NANO LETTERS

Letter

pubs.acs.org/NanoLett

# Detection of a Few Metallo-Protein Molecules Using Color Centers in Nanodiamonds

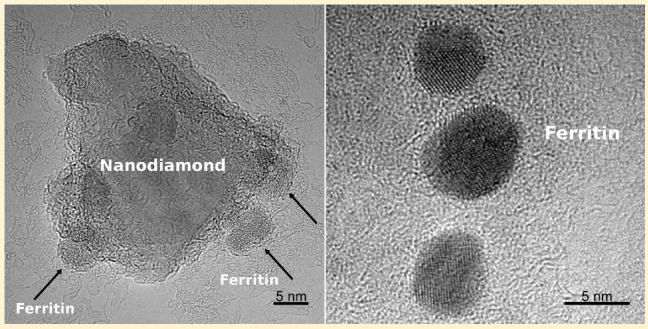
A. Ermakova,[†] G. Pramanik,[‡] J.-M. Cai,[§] G. Algara-Siller,[∥] U. Kaiser,[∥] T. Weil,[‡] Y.-K. Tzeng,[⊥] H. C. Chang,[⊥] L. P. McGuinness,[†] M. B. Plenio,[§] B. Naydenov,*[,†] and F. Jelezko[†]

[†]Institut für Quantenoptik and IQST, [‡]Institut für Organische Chemie III and IQST, [§]Institut für Theoretische Physik and IQST, and [∥]Materialwissenschaftliche Elektronenmikroskopie and IQST, Albert-Einstein Allee 11, Universität Ulm, 89069 Ulm, Germany

[⊥]Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106, Taiwan

Supporting Information

**ABSTRACT:** Nanometer-sized diamonds containing nitrogen-vacancy defect centers (NV) are promising nanosensors in biological environments due to their biocompatibility, bright fluorescence, and high magnetic sensitivity at ambient conditions. Here we report on the detection of ferritin molecules using magnetic noise induced by the inner paramagnetic iron as a contrast mechanism. We observe a significant reduction of both coherence and relaxation time due to the presence of ferritin on the surface of nanodiamonds. Our theoretical model is in excellent agreement with the experimental data and establishes this method as a novel sensing technology for proteins.




**KEYWORDS:** *Nanodiamonds, biomolecules, ferritin, nitrogen-vacancy centers, optically detected magnetic resonance*

The detection of single proteins in biological environments using noninvasive nanoprobes has a wide range of applications in all areas of life sciences. Currently the most common route to visualizing the interior of living cells involves using organic molecules (dyes and fluorescent proteins)[1] and quantum dots.[2] The main disadvantage of the former is that they tend to bleach after some illumination time, thus limiting their usefulness. Quantum dots do not bleach but can be toxic and have unstable fluorescence, resulting in photoblinking.[2] A further challenge remains to extend the functionality of biomarkers by linking their fluorescence to interesting events, such as the presence of a particular protein, and changes in pH or concentration.[3] Recently color centers in nanosized diamonds (nanodiamonds or ND) have been studied with this application in mind since they do not show the above limitations[4−6] and because it has been demonstrated that they can be introduced even in living multicellular organisms.[7] Their fluorescence depends on the electronic spin-state of the NV center, thus allowing the optical detection and coherent manipulation of single NV center spins even at room temperature.[8,9] Due to their long coherence time, these color centers are ideal magnetic field sensors at the atomic scale, and the first proof-of-principle experiments have already been demonstrated.[10−13] Other studies have proposed using NV centers to detect and resolve the structure of single biological molecules through dipolar coupling to nuclear spins of the protein.[14−16]

Here we report experiments toward the implementation of nanodiamonds as a biological sensor by demonstrating that the influence of ferritin metalloprotein on the spin properties of single NV centers in nanodiamonds can be detected and quantified. Thermal fluctuations of iron electron spins in the protein molecule create a strongly fluctuating spin bath which couples to the electron spin of the NV. This interaction leads to shortening of both the NV spin coherence time $T_2$ and relaxation time $T_1$ by an order of magnitude, when compared to the values for noncoated nanodiamonds. This effect is fully explained by our theoretical model.

Ferritins are a family of proteins found in many types of animals, plants, and prokaryotes, with the primary function being iron storage. Each ferritin molecule is made of 24 subunits, which are self-assembled, in a noncovalent fashion, into a cage-like nanostructure.[17] Their structure consists of a hollow protein shell (Apoferritin) with a thickness of 2 nm that can store up to 4500 iron atoms.[18] The cavity inside is approximately 8 nm in diameter and contains a single-domain antiferromagnetic iron-oxyhydroxide core $(FeO(OH)]_8[FeO(H_2PO_4)])$ with uniaxial anisotropy and a magnetic moment of about 300 $\mu_B$ (resulting from a small fraction of uncompensated $Fe^{3+}$ spins).[19] The ferritin concentration in blood serum is correlated with the total amount of iron stored in the body, making this protein critical for iron homeostasis and metabolism in mammals. Its alteration plays a significant role in anemias, such as iron aplastic anemia, and chronic hemolytic

**Received:** April 26, 2013

   dx.doi.org/10.1021/nl4015233 | *Nano Lett.* XXXX, XXX, XXX−XXX

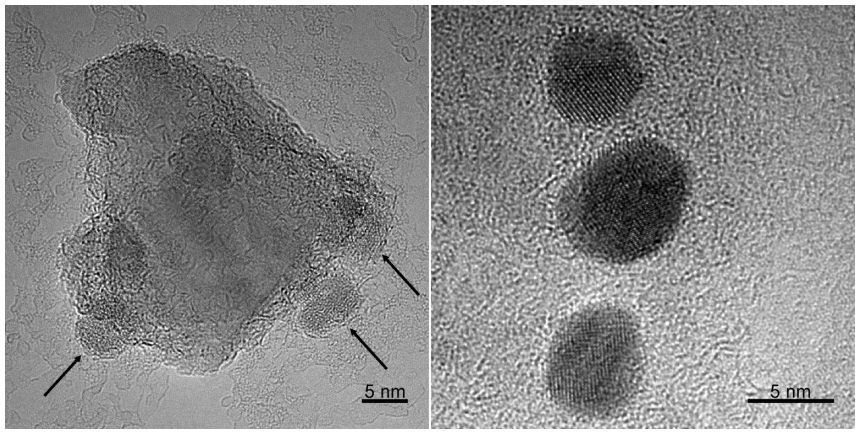



**Figure 1.** HRTEM images of ferritin molecules (right) showing the iron containing core. Single nanodiamond covered with ferritin (left). The arrows indicate the position of the metalloprotein on the surface.

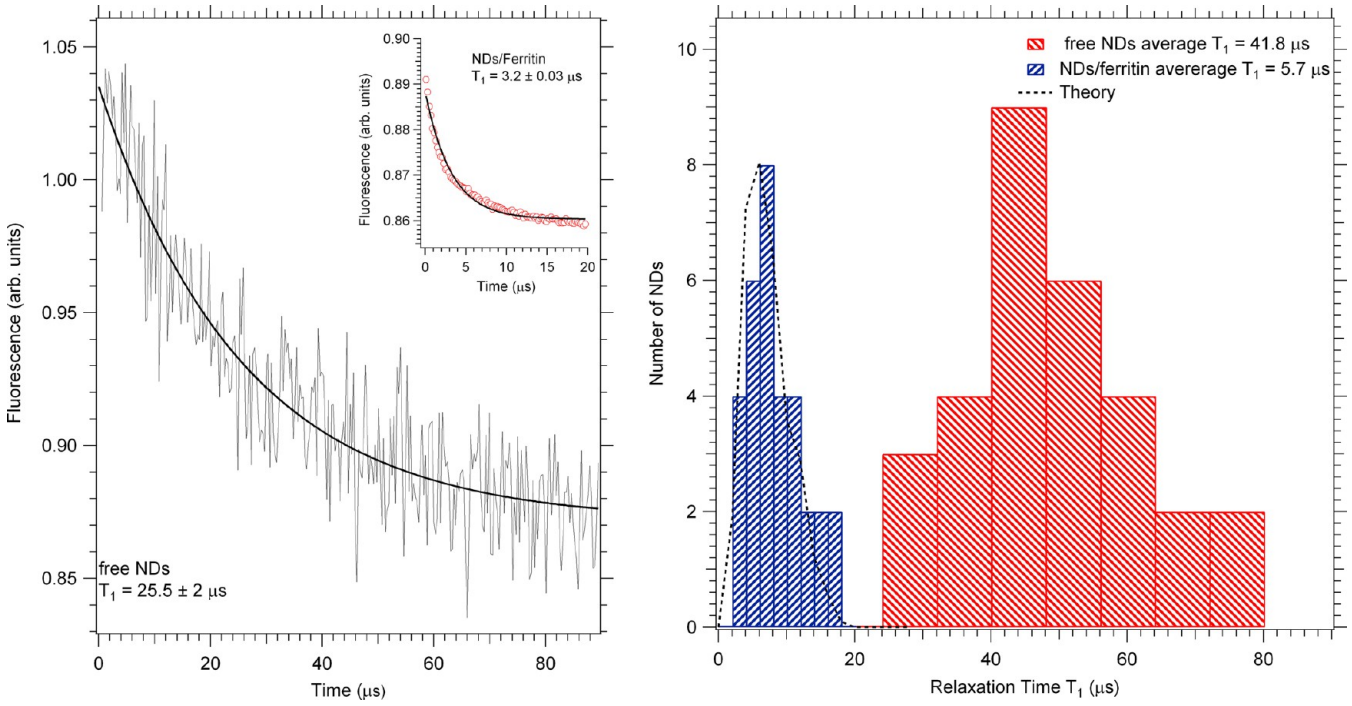



**Figure 2.** Inversion recovery measurement used to obtain the electron spin−lattice relaxation time $T_1$ of NV in NDs as received (left) and in ferritin coated NDs (left, inset). Statistical distribution of the $T_1$ for free nanodiamonds (right, blue bars) and for ferritin coated nanodiamonds (right, red bars). The decrease of $T_1$ in the presence of ferritin is clearly visible and observed in all measurements. The black dotted curve is a theoretical model using the fluctuation dynamics of the iron ions (see main text).

anemia, in addition to thalassemia, hepatitis, hepatoma, and chronic or acute hepatic injury.[20−22] Iron overload and overexpression of ferritin has also been implicated in neurodegeneration in a genetically engineered mouse model.[23] Hence, the determination of ferritin under physiological conditions is important for obtaining information on disease pathology and diagnosis.

To date, various methods have been applied for ferritin detection with atomic force microscopy (AFM)[24,25] and different immunoassays[26−30] being commonly used. Ferritin has even been used as a contrast agent for magnetic resonance imaging.[31] However, these techniques have some limitations. For example the contact mode and/or the vibrating motion of the samples in AFM imaging may lead to denaturation of the biological molecules.[25,32−34] Most of the other reported detectors for ferritin use a complicated biochemical assay which requires expensive antibodies and sophisticated instrumentation for immunochromatography. Additionally, the reproducibility of electrochemical immunoassays is inconsistent.[28,29] Therefore, a new, simple, sensitive, and noninvasive method for ferritin detection can be expected to find wide applicability.

NV spins in nanodiamonds have been proposed for use as noninvasive sensors in living cells,[11,15,16] and the first sensing protocols have been implemented.[10−13] However, the ability to detect single biomolecules requires linking them to nanodiamonds. If the latter are oxidized, they are good adsorbents for proteins or polypeptides.

To implement biosensing of metalloproteins, we first realized adsorption of ferritin onto the nanodiamond surface by using


dx.doi.org/10.1021/nl4015233 | Nano Lett. XXXX, XXX, XXX−XXX

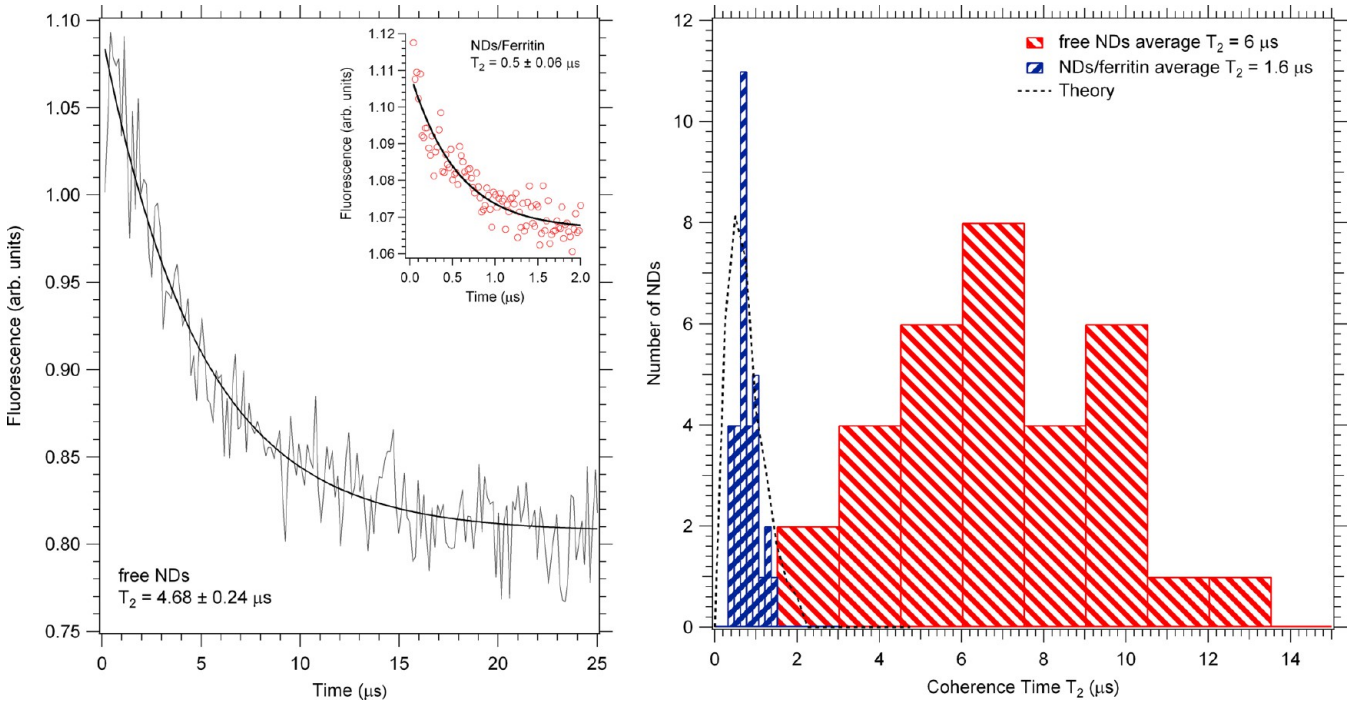



**Figure 3.** Electron spin Hahn echo decay for free NDs (left) and ferritin conjugated NDs (left, inset). A decrease of $T_2$ for ferritin-NDs is observed in all experiments. The dotted line is a theoretical model (see main text for more details).

electrostatic attraction between the anionic groups ($COO^-$) terminating the nanodiamond surface and the positively charged amino groups ($-NH^{3+}$) of the adsorbate (ferritin). Previous studies have shown that oxidized nanodiamonds adsorb various biomolecules such as proteins,[35−39] toxins,[40,41] antibodies,[42] and hormones.[43] In our experiments the attachment of ferritin to the surface of fluorescent nanodiamonds was performed by noncovalent binding to the amino groups of the protein. Nanodiamonds having an average size of 20 nm were produced according to the method described in ref 44; horse spleen ferritin was obtained from Sigma-Aldrich. Nanodiamond−ferritin complexes were synthesized by incubating the ingredients in boric acid buffer (pH = 8.5). The solution was purified from free ferritin by centrifugation and thorough rinsing with deionized water. Fourier transform infrared spectroscopy (FTIR), zeta potential measurements, X-ray photoelectron spectroscopy (XPS), and high-resolution transmission electron microscopy (HRTEM) were used for the characterization of the ND-ferritin complex and showed on average 10 ferritin molecules attached to each nanodiamond (see Supporting Information). The XPS spectrum of nanodiamond−ferritin complex shown in Figure S4 (Supporting Information) reveals the presence of iron. The 2p transition was observed at 711 eV and 3s, 3p exhibit binding energy peaks at 95 and 53 eV, respectively.

Figure 1 shows a HRTEM image of ferritin adsorbed on ND surface. Single ferritin molecules can be easily resolved and identified. The diameter of the iron core is determined to be around 5 nm, consistent with the values reported in the literature.[18]

The fluorescence and optically detected magnetic resonance (ODMR) measurements were performed on a home-built confocal microscope. An excitation wavelength of 532 nm was used, and the fluorescence was detected using an Avalanche photodiode (APD) after a 650 nm long pass filter. Nanodiamonds were spin-coated on a glass coverslip, with microstrip-lines fabricated on the surface for applying microwaves, produced by standard photolithography. The electron spin coherence time $T_2$ was measured with the ODMR version of the Hahn echo pulse sequence,[9] and for the relaxation time $T_1$ determination we used the adapted inversion recovery technique.[45]

We have investigated a total of 64 nanodiamonds divided in two groups—free (as received) and ferritin covered NDs. The $T_1$ and $T_2$ times for both groups are plotted in Figures 2 and 3.

In all experiments we observed a shorter $T_1$ and $T_2$ for ferritin conjugated nanodiamonds compared to free NDs, with an average reduction of about a factor of 7. We obtained $T_1$ = 5.7 $\mu$s and $T_2$ = 1.6 $\mu$s for ferritin conjugated nanodiamonds compared to $T_1$ = 41.8 $\mu$s and $T_2$ = 6.0 $\mu$s for free NDs. This result confirms that the presence of very few ferritin molecules have a strong and quantifiable impact on the spin properties of NV, thus demonstrating the feasibility of nanodiamonds as nanoscale magnetic field sensors. Moreover, the observed effect is so large, that even a single ferritin close to the nanodiamond could be detected using this novel sensor. In the following we present our theoretical model fully describes the observed effects.

If we neglect the hyperfine coupling to the nitrogen nuclear spin the electron spin properties of the NV's ground state are well-described by the following Hamiltonian:

$$H = S \cdot D \cdot S + g\mu_B B_0 S_z$$

where $S = (S_x, S_y, S_z)$ is the NV's electron spin operator with spin number $S = 1$, $D = 2.87$ GHz is its zero-field splitting, g is its g-factor, $\mu_B$ is the Bohr magneton, and $B_0 \sim 20$ $G$ is the applied constant magnetic field. The $Fe^{3+}$ stored in the ferritin core have an electron spin $S = 5/2$. The mutual flip-flops of these spins cause fluctuating magnetic field acting on the NV center inside the nanodiamond. At room temperature this


dx.doi.org/10.1021/nl4015233 | Nano Lett. XXXX, XXX, XXX−XXX

process can be described classically as a random exchange of polarization between iron spins at a rate that of 2.1 GHz which has been determined to match the line width of the electron spin resonance spectrum of ferritin.[46] The transversal component (perpendicular to the NV's quantization axis) of this fluctuating field induces flips of the NV's electron spin, thus leading to relaxation and accordingly to shortening the value of $T_1$. More precisely, the noise spectrum $S^{\perp}(\omega)$ at the frequency corresponding to the NV centers' zero field splitting (i.e., $\omega_0 \approx$ 2.87 GHz) provides a very accurate estimation of $T_1$ as $1/S^{\perp}(\omega = \omega_0)$. The value of $T_2$ is affected by the longitudinal component (parallel to the NV's quantization axis) of the fluctuating field. Using the longitudinal noise spectrum $S^{\parallel}(\omega)$ the decay of the spin echo signal SE (a direct measurement of the coherence) and thus the $T_2$ time can be calculated[47] as SE = $8\int_0^{\infty}(d\omega/\pi)S^{\parallel}(\omega)\ \sin^4(\omega t/4)(1/\omega^2)$. Note however that the nanodiamonds possess a size distribution and that the position of the NV inside is not precisely known. This results in a variation of the distance of the NV center to the ferritin and thus in a variation of the magnitude of the fluctuating magnetic field. Nevertheless we were able to take both factors into account by assuming a random position of 8−15 ferritins attached to the nanodiamond. The numerical results for $T_1$ and $T_2$ are shown as dashed lines in Figures 2 and 3, respectively. A more detailed explanation of the theory model can be found in the Supporting Information. There is an excellent agreement between the experimental results and the theoretical prediction.

In conclusion, we have demonstrated that single nanodiamonds containing NV centers can be used to detect individual ferritin molecules. Our method has the sensitivity required for single protein detection, can be easily applied to cellular environments, and may be developed further to resolve some important biological processes such as electron transfer.[48] Recently[49,50] two groups have demonstrated nuclear magnetic resonance on very small volumes containing about $10^4$ nuclei using NV centers in single crystal macroscopic diamonds. These experiments, together with our results presented here, prove the feasibility of nanoscopic magnetic field sensors based on single defects in diamonds.

Recently we became aware of the work by Ziem et al.[51] who report the detection of iron in ferritin and manganese ions in solution by using an ensemble of NVs as sensors.

## ■ ASSOCIATED CONTENT

### Supporting Information

Additional details about the synthesis and characterization of the nanodiamond/ferritin complex and about our theoretical model. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*E-mail: boris.naydenov@uni-ulm.de.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The research was supported by DFG (SPP1601 and SFB TR21), EU (DIAMANT), DARPA (QUASAR), and the Alexander von Humboldt Foundation. J.-M.C. acknowledges the support of Marie-Curie fellowship and FP7. We would like also to thank J. Wrachtrup and R. Walsworth for the fruitful discussions.

## ■ REFERENCES

(1) Weijer, C. J. *Science* **2003**, *300*, 96−100.
(2) Resch-Genger, U.; Grabolle, M.; Cavaliere-Jaricot, S.; Nitschke, R.; Nann, T. *Nat. Methods* **2008**, *5* (9), 763−775.
(3) Gomez, D. E.; Califano, M.; Mulvaney, P. *Phys. Chem. Chem. Phys.* **2006**, *8*, 4989−5011.
(4) Neugart, F.; Zappe, A.; Jelezko, F.; Tietz, C.; Boudou, J. P.; Krueger, A.; Wrachtrup, J. *Nano Lett.* **2007**, *7* (12), 3588−3591.
(5) Mohan, N.; Tzeng, Y.-K.; Yang, L.; Chen, Y.-Y.; Hui, Y. Y.; Fang, C.-Y.; Chang, H.-C. *Adv. Mater.* **2010**, *22*, 843−847.
(6) Tisler, J., Reuter, R., Lammle, A., Jelezko, F., Balasubramanian, G., Hemmer, P. R., Reinhard, F.; Wrachtrup J. 2011. *10*.
(7) Mohan, N.; Chen, C.-S.; Hsieh, H.-H.; Wu, Y.-C.; Chang, H.-C. *Nano Lett.* **2010**, *10*, 3692.
(8) Gruber, A.; Drabenstedt, A.; Tietz, C.; Fleury, L.; Wrachtrup, J.; von Borczyskowski, C. *Science* **1997**, *276* (5321), 2012−2014.
(9) Jelezko, F.; Gaebel, T.; Popa, I.; Gruber, A.; Wrachtrup, J. *Phys. Rev. Lett.* **2004**, *92* (7), 076401.
(10) Balasubramanian, G.; Chan, I. Y.; Kolesov, R.; Al-Hmoud, M.; Tisler, J.; Shin, C.; Kim, C.; Wojcik, A.; Hemmer, P. R.; Krueger, A.; et al. *Nature* **2008**, *455* (7213), 648.
(11) Maze, J. R.; Stanwix, P. L.; Hodges, J. S.; Hong, S.; Taylor, J. M.; Cappellaro, P.; Jiang, L.; Gurudev Dutt, M. V.; Togan, E.; Zibrov, A. S.; et al. *Nature* **2008**, *455*, 644−647.
(12) McGuinness, L. P., et al. *arXiv:1211.5749*, **2012**.
(13) McGuinness, L. P.; Yan, Y.; Stacey, A.; Simpson, D. A.; Hall, L. T.; Maclaurin, D.; Prawer, S.; Mulvaney, P.; Wrachtrup, J.; Caruso, F.; Scholten, R. E.; Hollenberg, L. C. L. *Nat. Nanotechnol.* **2011**, *6*, 359−363.
(14) Cai, J.-M.; Jelezko, F.; Plenio, M. B.; Retzker, A. *New J. Phys.* **2013**, *15*, 013020.
(15) Hall, L. T.; Cole, J. H.; Hill, C. D.; Hollenberg, L. C. L. *Phys. Rev. Lett.* **2009**, *103*, 220802.
(16) Cole, J. H.; Hollenberg, L. C. L. *Nanotechnology* **2009**, *20*, 495401.
(17) Uchida, M.; Kang, S.; Reichhardt, C.; Harlen, K.; Douglas, T. *Biochim. Biophys. Acta* **2010**, *1800*, 834−845.
(18) Theil, E. *Annu. Rev. Biochem.* **1987**, *56*, 289−315.
(19) Simon, K. H. L.; John, R. C.; Wenrong, Y. *Nanotechnology* **2011**, *22*, 455501.
(20) Yoneda, M.; Nozaki, Y.; Endo, H.; Matawari, H.; Iida, H.; Fujita, K.; Yoneda, K.; Takahashi, H.; Kirikoshi, H.; Inamori, M.; et al. *Dig. Dis. Sci.* **2010**, *55*, 808−814.
(21) Shalitin, S.; Carmi, D.; Weintrob, N.; Philip, M.; Miskin, H.; Kornreich, L.; Zilber, R.; Yaniv, I.; Tamary, H. *Eur. J. Haematol.* **2005**, *74*, 93−100.
(22) Knovich, M. A.; Storey, J. A.; Coffman, L. G.; Torti, S. V.; Torti, F. M. *Blood Rev.* **2009**, *23*, 95−104.
(23) Grabill, C.; Silva, A. C.; Smith, S. S.; Koretsky, A. P.; Rouault, T. A. *Brain Res.* **2003**, *971*, 95−106.
(24) Fernandez, B. N.; Galvez, N.; Sanchez, P.; Morales, J.; Santoyo, F.; Cuesta, R.; Dominguez-Vera, J. M. *Inorg. Chim. Acta* **2007**, *360*, 3951−3954.
(25) Fujikawa, S.; Muto, E.; Kunitake, T. *Langmuir* **2007**, *23*, 4629−4633.
(26) Shapiro, M. G.; Szablowski, J. O.; Langer, R.; Jasanoff, A. *J. Am. Chem. Soc.* **2009**, *131*, 2484−2486.
(27) Muhsen, K.; Barak, M.; Shifnaidel, L.; Nir, A.; Bassal, R.; Cohen, D. *J. Ped. Gastroenterol. Nutr.* **2009**, *49*, 262−264.
(28) Wang, X.; Tao, G.; Meng, Y. *Microchim. Acta* **2009**, *167*, 147−152.
(29) Zhang, X.; Wang, S.; Hu, M.; Xiao, Y. *Biosens. Bioelectron.* **2006**, *21*, 2180−2183.
(30) Wang, S.-F.; Tan, Y.-M. *Anal. Bioanal. Chem.* **2007**, *387*, 703−708.

(31) Naumova, A. V.; Reinecke, H.; Yarnykh, V.; Deem, J.; Yuan, C.; Murry, C. E. *J. Mol. Imaging* **2010**, *9*, 201.
(32) Daniels, S.; Ngunjiri, J.; Garno, J. *Anal. Bioanal. Chem.* **2009**, *394*, 215−223.
(33) Schon, P.; Gorlich, M.; Coenen, M. J. J.; Heus, H. A.; Speller, S. *Langmuir* **2007**, *23*, 9921−9923.
(34) Dobson, J. *FEBS Lett.* **2001**, *496*, 1−5.
(35) Huang, L. C. L.; Chang, H.-C. *Langmuir* **2004**, *20*, 5879−5884.
(36) Kong, X. L.; Huang, L. C. L.; Hsu, C.-M.; Chen, W.-H.; Han, C.-C.; Chang, H.-C. *Anal. Chem.* **2004**, *77*, 259−265.
(37) Puzyr, A.; Pozdnyakova, I.; Bondar, V. *Phys. Solid State* **2004**, No. 46, 761−763.
(38) Nguyen, T.T.-B.; Chang, H.-C.; Wu, V. W.-K. *Diamond Relat. Mater.* **2007**, *16*, 872−876.
(39) Tzeng, Y.-K.; et al. *Angew. Chem., Int. Ed.* **2011**, *50*, 2262−2265.
(40) Liu, K. K.; Chen, M.-F.; Chen, P.-Y.; Lee, T. J. F.; Cheng, C.-L.; Chang, C.-C.; Ho, Y.-P.; Chao, J.-I. *Nanotechnology* **2008**, *19*, 205102.
(41) Puzyr, A.; et al. *Dokl. Biochem. Biophys.* **2007**, *417*, 299−301.
(42) Shimkunas, R. A.; Robinson, E.; Lam, R.; Lu, S.; Xu, X.; Zhang, X.-Q.; Huang, H.; Osawa, E.; Ho, D. *Biomaterials* **2009**, *30*, 5720−5728.
(43) Purtov, K. V.; Petunin, A. I.; Burov, A. E.; Puzyr, A. P.; Bondar, V. S. *Nanoscale Res. Lett.* **2010**, *5*, 631−636.
(44) Chang, Y.; Lee, H.-Y.; Chen, K.; Chang, C.-C.; Tsai, D.-S.; Fu, C.-C.; Lim, T.-S.; Tzeng, Y.-K.; Fang, C.-Y.; Han, C.-C.; et al. *Nat. Nanotechnol.* **2008**, *3*, 284.
(45) Schweiger, A.; Jeschke, G. *Principles of Pulse Electron Paramagnetic Resonance*; Oxford University Press: New York, 2001.
(46) Wajnberg, E.; El-Jaick, L. J.; Linhares, M. P.; Esquivel, D. M. S. *J. Magn. Reson.* **2001**, *153*, 69−74.
(47) Cywiński, L.; Lutchyn, R. M.; Nave, C. P.; Das Sarma, S. *Phys. Rev. B* **2008**, *77*, 174509.
(48) DeVault, D. *Quart. Rev. Biophys.* **1980**, *13*, 387−564.
(49) Mamin, H. J.; Kim, M.; Sherwood, M. H.; Rettner, C. T.; Ohno, K.; Awschalom, D. D.; Rugar, D. *Science* **2013**, *339*, 557.
(50) Staudacher, T.; Shi, F.; Pezzagna, S.; Meijer, J.; Du, J.; Meriles, C. A.; Reinhard, F.; Wrachtrup, J. *Science* **2013**, *339*, 561.
(51) Ziem, F., et al., unpublished.


dx.doi.org/10.1021/nl4015233 | *Nano Lett.* XXXX, XXX, XXX−XXX