Biosensors

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A Detection System Based on Giant Magnetoresistive Sensors and High-Moment Magnetic Nanoparticles Demonstrates Zeptomole Sensitivity: Potential for Personalized Medicine**

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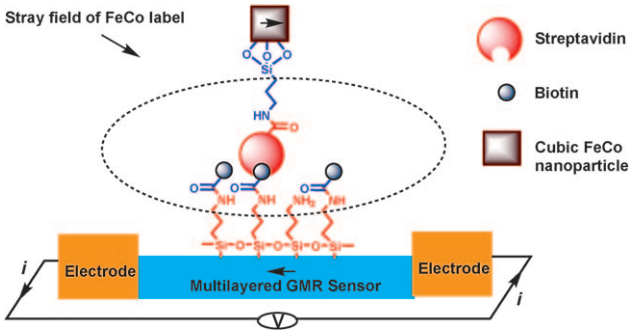
Longitudinally monitoring the changes of protein biomarkers (generally at concentrations $<$ pM)[1] is expected to help design medical treatment for specific individuals[2] and detect chronic diseases in their early stage.[3] This requires a highly sensitive detecting system that is of low cost, easy to use, and preferentially compatible with current electronic technologies. Since the late 1990s, magnetoelectronics,[4] including giant magnetoresistive (GMR) sensors,[5] has emerged as one promising platform to meet such requirements.[6,7] In principle, a GMR sensor would detect stray magnetic fields resulted from magnetic labels on the sensor surface (Scheme 1). Several studies have demonstrated the usage of magnetic biosensors to detect biomolecules with relatively large particle labels ($>$250 nm).[6,8] The high mass and size of the label in relation to the size of the biomolecule to be tethered for testing is expected to interfere with its natural movement, recognition, and binding; this effect would be less serious with smaller magnetic nanoparticles. However, magnetic nanoparticles, as labels, have a smaller magnetic moment than the larger ones, and they require more sensitive sensors and measurement systems. Although the detection of tens of 16 nm iron oxide nanoparticles by direct placement on the surface of a GMR sensor was achieved,[9] their usefulness in real biological systems has not been demonstrated. Therefore, the use of small magnetic nanoparticles for sensitive detection and quantification of biomolecule has yet to be realized.[6]



Scheme 1. GMR sensor for detection of molecular recognition between streptavidin and biotin.

We report herein the development of a highly sensitive detection system based on a GMR sensor and 12.8 nm high-moment cubic FeCo nanoparticles, which linearly detects 600–4500 copies of streptavidin based on the biotin–streptavidin interaction (Scheme 1). We also demonstrate the feasibility of this detecting system for real biological applications, with the example of the linear detection of human interleukin 6 (IL-6, a potential lung cancer biomarker) through a sandwich-based principle.

The GMR sensor consists of a multilayer structure: Ta (5 nm)/$Ir_{0.8}Mn_{0.2}$ (10 nm)/$Co_{0.9}Fe_{0.1}$ (2.5 nm)/Cu (3.3 nm)/$Co_{0.9}Fe_{0.1}$ (1 nm)/$Ni_{0.82}Fe_{0.12}$ (2 nm)/Ta (5 nm). The multilayered GMR sensors were patterned into rectangular shapes of different sizes using photolithography and ion milling (for detailed information, see the the Supporting Information). Figure 1a shows a scanning electron microscope (SEM) image of a GMR sensor with lateral dimensions of 80 μm × 40 μm after the patterning. GMR biochip design layout is shown in Figure 1b and c.

The GMR sensor surface was sequentially modified with 3-aminopropyltriethoxysilane (APTES) followed by Chromalink biotin *N*-hydroxysuccinimidyl ester (see Scheme S1 and Figure S1 in the Supporting Information). For each modification step, bound ligands were quantified using the following equation: amount of bound ligand on the surface = total amount of ligand added − amount of ligand recovered (see Figures S2 and S3, Tables S1 and S2 in the Supporting Information).

High-magnetic-moment FeCo nanoparticles with a composition of 70:30 were synthesized using a sputtering gas condensation technique.[10] By introducing a modification of the magnetic field for sputtering, precise control of particle morphology and size was realized. As shown in Figure 1d,e (see also Figure S5 in the Supporting Information), the shape

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zeptomole = 10^{-21} mol.

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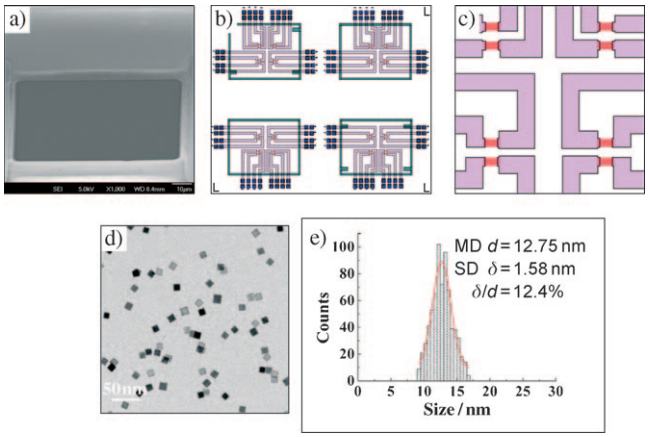

Figure 1. a) SEM image of a GMR sensor. b) Design of a GMR biochip. c) Expansion of one active area of the GMR biochip having eight GMR sensors (red). d) TEM image of cubic FeCo nanoparticles. e) Size distribution of FeCo nanoparticles MD d = mean diameter; SD δ = standard deviation.

(cubic) and size (12.8 nm in diameter) of the nanoparticles are of high homogeneity. This homogeneity is critical for accurate quantification, especially for samples with a small number of particles because of the limited power of minimizing the potential influence of heterogeneity. The FeCo nanoparticles have an oxidation layer 1.5 nm thick. For one particle, the ratio of oxidized volume to the total volume ($V_{oxidized}/V_{total}$) is estimated to be 55.1 %. Based on size, it is estimated that 1 gram of these 12.8 nm cubic FeCo nanoparticles will correspond to 6.77×10^{17} particles. With the same particle volume, the net magnetic moment of one FeCo nanoparticle is 1.77×10^{-17} emu, more than 8 times higher than that of one γ-Fe_2O_3 nanoparticle of the same size at an applied field of 100 Oe.[11]

FeCo nanoparticles were first modified with amino groups on the surface by using APTES (see Scheme S2 in the Supporting Information); this resulted in approximately 660 copies of APTES molecules on each nanoparticle (see Table S4 and Figure S2 in the Supporting Information). APTES-modified nanoparticles were subjected to streptavidin-AF488 modification using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling chemistry. It is estimated that each nanoparticle is modified by 1.3 streptavidin-AF488 molecules (see Figure S7 and Table S5 in the Supporting Information). There is no significant nonspecific binding of streptavidin to the magnetic nanoparticles (see Figure S8 in the Supporting Information). These data suggest that most particles are likely to be modified by one copy of the streptavidin molecule. TEM images of APTES-modified and streptavidin-modified nanoparticles are shown in Figure 2, demonstrating no significant particle aggregation. This is again critical for quantification of biomolecules, which is based on the magnitude of magnetic signals from the nanoparticles.

To explore the sensitivity of this detecting system based on GMR sensors and magnetic nanoparticles, varied quantities of streptavidin-AF488 modified magnetic nanoparticles were applied onto the surface of a GMR sensor modified by

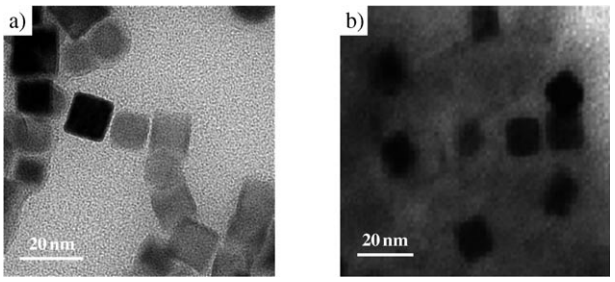

Figure 2. TEM images of FeCo nanoparticles modified by a) APTES and b) streptavidin-AF488.

Chromalink biotin. After thorough washing to remove the potentially unbound streptavidin, magnetic signals from the magnetic nanoparticles specifically retained on the GMR sensor through biotin–streptavidin interactions were measured by the GMR sensor.

As shown in Figure 3, the GMR sensor detected signals from as few as 600 copies ($<10^{-21}$ mol, zeptomol) of streptavidin; this sensitivity is expected to suffice for detection of all known potential biomarkers from body fluid samples of 10 nanoliters or less. More importantly, there is a linear dose–response relationship for the amount of streptavidin applied and the magnetic signals detected by the GMR sensors (saturation of signal was observed with more than 20000 copies of streptavidin; see Figure S13 in the Supporting Information). Such a dynamic range of linearity outstrips most other GMR-based detecting systems reported to date,[12] making accurate quantification possible. The signal detected on the biotin-modified GMR sensor is introduced through the biotin–streptavidin interaction as the control sensors with no biotin modification detected no resistance changes under identical experimental conditions (also see Tables S6 and S7 in the Supporting Information).

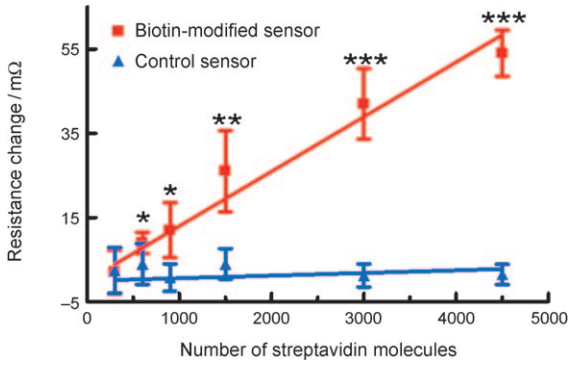

Figure 3. Dose–response curves of resistance change detected by sensors and the amount of magnetic nanoparticles on the GMR sensor. Red: sensors with surface modified by biotin. Blue: sensors with no biotin modification. Data points: mean; bars: standard deviation ($n=3$); *: $P<0.10$; **: $P<0.01$; ***: $P<0.001$. The comparisons are between the control sensor and the biotin-modified sensor. P = power (statistical).

To further explore the practical usage of this GMR-sensor- and magnetic-nanoparticle-based detecting system,

we evaluated the potential of this system to detect human IL-6 using a sandwich -based approach, which follows the same principle of enzyme-linked immunoSorbent assay (ELISA). Monoclonal anti-human IL-6 antibodies (capture antibodies) were covalently attached to the APTES-modified GMR sensor using EDC coupling chemistry. Recombinant human IL-6 was applied to capture antibodies on the GMR sensor surfaces. Polyclonal anti-human IL-6 antibodies (detection antibodies) were magnetically labeled with APTES-modified FeCo nanoparticles through covalent linkage using EDC coupling chemistry. Each nanoparticle was estimated to be modified by 1.19 copies of the detection antibody (see Figure S14 and Table S8 in the Supporting Information). The magnetic-particle-labeled detection antibodies were allowed to bind to IL-6, which was bound to the GMR sensor surface through the capture antibodies. After thorough washing to remove the potentially unbound detection antibodies, magnetic signals from the magnetic nanoparticles specifically retained on the GMR sensor through detection of antibody IL-6/capture antibody interactions were measured by the GMR sensor.

As shown in Figure 4, the GMR sensor detected signals from as low as 2.08×10^6 molecules of IL-6 molecules, which is 13 times more sensitive than the IL-6 ELISA assay (R&D).

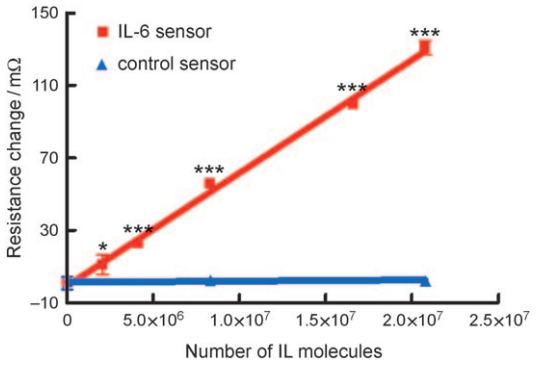

Figure 4. Dose–response curves of resistance change detected by sensors and IL-6 molecules. The same amount of capture antibody and detection antibody-modified magnetic nanoparticles were applied to each sensors with varied numbers of IL-6 molecules. Data points: mean; bars: standard deviation ($n=3$); *: $P<0.10$; **: $P<0.01$; ***: $P<0.001$.

More importantly, there is a linear dose–response relationship between the amount of IL-6 applied and the magnetic signals detected by the GMR sensor. Similar to the case of biotin–streptavidin, nonspecific noise is insignificant (Figure 4, control sensor). In current work in our laboratory we are optimizing the conditions of the sandwich assay to improve the sensitivity and dynamic detecting range.

In conclusion, we have established a GMR-sensor- and high-moment magnetic-nanoparticle-based detecting system that, for the first time, detects as few as 600 copies of streptavidin, which is modified by 12.8 nm cubic FeCo nanoparticle in a 1:1 ratio. We also demonstrated that this detecting system can adopt the principle of ELISA assay with improved sensitivity. This GMR- and magnetic-nanoparticle-based detecting system, therefore, is expected to be applicable to many other biological systems for detection and quantification of various biomolecules. The high sensitivity of this detecting system open new avenues for the detection of biomolecules involved in the etiology of various diseases, especially chronic ones, such as cancer, which is now under investigation. It can also be used to monitor residual disease and disease reoccurrence. Most importantly, because of the magnetic/electric nature of this detecting system, we expect that this detecting system will help the realization of personalized medicine.

Experimental Section

Sensor surface modification: The GMR biochip has four active areas which contain eight sensors each (Figure 1b and c). Step 1: APTES solution in DMSO (5%, w/v, 1 μL) was carefully applied to the sensor surface with the aid of a magnifying glass and kept for 4 h at RT. Step 2: Chromalink biotin solution (30 μg Chromalink biotin, 20 μg triethylamine in 4 μL DMSO) was applied and kept under inert atmosphere for 4 h. After each step, the sensor surface was thoroughly washed with water to remove unbound ligands, which were collected for quantification (for detailed procedures see the Supporting Information).

Modification of the surface of cubic FeCo nanoparticles: Step 1: A solution of APTES (100 μg in 500 μL ethanol) and ammonium hydroxide (5 μL, 29%) was added to cubic FeCo nanoparticles (100 μg) and sonicated for 4 h. Step 2: To a dispersion of APTES-modified nanoparticles (0.5 mL, PBS buffer, pH 7.2; 95 μg of FeCo), streptavidin-AF488 (5 μL, 2 mg mL^{-1} in PBS buffer, pH 7.4) and 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (EDC) hydrochloride (5 mg) was added and shaken overnight at 4°C. After each step, nanoparticles were harvested by an external magnet, and modified nanoparticles were thoroughly washed with water to remove unbound ligands, which were collected for quantification (for detailed procedures see the Supporting Information).

Binding of the streptavidin-AF488-modified FeCo nanoparticles to the biotinylated sensor surface: Streptavidin-AF488-modified cubic FeCo nanoparticles (ranging from 300–600 000 copies) were applied on biotinylated GMR sensor surface at 4°C and kept for 30 min (for detailed procedures see the Supporting Information). The sensor surface was thoroughly washed with water and dried with a stream of nitrogen.

Sandwich-based assay for human IL-6: A mixture of 4 μL (2 μg) capture antibody (500 μg mL^{-1} in PBS buffer, pH 7.4) and 1 μL (10 μg) EDC (10 mg mL^{-1} in PBS, pH 7.4) was added to APTES-modified GMR biochip areas and kept for 4 h at 4°C. After thorough washing, 2 μL of human IL-6 containing various numbers of copies of IL-6 molecules per sensor area ranging from 2.08×10^6–2.08×10^7 was added and allowed to bind for 1 h at 4°C followed by thorough washing. 2 μL of the detection-antibody-modified FeCo nanoparticle dispersion (each sensor will have 72 000 detection antibody–nanoparticle conjugates) was added to all human IL-6-modified GMR biochip areas and allowed to bind for 1 h at 4°C followed by thorough washing (for detailed procedures see the Supporting Information).

Magnetoresistance measurements: The magnetoresistance of GMR sensor was measured using a four-probe station, which used a sourcemeter (model Keithely 2400) in a four-wire configuration. The sensing current that ran through the GMR sensor is 1 mA. An external magnetic field generated by a magnetic coil was applied in parallel to the sensor surface to change the magnetization direction of the $Co_{0.9}Fe_{0.1}$ (1 nm)/$Ni_{0.82}Fe_{0.12}$ (2 nm) bilayer. The second function of the external magnetic field is to polarize the magnetic nanoparticles on the GMR sensor. A labview program (National instruments, USA) was used to control all the electronic components involved in the

resistance measurement by a central computer. A typical transfer curve of a single GMR sensor is shown in Figure S9 (see the Supporting Information). The transfer curve shows that the GMR sensor has a coercivity of 4 Oe. Most of the GMR sensors reported to date were fabricated with a linear transfer curve. We specially designed and fabricated the GMR sensor with a proper coercivity, which shows a larger sensitivity around the coercivity point because of its characteristic hysteresis loop. Our GMR sensor showing the hysteresis has a sensitivity of 0.15 Ohm Oe^{-1}, whereas most other GMR sensors showing linear transfer curve have a sensitivity of about 0.015 Ohm Oe^{-1}.[9] The magnetoresistnace ratio (MR) ratio for our GMR sensor is about 2.9% with a maximum sensitivity of 0.29 Ohm Oe^{-1} at 10 Oe field.

Detailed experimental information for surface modification and quantification protocols, nanoparticle preparation, characterization, and estimation, and GMR sensor fabrication and measurements are available in the Supporting Information.

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[1] a) N. L. Anderson, N. G. Anderson, *Mol. Cell. Proteomics* **2002**, *1*, 845; b) Z. J. Sahab, S. M. Semaan, Q.-X. A. Sang, *Biomarker Insights* **2007**, *2*, 21.
[2] E. M. Macy, T. E. Hayes, R. P. Tracy, *Clin. Chem.* **1997**, *43*, 52.
[3] M. Gomez, G. A. Silvestri, *Am. J. Med. Sci.* **2008**, *335*, 46.
[4] a) G. A. Prinz, *Science* **1998**, *282*, 1660; b) E. E. Fullerton, I. K. Schuller, *ACS Nano* **2007**, *1*, 384; c) A. Fert, *Angew. Chem.* **2008**, *120*, 6042; *Angew. Chem. Int. Ed.* **2008**, *47*, 5956.
[5] A. Sandhu, *Nat. Nanotechnol.* **2007**, *2*, 746.
[6] a) D. L. Graham, H. A. Ferreira, P. P. Freitas, *Trends Biotechnol.* **2004**, *22*, 455; b) C. R. Tamanaha, S. P. Mulvaney, J. C. Rife, L. J. Whitman, *Biosens. Bioelectron.* **2008**, *24*, 1; c) X. J. A. Janssen, L. J. van Ijzendoorn, M. W. J. Prins, *Biosens. Bioelectron.* **2008**, *23*, 833; d) L. Lagae, R. Wirix-Speetjens, J. Das, D. Graham, H. Ferreira, P. P. Freitas, G. Borghs, J. De Boeck, *J. Appl. Phys.* **2002**, *91*, 7445.
[7] a) D. R. Baselt, G. U. Lee, M. Natesan, S. W. Metzger, P. E. Sheehan, R. J. Colton, *Biosens. Bioelectron.* **1998**, *13*, 731; b) M. Tondra, M. Porter, R. J. Lipert, *J. Vac. Sci. Technol. A* **2000**, *18*, 1125.
[8] a) M. M. Miller, P. E. Sheehan, R. L. Edelstein, C. R. Tamanaha, L. Zhong, S. Bounnak, L. J. Whitman, R. J. Colton, *J. Magn. Magn. Mater.* **2001**, *225*, 138; b) R. DePalma, G. Reekmans, C. Liu, R. Wirix-Speetjens, W. Laureyn, O. Nilsson, L. Lagae, *Anal. Chem.* **2007**, *79*, 8669; c) W. Shen, B. D. Schrag, M. J. Carter, J. Xie, C. Xu, S. Sun, G. Xiao, *Appl. Phys. Lett.* **2008**, *103*, 07A306.
[9] G. Li, S. Sun, R. J. Wilson, R. L. White, N. Pourmand, S. X. Wang, *Sens. Actuators A* **2006**, *126*, 98.
[10] J. Bai, Y.-H. Xu, J.-P. Wang, *IEEE Trans. Magn.* **2007**, *43*, 3340.
[11] Y. H. Xu, J. Bai, J.-P. Wang, *J. Magn. Magn. Mater.* **2007**, *311*, 131.
[12] a) H. A. Ferreira, D. L. Graham, P. P. Freitas, J. M. S. Cabral, *J. Appl. Phys.* **2003**, *93*, 7281; b) H. Brückl, M. Panhorst, J. Schotter, P. B. Kamp, A. Becker, *IEE Proc. Nanobiotechnol.* **2005**, *152*, 41; c) T. Aytur, J. Foley, M. Anwar, B. Boser, E. Harris, P. R. Beatty, *J. Immunol. Methods* **2006**, *314*, 21; d) V. N. Morozov, S. Groves, M. J. Turell, C. Bailey, *J. Am. Chem. Soc.* **2007**, *129*, 12628; e) K. S. Kim, J. K. Park, *Lab Chip* **2005**, *5*, 657; f) B. M. de Boer, J. A. H. M. Kahlman, T. P. G. H. Jansen, H. Duric, J. Veen, *Biosens. Bioelectron.* **2007**, *22*, 2366; g) J. Schotter, P. B. Kamp, A. Becker, A. Puhler, D. Brinkmann, W. Schepper, H. Bruckl, G. Reiss, *IEEE Trans. Magn.* **2002**, *38*, 3365; h) J. C. Rife, M. M. Miller, P. E. Sheehan, C. R. Tamanaha, M. Tondra, L. J. Whitman, *Sens. Actuators A* **2003**, *107*, 209.

