Fabrication of worm-like Ag₂S nanocrystals under mediation of protein

DEZHI QIN¹, LI ZHANG^{1,*}, XIAN DU¹, GUANGRUI YANG² and QIUXIA ZHANG¹

¹College of Chemistry and Chemical Engineering, Pingdingshan University, Pingdingshan 467000, China

²Institute of Environmental and Municipal Engineering, North China University of Water Conservancy and Electric Power, Zhengzhou 450011, China

MS received 15 May 2015; accepted 24 June 2015

Abstract. A simple protein-assisted method was reported to synthesize pepsin-conjugated Ag₂S nanocrystals in aqueous solution. The morphology, composition and structure of the products were characterized by transmission electron microscopy, high-resolution transmission electron microscopy, energy-dispersive spectroscopy, selected area electron diffraction and X-ray diffraction measurements. The results showed that as-prepared monoclinic Ag₂S nanocrystals are worm-like nanochains in shape with sizes about 25 nm in diameter and up to hundreds of nanometres in length. The multiple coordinate bonds of pepsin molecules to the surface of Ag₂S nanocrystals make as-prepared samples have good colloidal stability and biocompatibility as elucidated by Fourier transform infrared examination. Thermogravimetry–differential scanning calorimetry analysis indicated that the obtained products are inorganic–organic nanocomposites and there is strong interaction between Ag₂S and pepsin. This interaction could result in the change of hydrophilic environment of pepsin and consequently intrinsic fluorescence of protein was quenched by Ag₂S nanocrystals. Furthermore, the nanochains assembly of particle–particle and rod–rod oriented attachment was discussed to investigate the growth mechanism.

Keywords. Silver sulphide; biomimetic; pepsin; nanocrystals.

1. Introduction

The preparation of semiconductor nanocrystals with different shapes has attracted considerable attention owing to their novel shape- and size-dependent electronic and optical properties.^{1,2} Chalcogenide nanocrystalline materials, especially I–VI and II–VI semiconductors have been widely applied in the fields of photovoltaics, electroluminescence devices, bioimaging agents, light-emitting devices, catalysis, sensors, solar energy cells, etc.^{3–6} It is evident that there is a relationship between specific morphology of nanocrystals and their corresponding unique performance in chemistry and materials science. In recent times, materials scientists have synthesized chalcogenide nanostructures with special morphology such as nanowire, nanrod, nanobelt, nanocube, nano-core–shell, nanochain, nanoflake, nanotube and hierarchical nano-structure via various methodologies.^{6–11} The preparation of these nanostructures usually needs harsh operating conditions such as high synthetic pressure and temperature or strong acid and alkali solutions; the products require toxic organic solvents or ligands such as thiols or polyphosphates as stabilizing agents for preventing agglomeration. The development of simple, mild and environment-friendly synthetic routes is of importance to nanotechnology and remains a key research challenge.

Biomaterialization research suggested biomolecules could be exploited to control nucleation and growth of inorganic

materials and thus manipulate their morphology, physical and chemical properties.^{12–14} Inspired by this theory, many kinds of biomolecules including proteins, DNAs, aminoacids, peptides, polysaccharides have been extensively used as soft matrix for the fabrication of chalcogenide nanocrystals with complicated structures and unique optical properties.^{15,16} In recent times, sea urchin-like γ -MnS, octahedron-like α -MnS nanocrystals and CdS nanotubes have been successfully synthesized by aminoacid-assisted biomimetic methods.^{17,18} Huang *et al.*¹⁹ prepared bovine serum albumin (BSA)-conjugated M_xSe_y (M = Ag, Cd, Pb, Cu) nanomaterials with different morphologies via solution-phase, green chemistry approach. Komarneni and co-workers²⁰ prepared multi-assembly architectures of complex PbS based on primary nanorods structure by a simple and general biomimetic route. The conjugation of biomolecules enables chalcogenide nanocrystals to have potential application in biology and medicine such as anti-neoplastic agents, immunoassay, biological sensors, biological labelling, etc. For example, biomolecules-conjugated CdTe, CuS, Ag₂S, ZnS nanoparticles and PbS/Ag₂S core/shell nano-composites have been proven to hold great promise for cancer imaging and therapy.^{6,21–24} Bioconjugates of HgS nanoparticles and protein were prepared by using antibody goat anti-human IgG as a scaffold and a new fluorescence immunoassay method was established for the ultrasensitive detection of the concentration of human IgG in human serum.²⁵ Kotov and co-workers⁷ designed biological assembly of nanocircuit from protein-modified CdTe

* Author for correspondence (lizhangpds@126.com)

nanowires in aqueous media. Yang *et al*²¹ synthesized NIR-II-emitting ultrasmall Ag₂S QDs with good biocompatibility directly in aqueous phase through a facile biomimetic sol-gel route at room temperature; as-prepared QDs could be as a new kind of fluorescent label for *in vivo* imaging. In biomimetic methodology, protein-assisted synthesis, due to nanoscale dimensions, distinctive molecular structures and fascinating self-assembly functions, has been found to be a mild, environmentally benign route to produce sulphide nanostructures.

Meanwhile, more studies on interaction between proteins and nanoparticles were investigated through various methods. According to modern biology point of view, proteins are the main building material basis of life, which have complicated spatial structure and a great many of functional groups such as N-H, O-H, S-H, S-S, C=O, etc.^{15,26} These features of proteins could control the growth rate of nanocrystals by retarding the heterogeneous reaction rate with a more strongly binding functional groups or steric hindrance, which is similar to amphiphilic surfactants. Generally, detailed knowledge of physical and chemical aspects associated with the behaviour of nanoparticles in biological systems has been recognized as critically important for exploring their nano-toxicology *in vivo* and application in biomedicine field.²⁷ Some researchers have reported the interaction of BSA and chalcogenide nanomaterials (CdS, CdSe, Ag₂S, HgS) via ultraviolet-visible (UV-vis), photoluminescence (PL), Fourier transform infrared (FT-IR) and circular dichroism (CD) spectrometry.^{4-6,8,28}

Due to direct, narrow bandgap and relatively large absorption coefficient, Ag₂S exhibits great potential in a wide range of applications, such as near-IR photon detectors, photovoltaic cells and superionic conductors.^{21,29,30} A growing number of studies suggested that nanomaterials might dissolve in the biological matrix, which could cause severe toxic effects for biological organisms and tissues.³¹⁻³³ Thus, the study of synthesizing water-soluble nanocrystals with low nano-toxicity is a hot spot in nanoscience. There have been studies already conducted on fluorescent labelling for *in vivo* imaging of Ag₂S QDs.²¹ Due to an ultra-low solubility product constant ($k_{sp} = 6.3 \times 10^{-50}$), Ag₂S ensures the minimum amount of Ag ions released into the biological surroundings; the results of some studies suggested negligible toxicity of Ag₂S in biosystems through the cytotoxicity and haemolysis examination.^{5,34,35} Biomimetic synthesis is appropriate method for fabricating Ag₂S nanostructure by using proteins as matrix, which could reduce nano-toxicity and improve biocompatibility of nanocrystals. The globular protein pepsin is a proteolytic enzyme; it can degrade food proteins into peptides in acidic environments, which has been used as soft template to prepare inorganic materials *in vitro*. For instance, Kato *et al*³⁶ reported pepsin enzyme-catalysed precipitation of silica, synthesized via a bio-inspired sol-gel chemistry route in an ionic liquid. Self-assembly of flower-like superstructures of CaCO₃ were synthesized at air-water interface regulated by pepsin Langmuir monolayers as the biomimetic matrix.³⁷ Herein, a facile, mild and green route

has been reported to the synthesis of Ag₂S nanocrystals conjugated with pepsin in aqueous solution; the influence of protein molecules on the formation of as-prepared products was also investigated through spectroscopic techniques.

2. Experimental

2.1 Materials

Silver nitrate (AgNO₃, >99%), thioacetamide (TAA, >99%) and other routine chemicals were purchased from Sinopharm Chemical Regent Co., Ltd., P.R. China. Pepsin was of electrophoretic purity, purchased from Sigma. All the reagents were used as-received without any further purification. Water was used after purification through double distillation.

2.2 Synthesis of pepsin-conjugated worm-like Ag₂S nanocrystals

In a typical process, 0.5 g pepsin was dissolved in 150 ml of distilled water to form a homogeneous solution, then 0.42 g AgNO₃ was added into pepsin aqueous solution and transferred to a 250 ml round-bottom flask. The mixture of Ag⁺-pepsin emulsion was stirred gently under N₂ atmosphere for 8 h at 37°C (physiological condition). Thereafter, 0.094 g TAA was dissolved in 50 ml water and added drop-wise into mixed solution of Ag⁺-pepsin with vigorous stirring at 37°C for 30 min; then the reaction system was kept static for 72 h. During the course of reaction, the colour of solution changed from colourless to greyish black rapidly and finally to black, indicating the formation of colloidal Ag₂S crystals. The resulting black products were collected by high speed centrifuging, washed several times with distilled water and ethanol, and then dried in a vacuum for 48 h. The whole process of experiment should be away from light.

2.3 Characterizations

The crystal structure and composition of sample were characterized powder X-ray diffraction (XRD), selected area electron diffraction (SAED), energy-dispersive X-ray spectrum (EDS) and thermogravimetry-differential scanning calorimetry (TGA-DSC) measurements. The size and morphology of nanocrystals were obtained by using transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) observations. The interaction between Ag₂S nanocrystals and protein molecules was investigated by FT-IR and PL spectroscopy. The optical property of samples was examined by UV-vis spectra. XRD pattern was carried out on a Bruker D8 Advance X-ray diffractometer using Cu K α ($\lambda = 0.15406$ nm) radiation. TEM, HRTEM, SAED and EDS measurements were observed by means of a JEOL JEL-2010 transmission electron microscope equipped with an X-ray energy-dispersive detector.

FT-IR spectra were taken on a Bruker Tensor-37 spectrophotometer in the wavenumber range of 4000–1000 cm⁻¹. UV-vis spectra were examined by a Shimadzu UV-2550 spectrophotometer equipped with a 10 mm quartz cell. PL spectra were acquired with a Hitachi F7000 spectrophotometer. TGA–DSC analysis was performed with a TA Q-600 thermogravimetric apparatus under a stream of N₂.

3. Results and discussion

XRD analysis was used to examine the structure phase of the Ag₂S nanocrystals. Figure 1a shows powder XRD pattern of the pepsin-conjugated Ag₂S nanocrystals. The position and relative intensity of diffraction peaks match well with monoclinic phase α -Ag₂S and a broad non-crystalline peak (20–25°) is assigned to pepsin biopolymer. Meanwhile, there are no impure peaks detected from XRD pattern, clearly indicating the high purity of as-prepared sample. The broadening of diffraction peaks may be attributed to the small size of nanoparticles. From XRD pattern, it is found that the Ag₂S nanocrystals are of poor crystallinity because of the coating of protein molecules. The EDS spectrum of Ag₂S nanocrystals is shown in figure 1b, which confirms the presence of elements Ag and S in the sample. Furthermore, the average ratio of atomic percentage of Ag/S is 68.6 : 31.4 showing that as-prepared products are Ag rich.

Figure 2a shows representative TEM image of protein-conjugated Ag₂S nanocrystals in pepsin solution from the typical experiment. It can be clearly observed that the Ag₂S crystals form parallel aligned worm-like nanochains with average sizes of about 15–25 nm in diameter and hundreds of nanometres in length. The worm-like nanochains are composed of several adjacent nanoparticles and short nanorods attached with connective interfaces. The as-prepared Ag₂S nanocrystals present narrow size distribution and good

dispersivity. The slow release of S ions could control the rate of nucleation and growth, which was the basis for the formation of Ag₂S nanocrystals. The control experiment was carried out using sodium sulphide instead of TAA as the sulphur source while keeping other reaction condition the same, only irregular particles and network structures were obtained, which have been proven in other studies.³⁸ In HRTEM picture (figure 2b), the regime with low contrast is probably a pepsin bio-film layer, which encapsulates the nanoparticles, as indicated by the arrows. Biomolecule coating on the Ag₂S nanocrystals makes the products water-dispersible, biocompatible, and reduces nano-toxicity of Ag₂S. Protein conjugation contributes not only to stabilizing and dispersing the Ag₂S nanocrystals, but also to controlling the formation of the regular geometry. The SAED pattern (figure 2d) of Ag₂S nanocrystals simultaneously obtained in the TEM measurement shows distinct diffraction rings of monoclinic α -Ag₂S phase and randomly oriented, which is consistent with the results of XRD. The dependence between pepsin concentration and sample's morphology was investigated through a group of control experiments. As the concentration of pepsin was increased from 5 to 8 g l⁻¹, the diameter of nanochains increase accordingly, and the morphology of products began to transform from worm-like structure to cross-linked networks with fractal geometry, as shown in figure 2c. It was speculated that a high pepsin concentration could reduce the amounts of free Ag ions in solution to form seed crystals; therefore, the fewer the seeds, the larger the final nanocrystals. When the concentration of pepsin was decreased to 2 g l⁻¹, only disordered aggregation of nanorods and nanoparticles was obtained rather than uniform worm-like nanochains, which is not shown in the figure. Lower concentration of pepsin might reduce the extent of protein coating on Ag₂S nanoparticles, so there has not been an intense electrostatic repulsive force against agglomeration of particles. As a result, over-high or low

Figure 1. (a) XRD pattern and (b) EDS spectrum of pepsin-conjugated Ag₂S nanocrystals.

Figure 2. Typical TEM images of Ag₂S nanocrystals synthesized at different concentrations of pepsin solution (**a.** 5 g l⁻¹, **c.** 8 g l⁻¹), (**b**) HRTEM image and (**d**) SAED pattern of Ag₂S nanocrystals ($c_{\text{pepsin}} = 5 \text{ g l}^{-1}$).

concentrations of pepsin are barely sufficient to effectively control the growth of Ag₂S crystals, which is not good for the formation of well-dispersed worm-like Ag₂S nanochains.

Interactions between nanomaterials and biomolecules are highly complex on a molecular scale. FT-IR spectroscopy is a useful technique to research the structural changes of protein in protein-ligand complex. To understand the formation of Ag₂S nanocrystals in pepsin aqueous solution, the FT-IR spectra of pure pepsin and pepsin-conjugated Ag₂S were determined; the corresponding spectra and data of main IR peaks are shown in figure 3 and table 1. The spectra analysis indicates that Ag₂S has no characteristic adsorption peak in this area, hence the interaction between pepsin and Ag₂S by comparing the IR peaks of pepsin before and after reacting with Ag₂S nanoparticles was studied. The IR frequency of O-H and C-H stretching vibration of pepsin-Ag₂S shifts to high wavenumber of 15 and 17 cm⁻¹, compared with those of pure pepsin. As a soft Lewis acid, Ag ion has a low affinity for O-H and C-H groups; there might be electrostatic attraction between them. The intensity of amide II band weakens in pepsin-conjugated Ag₂S nanocrystals; even there is no obvious infrared adsorption to observe in FT-IR spectrum. Amide II band is mainly induced by the coupling of bending vibrates of N-H and stretching vibrates of C-N, which suggests that there is strong coordination interaction between Ag₂S and functional groups of pepsin.

TGA-DSC curves of Ag₂S nanocrystals synthesized in pepsin solution are shown in figure 4. From the TGA curve, the weight of sample decreases with a rise of temperature. The DSC curve shows that there are three stages of weight loss. The first stage of the decomposition observed

Figure 3. FT-IR spectra of (**a**) pure pepsin and (**b**) pepsin-conjugated Ag₂S nanocrystals.

Table 1. The main peaks of pure pepsin, pepsin-Ag₂S reaction systems from FT-IR spectra.

Assignment	O-H	C-H	Amide I	Amide II	C-N
Pure pepsin	3385	2920	1642	1547	1386
Pepsin-Ag ₂ S	3400	2937	1638	—	1385

Figure 4. TGA-DSC curves for pepsin-conjugated Ag₂S nanocrystals.

up to 160°C is due to the loss of absorbed moisture in pepsin-conjugated Ag₂S nanocrystals. The second stage of the weight loss of 0.97% from 160 to 280°C with the DSC peak at 255°C is mainly owing to the escape of small molecules in protein. From 280 to 580°C, the mass of the sample decreased by about 4.85% and there was an exothermal process in DSC curve. The third weight loss corresponds to the pyrolysis of pepsin embedded into the nanocrystals. The results of thermo-analysis confirm the existence of pepsin in products after high speed centrifuging and the

Figure 5. TEM images of Ag₂S nanocrystals obtained in pepsin solution after different aging times: (a) and (b) 10 min, (d) 1 h, (e) and (f) 12 h, (g) 48 h and (h) 30 d, respectively; (c) SAED pattern of sample grown in pepsin solution for 10 min.

strong conjugate bonds between protein molecules and Ag₂S nanocrystals.

Further studies suggested that the morphology of products was strongly dependent on reaction time. In order to illuminate the formation mechanism of pepsin-conjugated worm-like Ag₂S nanocrystals, the real-time TEM observation experiments were performed to monitor the morphological evolution. Figure 5 presents the typical TEM images of Ag₂S nanocrystals obtained in pepsin solution for different treatment times. The morphology of the sample in the beginning stage of reaction is displayed in figure 5a. From this image, it can be observed that the products were composed of sheet-like materials and very small particles. There was no obvious lattice fringe could be observed in the corresponding HRTEM image (figure 5b), and the associated SAED pattern (figure 5c, inset of figure 5b) taken from these Ag₂S nanoparticles exhibits extremely weak diffraction rings, which are suggestive of the presence of quasicrystals or amorphous Ag₂S at the early stage. Prolonging the reaction time to 1 h, it was presented that most of amorphous materials transformed into nanocrystalline particles and the nanoparticles grew larger (~5–15 nm; figure 5d). Figure 5e shows TEM images of Ag₂S nanocrystals after aging time for 12 h. From TEM image, peanut-like nanoassemblies and short nanorods were generated during the growth process of Ag₂S nanocrystals. Moreover, the HRTEM image shown in figure 5f (inset of figure 5e) clearly demonstrates that these nanostructures were connected by spherical nanoparticles.

Yang and co-workers⁸ presented a particle–particle oriented growth mechanism of nanocrystals, which suggested that the adjacent nanoparticles partly shared a common crystallographic orientation; and these particles rotated to find the identical crystallographic plane and finally fused to form nanorods. With the reaction time increased to 48 h, these short nanorods could expand and eventually self-assemble themselves into interconnected worm-like continuous nanoarchitectures, as displayed in figure 5g. After storage for 30 days, the products remained worm-like nanochains suggesting the non-aggregation and high stability of Ag₂S colloidal solution under pepsin protection (figure 5h). Nonetheless, the diameter of nanochains became nonuniform (20–60 nm) and cavernous structure appeared inside the crystals. The strong contrast between the dark and pale areas in HRTEM image shown in figure 5i (inset of figure 5h) is the evidence of their porous structure. According to the above results, the coordination effect between Ag ions and protein molecules plays a crucial role in the formation of the Ag₂S nanocrystals. At the beginning, when the two solutions containing AgNO₃ and pepsin were mixed together under stirring, the initial precursor complex pepsin–Ag⁺ was formed through the coordinate bonds between Ag⁺ and functional groups of pepsin. The concentration of the Ag⁺ around pepsin molecule was very high because of this interaction; a slow spontaneous heterogeneous nucleation process of Ag₂S took place at the protein molecules interface when the TAA was added to pepsin–Ag⁺ solution. The initial amorphous

Figure 6. UV-vis absorption spectra of pepsin-conjugated Ag_2S nanocrystals: (a) 10 min, (b) 1 h and (c) 48 h.

Ag_2S crystallized into nanoparticles and then gradually grew to form short nanorods by particle-particle oriented attachment. Finally, the thermodynamically stable worm-like nanostructures were favoured through rod-rod connect process. Such morphology of nanocrystals may be related to the secondary structure of pepsin, primarily dominated by β -sheet configuration.³⁹ More hydrophilic groups of protein molecules could be exposed to conjugate with Ag_2S and provided great steric hindrance to control the shape of nanocrystals. When the reaction duration was prolonged enough, the dissolution of nanocrystals interior occurred and transformed to the external interface via Ostwald ripening due to thermodynamic difference,⁴⁰ which resulted in the porous structure and inhomogeneous sizes of Ag_2S nanochains. More interestingly, Cui and co-workers³⁵ reported a novel transformation of Ag_2S nanoparticles from tiny solid particles to hollow particles and finally to solid spheres in RNase A solution. The exact mechanism for the interfacial molecular recognition of pepsin and Ag_2S nanocrystals is not clear at the moment and the influences of protein sequence, size, configuration, charge and self-organization are still being investigated.

In addition, the growth of worm-like nanochains was tracked by UV-vis spectra at several time intervals. The evolution of the UV-vis absorption peak of pepsin-conjugated Ag_2S nanocrystals is shown in figure 6. The UV-vis absorption spectra at different aging times present structureless curves with gradually increasing absorbance towards shorter wavelengths. The absorption edges of Ag_2S nanocrystals are located at 500–550 nm, which are blue-shifted considerably relative to the adsorption onset of bulk Ag_2S . Such a blue shift is associated with quantum size effect due to confinement of electron and hole in a small volume. The direction of the arrow indicates the absorption edges red shift during the course of the Ostwald ripening with the increase of reaction time.⁴¹

Figure 7 shows the PL spectra of pepsin before and after conjugating with Ag_2S nanocrystals. For pepsin molecules, the presence of Trp, Tyr and Phe residues gives it intrinsic fluorescence emission, which can be as a probe to detect the conformational transition of protein.⁴² As seen in figure 7a, pepsin has a strong fluorescence emission band at the range

Figure 7. Photoluminescence (PL) spectra of (a) pepsin and (b) pepsin-conjugated Ag_2S nanocrystals.

of 300–400 nm by fixing the excitation at 295 nm. However, as the Ag_2S nanocrystals generated, the fluorescence intensity of pepsin drastically decreased, which is interpreted that Ag_2S could interact with pepsin and quench its intrinsic fluorescence. A red shift (from 348 to 360 nm) was also observed for the maximum emission wavelength, which suggested the formation of nanoparticles had influence on microenvironment of pepsin.

4. Conclusion

In summary, hybrid protein-conjugated worm-like Ag_2S nanochains have been successfully fabricated in the presence of pepsin followed by a particle-particle and rod-rod assembly process. The whole synthetic process is facile, effectively, and environmentally friendly, and the obtained Ag_2S nanoparticles have the characteristics of low-toxicity and bio-compatibility which are important issues in biomedical applications. The protein molecules play a crucial role in the formation of Ag_2S nanochains, as primary secondary structure of pepsin, β -sheet conformation presents steric hindrance to regulate the morphology of the products. The Ag_2S nanocrystals coordinated with functional groups of pepsin, which could change microenvironment of protein and quench its intrinsic fluorescence.

Acknowledgements

This work was supported by the Key Project of Henan Province Higher Educational Science and Technology Program (15A150070) and the Key Nature Science Foundation of Pingdingshan University (PDSU-QNJJ-2013003). We also thank the referees for helpful comments.

References

1. Sapra S, Mayilo S, Klar T A, Rogach A L and Feldmann J 2007 *Adv. Mater.* **19** 569
2. Pan D C, Wang X L, Zhou Z H, Chen W, Xu C L and Lu Y F 2009 *Chem. Mater.* **21** 2489
3. Rai S and Bokatial L 2011 *Bull. Mater. Sci.* **34** 227
4. Ghosh D, Mondal S, Ghosh S and Saha A 2012 *J. Mater. Chem.* **22** 699
5. Priya R and Kanmani S 2010 *Bull. Mater. Sci.* **33** 85
6. Wang H J, Yu X H, Cao Y, Zhou B and Wang C F 2012 *J. Inorg. Biochem.* **113** 40
7. Wang Y, Tang Z, Tan S and Kotov N A 2005 *Nano Lett.* **5** 243
8. Yang L, Yang H, Yang Z, Cao Y, Ma X, Lu Z and Zheng Z 2008 *J. Phys. Chem. B* **112** 9795
9. Zhang X, Yang L, Jiang Y, Yu B B, Zou Y G, Fang Y, Hu J S and Wan L J 2013 *Chem. Asian J.* **8** 2483
10. Qin D, Zhang L, He G and Liu Q 2011 *Mater. Lett.* **66** 7
11. Mahdi M A, Hassan J J, Kasim S J, Ng S S and Hassan Z 2014 *Bull. Mater. Sci.* **37** 337
12. Mann S 1993 *Nature* **365** 499
13. Boskey L 1998 *J. Cell. Biochem.* **72** 83
14. Aizenberg J 2004 *Adv. Mater.* **16** 1295
15. Dickerson M B, Sandhage K H and Naik R R 2008 *Chem. Rev.* **108** 4935
16. Briggs B D and Knecht M R 2012 *Phys. Chem. Lett.* **3** 405
17. Jiang J, Yu R, Zhu J, Yi R, Qiu G, He Y and Liu X 2009 *Mater. Chem. Phys.* **115** 502
18. Liu X L, Zhu Y J, Zhang Q, Li Z F and Yang B 2012 *Mater. Res. Bull.* **47** 4263
19. Huang P, Bao L, Yang D, Gao G, Lin J, Li Z, Zhang C and Cui D 2012 *Chem. Asian J.* **6** 1156
20. Lu Q, Gao F and Komarneni S 2006 *Nanotechnol.* **17** 2574
21. Yang H Y, Zhao Y W, Zhang Z Y, Xiong H M and Yu S N 2013 *Nanotechnol.* **24** 055706
22. Kong Y, Chen J, Gao F, Li W, Xu X, Pandoli O, Yang H, Ji J and Cui D 2010 *Small* **6** 2367
23. Guo Y, Zhang J, Yang L, Wang H, Wang F and Zheng Z 2010 *Chem. Commun.* **46** 3493
24. Cao Y, Wang H J, Gao C, Sun Y Y, Yang L, Wang B Q and Zhou J G 2011 *J. Nanopart. Res.* **13** 2759
25. Liu X, Liu R, Tang Y, Zhang L, Hou X and Lv Y 2012 *Analyst* **137** 1473
26. Gray J J 2004 *Curr. Opin. Struct. Biol.* **14** 110
27. Treuel L, Eslahian K A, Docter D, Lang T, Zellner R, Nienhaus K, Nienhaus G U, Stauber R H and Maskos M 2014 *Phys. Chem. Chem. Phys.* **16** 15053
28. Ghosh D, Mondal S, Roy C N and Saha A 2013 *Phys. Chem. Chem. Phys.* **15** 20354
29. Pathan H M, Salunkhe P V, Sankapal B R and Lokhande C D 2011 *Mater. Chem. Phys.* **72** 105
30. Xu Z, Bando Y, Wang W, Bai X and Golberg D 2010 *ACS Nano* **4** 2515
31. Misra S K, Dybowska A, Berhanu D, Luoma S N and Valsami-Jones E 2012 *Sci. Total Environ.* **438** 225
32. Wataha J C, Hanks C T and Craig R G 1993 *J. Biomed. Mater. Res.* **27** 227
33. Xu C, Yuan Z, Kohler N, Kim J, Chung M A and Sun S 2009 *J. Am. Chem. Soc.* **131** 15346
34. Chen J, Zhang T, Feng L, Zhang M, Zhang X, Su H and Cui D 2013 *Mater. Lett.* **96** 224
35. Chen J, Kong Y, Ji J, Ruan J, Wang K, Gao F and Cui D 2012 *Nanoscale* **4** 4455
36. Kato K, Kawachi Y and Nakamura H 2014 *J. Asian Ceram. Soc.* **2** 33
37. Xue Z, Hu B, Dai S and Du Z 2012 *Mater. Chem. Phys.* **136** 771
38. Kuang D, Xu A, Fang Y, Liu H, Frommen C and Fenske D 2003 *Adv. Mater.* **15** 1747
39. Kamatari Y O, Dobson C M and Konno T 2003 *Prot. Sci.* **12** 717
40. Liu B and Zeng H C 2005 *Small* **1** 566
41. Priyam A, Chatterjee A, Das S K and Saha A 2005 *Res. Chem. Intermed.* **31** 691
42. Chatterjee A, Priyam A, Ghosh D, Mondal S, Bhattacharya S C and Saha A 2012 *J. Lumin.* **132** 545