

Fabrication and characterization of crystallized magnetoferritin as an artificial magnetic metamaterial

T. Schwarze¹, M. Okuda², R. Huber¹, J.-C. Eloi², F. Brandl¹, L. Dreher³, M. S. Brandt³, D. Grundler¹, and W. Schwarzacher²

¹Lehrstuhl für Physik funktionaler Schichtsysteme, Physik Department E10, Technische Universität München, James-Franck-Str. 1, D-85748 Garching b. München, Germany

²H. H. Wills Physics Laboratory, Tyndall Avenue, Bristol BS8 1TL, U.K.

³Walter Schottky Institut, Technische Universität München, Am Coulombwall 3, D-85748 Garching b. München, Germany

Abstract

We synthesized magnetic nanoparticles in the central cavity of a cage-shaped protein and fabricated periodic three-dimensional arrays using the protein's ability to self-assemble. The crystal's dynamic magnetic properties were observed by electron paramagnetic resonance in the GHz frequency regime. The crystals are superparamagnetic at higher temperatures but an isotropic resonance is observed at 5 Kelvin.

1. Introduction

Ferromagnetic materials periodically patterned on the nanoscale suggest to form interesting band gap metamaterials [1,2,3]. The tailored dynamic response of artificial magnetic materials might also give rise to effectively continuous metamaterial properties due to the modified magnetic permeability [4]. So far, however, it is not so clear how to prepare an artificial structure where the magnetic constituents are periodically patterned, in all three spatial directions and on a length scale much smaller than the wavelength of the electromagnetic waves. We report here the assembly of magnetic nanoparticles by a novel method to create a magnetic material with the required structural properties. We also show preliminary absorption data taken at a microwave frequency of 9.35 GHz for different temperatures and magnetic field orientations .

2. Fabrication process

Ferritin is a ubiquitous cage-shaped protein that plays a pivotal role in the physiological regulation of iron by storing it as nanoparticles of ferric oxyhydroxide. The protein shell (apoferritin) has an outer diameter of 12 nm and an inner diameter of 7 nm. It is well known that proteins crystallize to form three-dimensional crystals in appropriate conditions. In this paper we report the fabrication of artificial ferrimagnetic nanoparticles inside the apoferritin cavity and their assembly into regular periodic three-dimensional arrays by protein crystallization. To fabricate magnetic (Fe_3O_4 - γ - Fe_2O_3) nanoparticles in the apoferritin cavity (apoferritin containing Fe_3O_4 - γ - Fe_2O_3 nanoparticles is known as magnetoferritin), we have modified the synthesis method described in a previous paper [5] as follows. A degassed buffer solution (10 mL of 50 mM HEPES-NaOH pH 8.6) was added to a reaction vessel under a N_2 atmosphere followed by the addition of horse spleen apoferritin (20mg; Sigma Aldrich). Fe(II) (0.75 mL of 13.4 mM ammonium iron sulphate solution) and oxidizer (4.93 mM, trimethylamine N-oxide dehydrate) were added to the vessel at 333 Kelvin, 16 times. To separate magnetoferritin from

the synthesis solution, the solution was purified by ion exchange chromatography, magnetic separation (steel powder, applied field: ~ 0.8 T) and gel filtration chromatography to eliminate non-protein material, empty apoferritin or apoferritin containing a non-magnetic nanoparticle, and oligomeric apoferritin respectively. The purified monomeric magnetoferritin was observed by transmission electron microscopy (TEM) at 200 kV with and without aurothioglucose staining.

Purified magnetoferritin or apoferritin (10 μ L: 1-10 mg/mL in 200 mM acetate buffer (pH 5.5)) was mixed with crystallization solution (10 μ L of 153 mM CdSO₄ in 200 mM acetate buffer (pH 5.5)). The crystals were observed by microscopy after 24 hours.

The TEM image (Fig. 1(a)) shows that the purified solution contains monodisperse nanoparticles. The TEM image of the stained sample (Fig. 1(b)) shows that magnetic nanoparticles are surrounded by white rings with approximate diameter 12 nm. Since aurothioglucose only stains material outside the apoferritin, the white rings are the apoferritin shells (only protein) and the darker regions in the centre are magnetic nanoparticles formed in the apoferritin cavity. Figure 1(c) shows that magnetoferritin crystals have a brown colour due to their containing Fe₃O₄- γ -Fe₂O₃ nanoparticles, and have an octahedral morphology. The morphology is the same as that of apoferritin crystals (Fig. 1(d)).

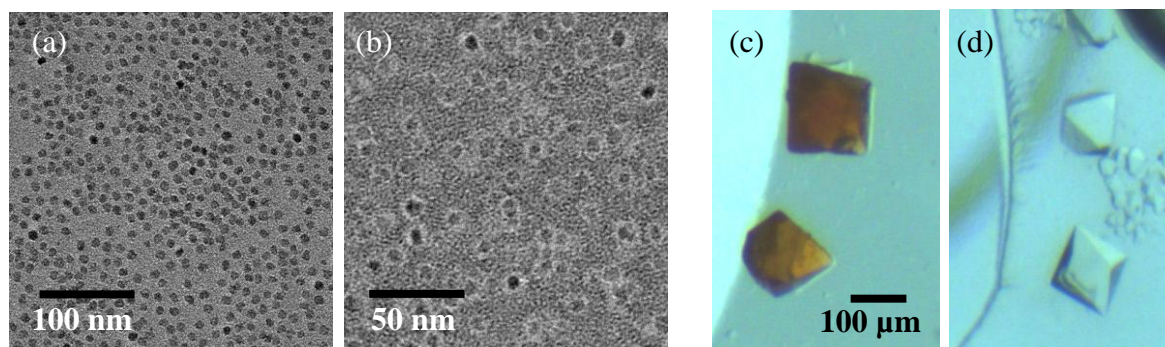


Fig. 1: TEM image of magnetoferritin after purification with (a) and without (b) aurothioglucose staining. The unstained sample shows well-separated uniform nanoparticles while the stained sample shows the protein shells. Optical microscopy images show (c) magnetoferritin crystals and (d) apoferritin crystals crystallized under identical conditions. Both images have the same magnification.

3. Magnetic properties and dynamic response

To explore the dynamic response of the magnetoferritin crystals we make use of electron paramagnetic resonance (EPR) spectroscopy based on a microwave cavity operated in the X band, i.e., at 9.35 GHz. The quality factor of the cavity was approximately $Q = 3000$. The spectrometer allows us to vary the temperature between 300 and 4.2 Kelvin. To enhance the signal-to-noise ratio (SNR) we simultaneously measure three crystals (Fig. 1(c)) supported by a GaAs substrate.

Figure 2(a) shows data for the microwave power reflected at the cavity as a function of the magnetic field. Here the orientation of the field H is fixed. The field strength is modulated to further enhance the SNR by lock-in detection. The data thus represent the derivative of the absorption with respect to H . We show data obtained at room temperature (RT) and at 5 Kelvin. All curves are obtained by subtracting the background signal of the empty cavity from the raw measurement signals. At RT the curve exhibits only a very weak variation with H . This is different for 5 Kelvin. Here we find a resonant behavior near 3200 Oe. Analyzing the peak-to-peak linewidth we obtain $\Delta H_{pp} = 150$ Oe. This value corresponds to $\Delta f = 0.73$ GHz as the resonance linewidth. Following Ref. [6], we attribute this resonance to the magnetoferritin crystals. Li *et al.* found a resonance of comparable shape and resonance field in the

case of non-crystallized samples. Importantly we find that at around 10 Kelvin the resonance has disappeared. This suggests that the magnetic constituents of the crystals behave as superparamagnets.

A detailed analysis of the spectra in Ref. [6] revealed the superposition of two resonances of significantly different intensity and linewidth at the same resonance field. This might point to inhomogeneities or a distribution of anisotropy fields. To investigate this further we performed measurements for different orientations of the external field. In Fig. 2(b) we show data at 5 Kelvin where the angle of the external magnetic field is oriented in such a way that at 0 (90) degrees the magnetic field is in the plane of (perpendicular to) the supporting substrate. The two curves do not show a variation in the shape and resonance field, thus suggesting that the ensemble of three crystals behaves isotropically.

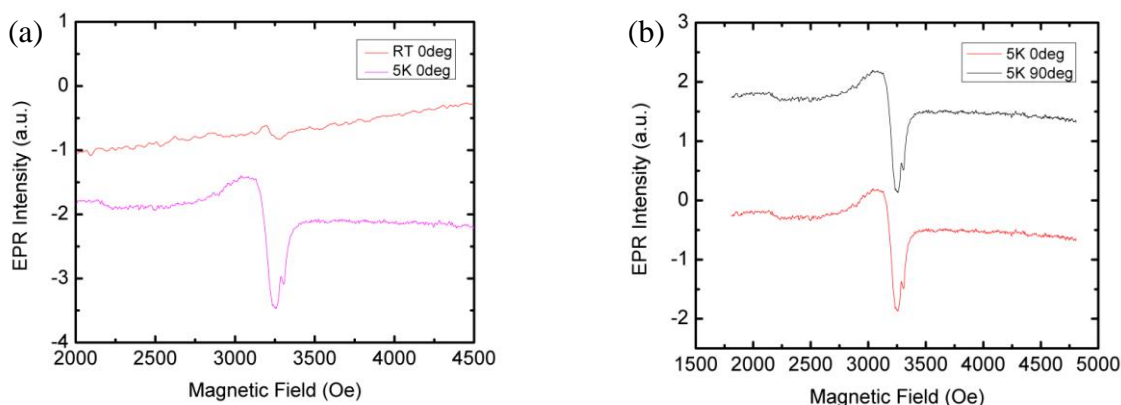


Fig. 2: (a) EPR data obtained on an ensemble of three magnetoferritin crystals at room temperature (upper curve) and 5 Kelvin (lower curve). (b) EPR data at 5 Kelvin taken for two different orientations of the magnetic field. The field is applied in the plane of and perpendicular to the plane of the supporting substrate for the lower and upper curve, respectively. The background signal of the empty cavity is subtracted from the raw dataset for each curve shown.

4. Conclusion

Magnetoferritin crystals have been prepared which exhibited resonant magnetic absorption at low temperature. The temperature dependence of the absorption suggests superparamagnetic behaviour at higher temperatures. Exhibiting lattice constants of about 10 nm artificial crystals which are self-assembled through protein crystallization suggest to provide metamaterials offering tailored effectively continuous properties over a broad wavelength regime due to their small unit cell or building block. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant No. 228673 (MAGNONICS).

References

- [1] M. Krawczyk and H. Puszkarski. *Cryst. Res. Technol.*, Vol. 41, p. 547, 2006.
- [2] J. Topp, D. Heitmann, M.P. Kostylev, and D. Grundler. *Phys. Rev. Lett.*, Vol. 104, p. 207205, 2010.
- [3] S. Neusser, G. Duerr, S. Tacchi, M. Madami, M.L. Sokolovskyy, G. Gubbiotti, M. Krawczyk, and D. Grundler. *Submitted*.
- [4] R. V. Mikhaylovskiy, E. Hendry, and V. V. Kruglyak. *Phys. Rev. B*, Vol. 82, p. 195446, 2010.
- [5] K. K. W. Wong, T. Douglas, S. Gider, D. D. Awschalom, and S. Mann. *Chem. Mater.* Vol. 10, p. 279, 1998.
- [6] H. Li, M. T. Klem, K. B. Sebby, D. J. Singel, M. Young, T. Douglas, and Y. U. Idzerda. *J. Magn. Mat.*, Vol. 321, p. 175, 2009.