing agent, such as NaBH<sub>4</sub>.<sup>[24]</sup> To the best of our knowledge, there is no report of the synthesis of fluorescent Au/Ag nanoclusters by using a supramolecular hydrogel in the presence of sunlight. Herein, we have successfully demonstrated the in situ preparation of fluorescent Ag nanoclusters within a short-peptidebased supramolecular hydrogel in presence of only sunlight.

In a typical experiment, the solid peptide Fmoc-Val-Asp-OH (4.5 mg,  $10^{-2}$  mmol) was dissolved in dimethyl sulfoxide (200 µL) by gentle heating, and then cooled to room temperature. Freshly prepared dilute aqueous AgNO<sub>3</sub> (3.8 mL, pH 7.46) was added to this solution in a molar ratio of 1:1 [Ag<sup>+</sup>]/COOH to give a final gelator concentration of 0.2 % w/v (2.5 mM) and [Ag<sup>+</sup>]=5 mM. Note that the each gelator peptide has two free COOH groups. An Ag<sup>+</sup>-encapsulating transparent hydrogel is immediately formed. A light violet colouration started to appear if the gel was exposed to bright sunlight (Figure 4a) and the reaction process was



Figure 4. a) Photograph of silver ions encapsulated by the hydrogel after irradiation by bright sunlight for a few minutes. b) Time-dependent UV/ Vis spectra during Ag nanocluster formation within the hydrogel.

complete within a few (3–4) minutes. The violet colouration indicates the reduction of silver ions to silver clusters  $(Ag^0)$ ; this type of colour change was not observed in the absence of sunlight or in the absence of silver or gelator peptide. This suggests that both the peptide molecule and sunlight have a role in silver-ion reduction. Transiently stable silver clusters were formed in an aqueous solution of gelator peptide (not in the gel state) that contained AgNO<sub>3</sub> in the presence of sunlight; these clusters aggregate to form bigger particles and are ultimately precipitated out from solution within 2 h. Thus, it can be envisaged that the three-dimensional gel network structure provides good stability for silver nanoclusters and prevents further growth of nanoclusters to form larger non-fluorescent nanoparticles.

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It is important to note that peptide-controlled mineralisation of silver nanoparticles has so far been achieved by redox-active peptides based on tyrosine or tryptophan.<sup>[25]</sup> However, herein we present the spontaneous reduction of silver ions by using a short-peptide-based hydrogel (containing aspartic acid residue) in the presence of bright sunlight to facilitate the production of templated fluorescent silver nanostructures. Interestingly, in the absence of any conventional reducing agent in medium, silver ions were (bio)reduced in the presence of bright sunlight by the carboxylate group of the gelator peptide that contained an aspartic acid residue. Generally, the deprotonated carboxylic acid groups in block copolymer systems have been utilised for silver-ion reduction because carboxylic acid has excellent binding affinity towards Ag<sup>+</sup> ions.<sup>[9c, 12, 13, 26]</sup> However, in all these above-mentioned cases, the presence of a reducing agent, such as  $H_2~gas,^{[26]}~NaBH_4,^{[9c]}~UV~radiation^{[12,13]}~or~\gamma$  radiation,<sup>[5a]</sup> was required. There is a single report for the reduction of silver ions into non-fluorescent nanoparticles by the polycarboxylic acid group of peptides (E<sub>6</sub> or D<sub>6</sub>) that contain glutamic acid (E) or aspartic acid (D) and by using ambient light.<sup>[27]</sup> Herein we have used the aspartic acid residue for the reduction of silver ions into nanoclusters  $(Ag^0)$ within a hydrogel medium, and the carboxylate groups of the aspartic acid unit play a vital role in the reduction under bright sunlight. Sunlight-induced reduction of Ag<sup>+</sup> to Ag nanoparticles by using DNA templates has been recently reported.<sup>[28]</sup> Another report includes the formation of silver nanoparticles in the presence of light by using lysozyme as a template.[29]

Herein we have attempted to determine the mechanism involved in this reduction. We conducted several extensive experiments to probe whether any structural change in the gelator peptide occurs during the process of reduction. FT-IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopies were performed for the peptide before and after silver mineralisation (given in the Supporting Information). However, no structural change in the gelator peptide (or aspartic acid residue) was observed during the reduction. It is necessary to judge the possibility of Ag<sup>+</sup> ion complexation during reduction. Ag<sup>+</sup> ions are known to complex with the carboxylic acid groups of the aspartic acid residues. The standard electrode potential of the  $Ag^+/Ag$  redox system is +0.8 V for bulk material. However, this value can be drastically different when the Ag<sup>+</sup> ions are involved in complexation. Usually, complexation decreases the redox potential and in that condition it is easier to reduce the Ag+ ions. Examples include the reduction potential of the Ag<sup>+</sup>/Ag system in Ag halides (AgBr) used in photography (+0.07 V), in Ag diamine complexes  $[Ag(NH_3)_2]$  (+0.37 V), in Ag benzoate (+0.52 V), in  $Ag_2CO_3$  (+0.47 V) and in AgOH (+0.24 V).<sup>[25a]</sup> Herein, the energy barrier for silver-ion reduction is significantly decreased when silver ions are complexed with the carboxylic groups of the aspartic acid residue present in the gelator peptide. The combination of carboxylic-acid-containing dipeptide and sunlight make it possible to reduce silver ions to Ag<sup>0</sup> to form nanoclusters. Because carboxylic groups are

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nucleophilic, their binding with silver ions induces partial electron transfer that displaces the Fermi level of silver cluster toward more negative potentials. We also believe that water could be a source of electrons to initiate the reduction process, and it is evident that the presence of solvated electrons in water are possible.<sup>[30]</sup> The carboxylate radical activated by visible light could possibly be involved in photochemical reductions of Fe<sup>III</sup> complexes of polycarboxylates.<sup>[31]</sup> Considering the effect of complexation, silver ions can be photoreduced by the carboxylic acid group of the gelator peptide even under sunlight.

#### Characterisation of the hydrogel-nanocluster composite

Optical study: We have investigated the spontaneous reduction of silver ions by using UV/Vis absorption spectroscopy. At first, the Ag<sup>+</sup>-encapsulating hydrogel is colourless and transparent and the UV/Vis spectrum showed no absorption within the range of  $\lambda = 300$  to 800 nm. Subsequent exposure to bright sunlight resulted in a gradual colour change from colourless to violet, and after 1 min of the exposure to sunlight a broad surface plasmon resonance band appeared around  $\lambda = 530$  nm with a shoulder around  $\lambda = 390$  nm (Figure 4b). With increased exposure time of up to 3 min, the intensities of these peaks gradually increased. However, no significant change in the peak intensity or wavelength was observed upon further irradiation. Fluorescent silver clusters in the polymer microgel template exhibit a shoulder at  $\lambda =$ 330 to 360 nm with an intense peak at  $\lambda = 490$  to 520 nm in UV/Vis absorption spectra.<sup>[12]</sup> Herein, the obtained absorption band may be responsible for in situ formation of Ag clusters within the hydrogel.

It is important to note that in our investigation, hydrogel alone (without any silver ions/Ag clusters) gives an emission maximum at  $\lambda = 415$  nm upon the excitation at  $\lambda = 300$  nm (Figure S1 in the Supporting Information). This photoluminescence (PL) emission comes from self-assembled gelator peptides that contain an aromatic Fmoc group. It can be stated that no other PL emission was obtained in the range of  $\lambda = 300$  to 800 nm. PL and photoluminescence excitation (PLE) spectra of the Ag+-encapsulating hydrogel after irradiation with bright sunlight are shown in Figure 5. The PL spectrum shows an emission maximum at  $\lambda = 634$  nm upon excitation at  $\lambda = 530$  nm. As the reaction progresses, the fluorescence intensity steadily increases with the increase in irradiation time and is ultimately saturated after 3 min (Figure S2 in the Supporting Information). This emission is due to the formation of fluorescent silver nanoclusters within the hydrogel and it does not come from the gelator molecules. Other researchers have reported that the oxidised form of dendrimers, which are used as templates in the formation of Au/Ag nanoclusters,[4j] are fluorescent.[32] To explore the possibility of whether the fluorescence originates from metal-assisted oxidation of the peptide, experiments were carried out in which AgNO3 was replaced by oxidising agents such as H<sub>2</sub>O<sub>2</sub>, Fe<sup>III</sup> or persulfate under similar conditions.<sup>[4k,32]</sup> However, no fluorescence emission around  $\lambda =$ 

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Figure 5. Normalised a) PL and b) PLE spectra of the silver nanocluster within the hydrogel.

634 nm was observed from the peptide hydrogel in the presence of any of these above-mentioned oxidants upon excitation at  $\lambda = 530$  nm (Figure S3 in the Supporting Information). UV/Vis spectra also show a small shoulder at  $\lambda =$ 390 nm. Excitation at this wavelength did not produce any emission maximum at  $\lambda = 634$  nm, which indicates that the absorption band at  $\lambda = 530$  nm is responsible for the PL property. The quantum yield (Q) of the reported fluorescent Ag nanoclusters was found to be 1.3% in water by using the Atto 520 dye (Q = 90% in water) as a reference. Our fluorescent Ag nanocluster-hydrogel composite shows good optical properties. Firstly, it exhibits an emission band width with a full width at half maximum (FWHM) of  $36\pm1$  nm, which is narrower than all previously reported Ag nanoclusters and indicates the narrow size distribution of Ag nanoclusters.<sup>[1d]</sup> Secondly, the as-prepared Ag nanoclusters within the hydrogel have a large Stokes shift (104 nm); this is a consequence of a small overlap between the absorption and emission spectra. The sunlight-induced formation of fluorescent Ag clusters within hydrogel is very stable. Both PL intensity and wavelength of the Ag clusters do not change even after storage of this nanomaterial for 6 months in the dark at 4°C.

*Electron microscopy studies*: The size of the nanoclusters has been investigated through high-resolution transmission electron microscopy (HR-TEM). HR-TEM images of hydrogel–Ag nanocomposite showed the formation of silver nanoclusters, with the majority of nanoclusters being within 1 and 3 nm (Figure 6). The particle size distribution from HR-TEM (Figure S4 in the Supporting Information) shows that a significant amount of particles are below 2 nm, which is responsible for the fluorescence property. Interestingly, most of the nanoclusters are on the gel fibres (Figure S5 in the Supporting Information). These nanoclusters are crystalline in nature. The HR-TEM image (Figure 6b) indicates the presence of a lattice plane that has an interfringe distance of 2.34 Å, which corresponds to the (111) plane of fcc Ag. A selected-area electron diffraction study (SAED)



Figure 6. a) HR-TEM image of small Ag nanoclusters within the hydrogel. b) Enlarged version of the HR-TEM image of the Ag nanoclusters, clearly showing the lattice fringes.

shows the presence of a (111) plane for silver (Figure S6 in the Supporting Information). This result of SAED and HR-TEM are in consistent with that of X-ray diffraction study. Atomic force microscopic (AFM) images of hydrogel–Ag cluster nanocomposite exhibited the presence of very small spherical nanoclusters with an average diameter of 1.53 nm, and these nanoclusters are attached to the larger gel fibres of average diameter 50 nm (Figure 7). Thus, this hydrogel has been shown to be an excellent medium for the formation of fluorescent silver nanoclusters.

The hydrogel is stable at this high concentration of silver (a ratio of COOH/Ag<sup>+</sup>=1:1). Morphological studies (HR-TEM, AFM) and FT-IR (Figure S7 in the Supporting Information) suggest that the supramolecular structure of the hydrogel is similar before and after silver mineralisation. In this study it is clear that the hydrogel can endure the presence of a considerable amount of silver nanoclusters, without having any adverse effect on gelation and its supramolecular structure. This may be due to the fact that the size of nanoclusters is very small compared to the gel fibres and this is evident from TEM and AFM images.

X-ray diffraction studies: The XRD pattern for the hydrogel-Ag nanocomposite showed diffraction peaks at  $2\theta =$ 38.2, 44.2, 64.5, 77.4 and 81.6°, all of which are consistent with those for Ag (Figure 8). These diffraction peaks correspond to the (111), (200), (220), (311) and (222) Miller indices of fcc Ag, respectively. Generally the intensity ratio between the peaks corresponding to (111) versus (200) planes is 0.40 versus 0.24.<sup>[33]</sup> However, in this study the intensity ratio was found to be 2.92 versus 0.73; this is higher than the conventional value. This observation suggests that the silver nanoclusters are primarily dominated by (111) facets. The reduction of silver ions growing along particular facets (111) may be explained by using a model suggested by Naik and co-workers.<sup>[34]</sup> According to this model, peptides modify crystal growth by allowing accumulation of the silver atoms on the lowest-surface-energy face. Furthermore, the surface energy of different crystal faces in face-centred-cubic metals is lower for {111} faces than that of other planes.<sup>[34]</sup> As a result, there is a preferential interaction of peptide gel fibres with the {111} faces of Ag clusters. This is demonstrated by the TEM and SAED data. We determined the silver cluster

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Figure 7. AFM images of a) the Ag nanocluster–hydrogel composite, b) an enlarged image showing a few clusters clearly and c) a 3D image of clusters.



Figure 8. XRD pattern of dried silver clusters, with the lattice planes indicated.

size from XRD peaks by using the Debye–Scherrer equation. By considering the (111), (200), (220) and (311) planes, particle diameters of 8.9, 5.17, 4 and 6.2 nm, respectively, were obtained. These values are higher than the average value obtained from the HR-TEM measurement. It is evident from the HR-TEM data that >2 nm particles are also present. It is possible that XRD picks up larger particles because larger particles scatter X-rays much more strongly than smaller clusters do.

MALDI mass spectrometric study: The formation of nanoclusters within the hydrogel medium was also confirmed by MALDI mass analysis. The spectrum (Figure 9) shows a



Figure 9. MALDI mass spectrum of silver nanoclusters within the hydrogel.

peak at 215.93 that corresponds to nanoclusters that contain only  $Ag_2$ . A peak at 454.77 is also present and is assigned to the gelator peptide. By using MALDI mass spectrometry, Dickson and co-workers reported the presence of  $Ag_1$  to  $Ag_5$  clusters that results in the observation of a broad emission band.<sup>[1e]</sup> Ras et al. showed the presence of clusters containing a small number of Ag atoms, such as  $Ag_1$ ,  $Ag_2$  and some  $Ag_5$ , from the MALDI-TOF mass spectrum reported in the literature.<sup>[15]</sup> Mass analysis also confirms the relatively narrower size distribution of our reported Ag nanoclusters within the hydrogel and this result is in good agreement with the relatively smaller emission band width obtained from the photoluminescence spectrum (Figure 5).

### Conclusion

A short-peptide-based hydrogel has been successfully utilised to make and stabilise fluorescent silver nanoclusters. Interestingly, in the absence of any conventional reducing agent in the medium, silver ions can complex with the carboxylate group of the aspartic acid residue of the gelator peptide and are spontaneously reduced under bright sunlight. The three-dimensional network structure of the hydrogel facilitates the stabilisation of the newly formed silver clusters within the hydrogel. The as-prepared hydrogel– nanoclusters are stable for a long time in the gel phase. The fluorescent silver clusters have interesting fluorescent properties with a narrow emission profile and large Stokes shift.

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The peptide-based hydrogel template is a biocompatible and eco-friendly system because this peptide is only composed of protein amino acid residues. The synthetic procedure for fluorescent silver clusters is a green chemistry approach because the preparation takes place at physiological pH and room temperature and does not require any toxic reducing agent. Moreover, silver is considered to be a non-toxic and environmentally friendly antibacterial agent.<sup>[29]</sup> Therefore, our fluorescent silver cluster–hydrogel conjugate may be utilised as an antimicrobial agent and for bioimaging purposes.

### **Experimental Section**

**Materials**: L-Valine and L-aspartic acid were purchased from Aldrich. HOBt (1-hydroxybenzotriazole), DCC (dicyclohexylcarbodiimide), Fmoc-chloride, silver nitrate (AgNO<sub>3</sub>), hydrogen peroxide and ferric chloride were purchased from Merck. The fluorescent dye Atto 520 was purchased from Sigma-Aldrich. The water used in all experiments was of Millipore Milli-Q grade.

**Peptide synthesis:** Peptide Fmoc-Val-Asp-OH was synthesised by conventional solution-phase methodology by using a racemisation-free fragment condensation strategy. The N terminus was protected by a Boc group and the C terminus was protected as a methyl ester. Coupling was mediated by using dicyclohexyl carbodiimide/1-hydroxybenzotriazole. Deprotection of the methyl ester was performed by using the saponification method. Deprotection of the Boc group was conducted by treatment with TFA. Finally, the Fmoc group was introduced into the deprotected dipeptide. All the compounds were fully characterised by mass spectrometry, <sup>1</sup>H (300 MHz) and <sup>13</sup>C NMR spectroscopy (75 MHz; for the spectra see the Supporting Information).

#### Instrumentation

*UV/Vis spectroscopy*: UV/Vis absorption spectra of the Ag<sup>+</sup>-containing hydrogel and silver nanocluster-encapsulating hydrogel were recorded by using a Varian Cary 50 Bio UV/Vis spectrophotometer.

*Photoluminescence (PL) spectroscopy:* All photoluminescence spectra were recorded by using a Horiba Jobin Yvon Fluoromax 3 instrument with a 1 cm path length quartz cell. The slit width for the excitation and emission was set at 5 nm.

*FT-IR spectroscopy*: All FT-IR spectra of dried gels were recorded by using the KBr pellet technique and a Nicolet 380 FT-IR spectrophotometer (Thermo Scientific).

High-resolution transmission electron microscopy (HR-TEM): HR-TEM images were recorded by using a JEOL electron microscope operated at an accelerating voltage of 200 kV. Dilute solutions of the hydrogel and the Ag nanocluster-hydrogel composite were dried on carbon-coated copper grids (300 mesh) by slow evaporation in air, then allowed to dry separately in a vacuum at 25 °C for 2 d. The average size of nanoclusters was determined from the HR-TEM images by using the ImageJ software.<sup>[35]</sup>

*Field-emission scanning electron microscopy (FE-SEM)*: For SEM study, the hydrogel was dried on a glass slide and coated with platinum. Then the micrographs were recorded by using a SEM apparatus (Jeol Scanning Microscope-JSM-6700F).

Atomic force microscopy (AFM): Tapping-mode atomic force microscopy studies were done by placing a small amount of Ag nanocluster-encapsulating hydrogel material on a microscope cover glass and then dried in air by slow evaporation. The material was then allowed to dry under vacuum at RT for 2 d. Images were recorded by using an Autoprobe CP Base Unit di CP-II instrument (model no. AP-0100).

*X-ray diffraction (XRD)*: The experiment was carried out by using a dried sample of fluorescent hydrogel–Ag nanocomposite by using an X-ray diffractometer (Bruker D8 Advance) equipped with a conventional

Cu<sub>Ka</sub> X-ray radiation ( $\lambda$ =1.54 Å) source and Bragg diffraction setup (Seifert 3000P).

*MALDI mass spectrometry*: Mass spectrum of silver nanoclusters encapsulated hydrogel was recorded on a MALDI-TOF mass spectrometer (Applied Biosystems 4700 Proteomics Analyser 170).

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