Cobalt oxide hollow nanoparticles derived by bio-templating

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We present here the first fabrication of hollow cobalt oxide nanoparticles produced by a protein-regulated site-specific reconstitution process in aqueous solution and describe the metal growth mechanism in the ferritin interior.

There has been great interest in the synthesis and characterization of hollow metal nanostructures due to their surface plasmonic properties and catalytic activities, which are different from their solid counterparts.[1–3] Most of the previous works utilize large hollow nanostructures of several hundreds of nanometers. Very recently, Xia and co-workers[2] and Alivisatos and co-workers[3] demonstrated the preparation of hollow nanostructures on the scale of tens of nanometers by a replacement reaction with a solid Ag template and a nanoscale Kirkendall effect, respectively. However, the fabrication process is complicated and requires organic solvents. Here we report the fabrication of hollow transition-metal oxide nanoparticles using ferritin protein in an aqueous solution and propose a mechanism for the metal growth in ferritin. The ferritin protein cage serves as a biocompatible and protective layer of the magnetic nano-material, making it suitable for a large variety of biomedical applications. The potential applications of magnetic nano-materials include enhanced MRI diagnostic contrast agents,[4] tumor hyperthermia therapies,[5] retinal detachment therapies,[6] and magnetic field-guided drug delivery system and radioactive therapies.[7]

It is difficult to form stable hollow nanoparticles and even solid nanoparticles due to the strong magnetic interactions in magnetic transition-metal particle systems. The magnetic properties of the nanoparticles strongly depend on the particle size, the precise crystal structure, and the presence of defects. Despite their weaker magnetic properties, metal oxide nanoparticles were used in many applications due to the air sensitivity of the magnetic transition-metal nanoparticles.[8] In this work, ferritin is used as a biotemplate to produce hollow nanoparticles and as a separator between the hollow nanoparticles. The horse spleen ferritin (HoSF, Sigma) used in this work is a natural iron storage protein that is composed of 24 subunits that form a segmented hollow protein shell with an outer diameter of 12 nm and an inner diameter of 8 nm.[9] Ferritin has hydrophobic and hydrophilic molecular channels through the protein shell which enable the removal of the inorganic phase *in vitro* by reduction and chelation. Through these molecular channels we can easily replace the metal core with different metals. Site-specific reconstitution of ferritin molecules with other metals[10,11] and semiconductors[12] in the ferritin cavity has been reported. Also, fabrication of nanoparticles has been demonstrated previously using different types of biotemplates such as viruses, heat shock proteins, and enzymes.[13] The protein cage of ferritin is very stable and robust which allows constrained material synthesis. However, previous studies using the ferritin cage were focused on demonstrating the fabrication of solid nanoparticles through reconstitution of the ferritin iron core with different metals rather than investigating the mechanism of the metal growth.

In this contribution, we report on the mechanism of cobalt oxide growth in apoHoSF (Sigma), which has a protein shell without a core, and the fabrication of hollow nanoparticles by controlling the number of metal atoms in the ferritin. The procedure of ferritin reconstitution with cobalt oxide is based on a method described by Douglas and Stark[11] with a modification to control the number of cobalt ions added to the ferritin. ApoHoSF solution (1 mg ml^{-1}) was adjusted to pH 8.5 in 25 mM MOPS buffer with 50 mM NaCl. $CoSO_4$ (50 mM) was used as a cobalt source and added to apoHoSF solution, followed by addition of an excess amount of H_2O_2 (3 vol%). Cobalt was added very slowly to achieve a certain loading of metal atoms evenly distributed among the ferritins. Reconstituted ferritins were prepared with 200 (Co_{200}) to 2000 Co (Co_{2000}) atoms per ferritin in increments of 200 atoms.

Field emission-scanning electron microscopy (FE-SEM, Hitachi S-5200) equipped with scanning transmission electron microscopy (STEM) and transmission electron microscopy (TEM, Hitachi HF-2000) were used to characterize the reconstituted Co-cored ferritins. The specimen of Co-cored ferritins immobilized on a holey carbon coated copper calibration grid (Ted Pella, Inc.) was thoroughly rinsed with doubly distilled, deionized water, dried in a vacuum atmosphere, and then subjected to the microscopic analysis. The operation of STEM and TEM was carried out at an acceleration voltage of 25 keV and 200 keV, respectively, in order to obtain the images. Fig. 1 shows STEM and TEM images of reconstituted Co-cored ferritins with 200 Co atoms on the TEM grid. When the ferritin has 200 Co atoms in the interior, several nanoparticles of cobalt oxide with mean diameters of 1.8 nm are formed in the interior (Fig. 1(a), (b) and (c)). Fig. 1(d) shows a nanoparticle diameter histogram analyzed with Fig. 1(a) by Image Pro Plus 5.0 software. STEM imaging allows us to see only the metal cores inside the ferritin cage due to the relatively low density of the protein shell. We believe the metal ions enter through the hydrophilic channels along the three-fold symmetric axis of the protein[9] and combine with the carboxylate groups of glutamic acid residues on the ferritin interior wall, forming discrete nanoparticles during the oxidative reaction. Once these seeds of metal oxides

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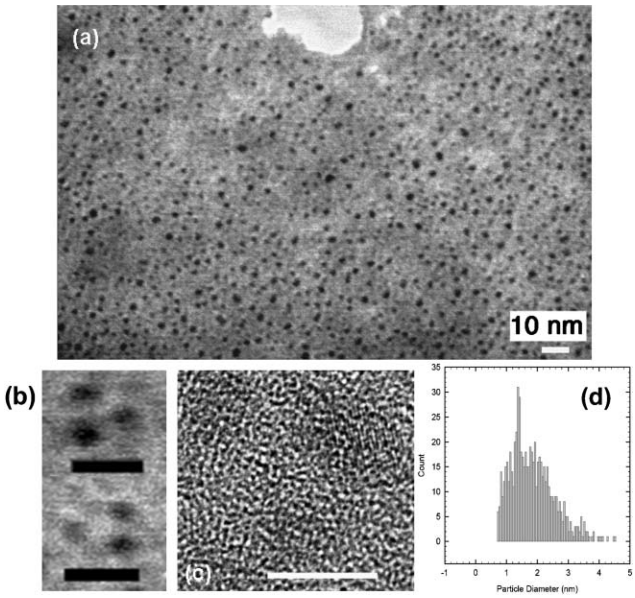

Fig. 1 STEM images of (a and b) Co_{200}-ferritins. (b) Magnified images of Co_{200} metal oxide core in a ferritin. (c) High-resolution lattice image of Co_{200}-cored ferritin. Scale bars in both (b) and (c) are 5 nm. (d) Histogram of cobalt oxide nanoparticle size distribution in the Co_{200}-ferritins.

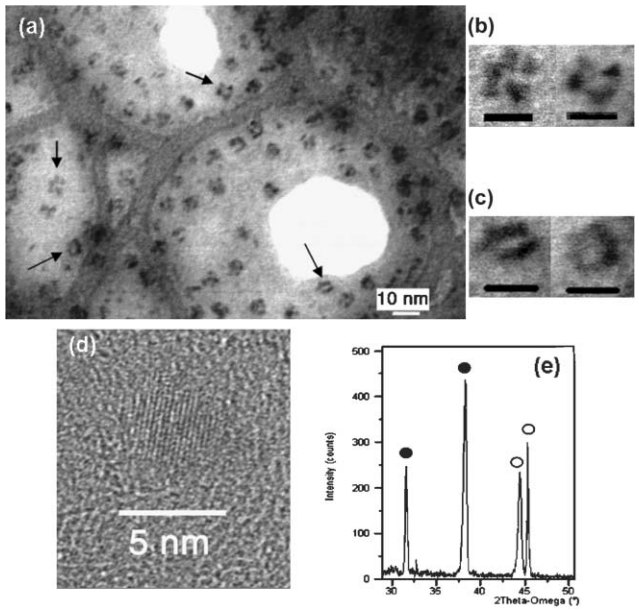

Fig. 2 STEM image of (a) Co_{1000}-cored ferritins. (b) and (c) Magnified images of Co_{1000} (arrowed particle in a image) metal oxide core in a ferritin. Scale bar is 5 nm. (d) High-resolution lattice image of Co_{1000}-cored ferritin. (e) Wide-angle XRD patterns of Co_{1000}-cored ferritins on the Si (004) substrate. ●: Co_3O_4, ○: CoOOH.

form the nanoparticles inside the ferritin, they grow autocatalytically along the ferritin interior wall and then merge together upon further addition of Co(II) and H_2O_2. When the ferritin contains over 1000 Co atoms, the cobalt oxide forms a hollow nanoparticle (Fig. 2(a), (b) and (c)). The phase of the Co_{1000}-cored ferritin film on a Si (004) substrate was characterized by powder X-ray diffraction (XRD, PANalytical X'pert PRO MRD) and a typical XRD pattern is shown in Fig. 2(e). XRD analysis shows the presence of cobalt oxide mixtures indexed to the Co_3O_4 (220), Co_3O_4 (311) (indexed from the Joint Committee on Powder Diffraction Standard (JCPDS) card No. 78-1970), CoOOH (101), and CoOOH (012) (JCPDS card No. 78-11213) consistent with previously reported cobalt oxide minerals.[14] The high-resolution TEM (Fig. 2(d)) shows clear cobalt oxide lattice planes with a spacing of 2.41 Å, close to d_{311} = 2.35 Å spacing expected for Co_3O_4 from XRD data. The STEM images of 2000 Co atoms contained in the ferritin show a clear circle with a hollow core, indicating merging between the nanoparticles (Fig. 3). The wall thickness and the size of hollow Co_{2000} core are somewhat larger than those of the Co_{1000} core. Finally, we note that the size of the nanoparticles ($\sim$6 nm) is less than the inner diameter of the ferritin core. This observation excludes the possibility that the hollow nanoparticle forms on the exterior surface of the ferritin.

The ferritin solution mineralized with Co(II) and H_2O_2 showed a homogeneous olive-green color from the specific oxidative mineralization process at pH 8.5. UV-Vis spectra were obtained on a Perkin-Elmer Lambda 900. The ferritin protein in microquartz cuvettes was adjusted to the final concentration of 0.33 mg ml^{-1} by dilution with 25 mM MOPS buffer at pH 8.5. The spectra were measured from 900 to 200 nm at a scan speed of 150 nm min^{-1}. The UV-Vis absorption spectra exhibited well-defined peaks at 280 nm (protein) and 350 nm corresponding with cobalt oxides, most likely cobalt oxyhydroxide (CoOOH) (Fig. 4).[11,15] Addition of Co(II) and H_2O_2 to the apoHoSF solution

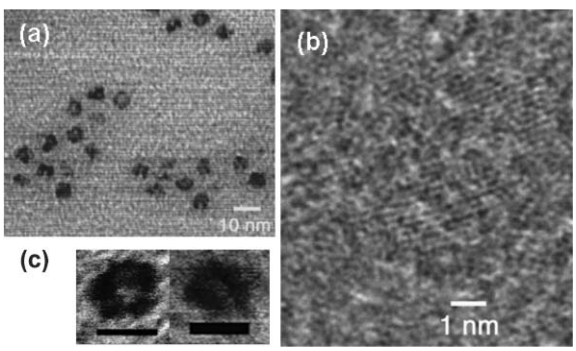

Fig. 3 STEM image of (a) Co_{2000}-cored ferritins. (b) and (c) Magnified TEM and STEM images of Co_{2000} metal core in a ferritin, respectively. Scale bar in (c) is 5 nm.

resulted in the convolution of protein absorption band at 280 nm with an absorption band at 225 nm with a new absorption band at 350 nm. These two peaks are related to the core material, which is shifted to higher wavelengths as the number of metal atoms in the ferritin increases.

Based on the results obtained in this study, we propose a metal growth mechanism in ferritin as outlined in Fig. 5. The first step of metal core formation is the nucleation of small nanoparticles in the interior of the ferritin protein shell. Normally, eight hydrophilic channels along the three-fold symmetric axis of the protein shell are considered to be the pathways of metal ions into the ferritin interior.[9] Once the metal ions enter the ferritin interior, they form chemical bonds with the functional groups in the interior wall of the ferritin during the oxidation process of the metal ions. The most likely scenario is that oxidized metal oxides are associated with the carboxylate groups of glutamic acid residues on the interior protein wall.[9] The cobalt mineral core is thus attached to

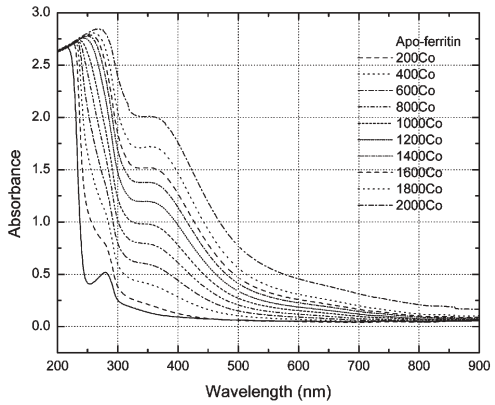

Fig. 4 UV-Vis absorption spectra of apoferritin and various cobalt oxide containing ferritins.

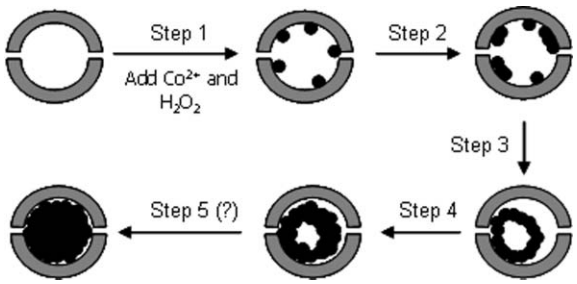

Fig. 5 Cartoon of the cobalt oxide growth mechanism in the ferritin.

the inside of the protein wall. In the absence of interaction with the interior protein wall, a single nanoparticle would be formed in order to reduce surface tension (step 1). The discrete nanoparticles continue to grow along the ferritin interior wall (step 2). In the merging step, a hollow nanoparticle forms through the combination of discrete nanoparticles (step 3). The size of the hollow nanoparticle is somewhat reduced due to the formation of metallic bonds. Finally, further Co addition results in hollow nanoparticles with thicker metal oxide walls (step 4). Apparently, the nanoparticles formed have an empty core even at step 4. This explains why we can not completely fill the ferritin interior with the theoretical number of Co atoms through the reconstitution process. We emphasize that the use of this metal growth mechanism in ferritin permits the synthesis of hollow metal oxide nanoparticles by controlling the number of metal atoms added to ferritin during the mineral core reconstitution process.

In summary, we present here the first fabrication of hollow cobalt oxide nanoparticles using a bio-template in aqueous solution and describe the metal growth mechanism in the ferritin interior. This growth mechanism facilitates the preparation of hollow metal oxide nanoparticles by controlling the number of metal atoms inserted in the ferritin interior.

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Notes and references

1 (*a*) S. J. Oldenburg, R. D. Averitt, S. L. Westcott and N. J. Halas, *Chem. Phys. Lett.*, 1998, **288**, 243; (*b*) F. Caruso, R. A. Caruso and H. Möhwald, *Science*, 1998, **282**, 1111; (*c*) S. J. Oldenburg, G. D. Hale, J. B. Jackson and N. J. Halas, *Appl. Phys. Lett.*, 1999, **75**, 1063; (*d*) F. Caruso, M. Spasova, A. Susha, M. Giersig and R. A. Caruso, *Chem. Mater.*, 2001, **13**, 109; (*e*) C. Graf and A. van Blaaderen, *Langmuir*, 2002, **18**, 524; (*f*) S.-W. Kim, M. Kim, W. Y. Lee and T. Hyeon, *J. Am. Chem. Soc.*, 2002, **124**, 7642; (*g*) E. Prodan, C. Radloff, N. J. Halas and P. Nordlander, *Science*, 2003, **302**, 419.

2 (*a*) Y. Sun, B. Mayers and Y. Xia, *Nano Lett.*, 2002, **2**, 481; (*b*) Y. Sun and Y. Xia, *Anal. Chem.*, 2002, **74**, 5297; (*c*) Y. Sun, B. Mayers and Y. Xia, *Adv. Mater.*, 2003, **15**, 641; (*d*) Y. Sun and Y. Xia, *J. Am. Chem. Soc.*, 2004, **126**, 3892.

3 Y. Yin, R. M. Rioux, C. K. Erdonmez, S. Hughes, G. A. Somorjai and A. P. Alivisatos, *Science*, 2004, **304**, 711.

4 (*a*) F. Liao and N. Chu, *J. Appl. Polym. Sci.*, 1988, **35**, 797; (*b*) J. S. Ullett and R. P. Chartoff, *Polym. Eng. Sci.*, 1995, **35**, 1086.

5 E. Dreerman, M. Narkis, A. Siegmann, R. Joseph, H. Dodiuk and A. T. Dibenedetto, *J. Appl. Polym. Sci.*, 1999, **72**, 647.

6 J. Connolly, T. G. St. Pierre, M. Rutnakornpituk and J. S. Riffle, *J. Phys. D: Appl. Phys.*, 2004, **37**, 2475.

7 (*a*) P. J. Pearce, A. R. Siebert, D. R. Egan, C. D. Guiley and R. S. Drake, *J. Adhes.*, 1995, **49**, 245; (*b*) U. O. Häfeli, *Int. J. Pharm.*, 2004, **277**, 19.

8 M. D. Bentzon and A. Tholen, *Ultramicroscopy*, 1990, **38**, 105.

9 (*a*) E. C. Theil, *Annu. Rev. Biochem.*, 1987, **56**, 289; (*b*) P. M. Harrison, S. C. Andrews, P. J. Artymiuk, G. C. Ford, J. R. Guest, J. Hirzmann, D. M. Lawson, J. C. Livingstone, J. M. A. Smith, A. Treffry and S. J. Yewdall, in *Iron Transport and Storage*, ed. P. Ponka, H. M. Schulman and R. C. Woodworth, CRC, Boca Raton, FL, 1990, pp. 81–101; (*c*) D. M. Lawson, P. J. Artymiuk, S. J. Yewdall, J. M. A. Smith, J. C. Livingstone, A. Treffry, A. Luzzago, S. Levi, P. Arosio, G. Cesareni, C. D. Thomas, W. V. Shaw and P. M. Harrison, *Nature*, 1991, **349**, 541; (*d*) P. M. Harrison and P. Arosio, *Biochim. Biophys. Acta*, 1996, **1275**, 161; (*e*) T. Douglas and D. R. Ripoll, *Protein Sci.*, 1998, **7**, 1083.

10 (*a*) F. C. Meldrum, V. J. Wade, D. L. Nimmo, B. R. Heywood and S. Mann, *Nature*, 1991, **349**, 684; (*b*) F. C. Meldrum, B. R. Heywood and S. Mann, *Science*, 1992, **257**, 522; (*c*) J. F. Hainfeld, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 11064; (*d*) T. Douglas, D. P. E. Dickson, S. Betteridge, J. Charnock, C. D. Garner and S. Mann, *Science*, 1995, **269**, 54; (*e*) F. C. Meldrum, T. Douglas, S. Levi, P. Arosio and S. Mann, *J. Inorg. Biochem.*, 1995, **58**, 59; (*f*) K. K. W. Wong, T. Douglas, S. Gider, D. D. Awschalom and S. Mann, *Chem. Mater.*, 1998, **10**, 279; (*g*) E. Mayers, A. Bewick, D. Gleeson, J. Hoinville, R. Jones, O. Kasyutich, A. Nartowski, B. Warne, J. Wiggins and K. K. W. Wong, *IEEE Trans. Magn.*, 2003, **39**, 624; (*h*) M. Okuda, K. Iwahori, I. Yamashita and H. Yoshimura, *Biotechnol. Bioeng.*, 2003, **84**, 187; (*i*) J. M. Dominguez-Vera and E. Colacio, *Inorg. Chem.*, 2003, **42**, 6983; (*j*) D. Ensign, M. Young and T. Douglas, *Inorg. Chem.*, 2004, **43**, 3441; (*k*) B. Zhang, J. N. Harb, R. C. Davis, J.-W. Kim, S.-H. Chu, S. Choi, T. Miller and G. D. Watt, *Inorg. Chem.*, 2005, **44**, 3738.

11 T. Douglas and V. T. Stark, *Inorg. Chem.*, 2000, **39**, 1828.

12 K. K. W. Wong and S. Mann, *Adv. Mater.*, 1996, **8**, 928.

13 (*a*) T. Douglas and M. J. Young, *Nature*, 1998, **393**, 152; (*b*) W. Shenton, S. Mann, H. Colfen, A. Bacher and M. Fischer, *Angew. Chem., Int. Ed.*, 2001, **40**, 442; (*c*) T. Douglas, E. Strable, D. Willits, A. Aitouchen, M. Libera and M. Young, *Adv. Mater.*, 2002, **14**, 415; (*d*) R. A. McMillan, C. D. Paavola, J. Howard, S. L. Chan, N. J. Zaluzec and J. D. Trent, *Nat. Mater.*, 2002, **1**, 247; (*e*) M. Allen, D. Willits, J. Mosolf, M. Young and T. Douglas, *Adv. Mater.*, 2002, **14**, 1562; (*f*) B. Dragnea, C. Chen, E.-S. Kwak, B. Stein and C. C. Kao, *J. Am. Chem. Soc.*, 2003, **125**, 6374; (*g*) M. Allen, D. Willits, M. Young and T. Douglas, *Inorg. Chem.*, 2003, **42**, 6300; (*h*) M. L. Flenniken, D. Willits, S. Brumfiled, M. Young and T. Douglas, *Nano Lett.*, 2003, **3**, 1573.

14 (*a*) Y. Matsumoto, M. Fujisue, T. Sasaki and J. Hombo, *J. Electroanal. Chem.*, 1994, **369**, 251; (*b*) T. Pauporte, L. Mendoza, M. Cassir, M. C. Bernard and J. Chlvot, *J. Electrochem. Soc.*, 2005, **152**, C49; (*c*) Y. Wang, C.-M. Yang, W. Schmidt, B. Spliethoff, E. Bill and F. Schüth, *Adv. Mater.*, 2005, **17**, 53.

15 (*a*) A. J. Varkey and A. F. Fort, *Sol. Energy Mater. Sol. Cells*, 1993, **31**, 277; (*b*) B. G. Ershov, N. L. Sukhov and E. Janata, *J. Phys. Chem. B*, 2000, **104**, 6138; (*c*) G. X. Chen, M. H. Hong, B. Lan, Z. B. Wang, Y. F. Lu and T. C. Chong, *Appl. Surf. Sci.*, 2004, **228**, 169; (*d*) L. G. Jacobsohn, M. E. Hawley, D. W. Cooke, M. F. Hundley, J. D. Thompson, R. K. Schulze and M. Nastasi, *J. Appl. Phys.*, 2004, **96**, 4444.