DOI: 10.1002/cphc.200800237

Magnetically Enhanced Dielectrophoretic Assembly of Horseradish Peroxidase Molecules: Chaining and Molecular Monolayers

Jianfei Sun,*[a, b, c] Haoyan Zhou,[c] Yonglong Jin,[a, b, c] Meng Wang,[a, b, c] Yifei Li,[c] and Ning Gu*[a, b, c]

Recently, interest in the controlled assembly of colloids has increased due to its scientific importance and widespread applications in nanoscience and nanotechnology.[1–3] Electric and magnetic fields are common tools to direct the self-assembly process under which nanoparticles often form one-dimensional ensembles.[4–5] Resulting from high-order damping with particulate size, the electromagnetic force is traditionally considered too weak to assemble very small objects. Although there are recent reports on electromagnetically controlled colloidal aggregation, showing the possibility that an electromagnetic field can manipulate nanoparticle aggregates,[6–8] the electromagnetically mediated assembly of several-nanometres-sized biological macromolecules has remained unexplored. This is mainly due to the small size of biological molecules and the possible destructive effect caused by planar lithographic electrodes. However, there are at least two driving forces in studying the field-controlled assembly of biological macromolecules. Firstly, proteins are greatly involved in many problems concerning life and health.[9–11] Thus, the controlled assembly of proteins may well play an important role in fundamental research and clinical therapy. Secondly, biological macromolecules are also considered as promising components and elementary frameworks in next-generation bioelectronics and devices.[12–14] The controllable assembly of biological macromolecules may be employed as a fabrication method of these components.

Herein, we show a novel experimental design to assemble horseradish peroxidase (HRP) molecules without using lithographical electrodes. Our design is able to generate a magnetostatic field and an alternating electric field simultaneously. The HRP molecules form linear assemblies in the synergistic presence of the magnetostatic and alternating electric fields. More interestingly, the enzyme molecules aggregate into a molecular monolayer on the substrate and their catalytic activity is scarcely deactivated after electromagnetic treatment. Our results demonstrate that the electromagnetic force is also capable of manipulating biological objects smaller than 10 nm without deactivation of biological function.

An alternating electric field was extensively employed in colloidal assembly, often called dielectrophoresis. The dielectrophoretic force can be expressed by Equation (1):

$$\langle \bar{F}_{DEP}(t) \rangle = 2\pi\varepsilon_m r^3 \mathrm{Re}[K(\omega)]\nabla|\bar{E}|^2 \quad (1)$$

where F_{DEP} is the dielectrophoretic force, ε_m is the permittivity of the medium, r is the radius of the particles, $\bar{E}$ is the intensity of electric field and $\mathrm{Re}[K(\omega)]$is the real part of the effective polarizability (Clausius–Mossotti factor) that is determined by the intrinsic properties of the material.[15] If the particles to be assembled were inorganic, the adjustable parameter would just be $\bar{E}$, including field intensity and frequency. For the assembly of proteins, the factor $\mathrm{Re}[K(\omega)]$ becomes adjustable as well, since the protein molecules are able to self-adapt to stimulation induced by an external field. Herein, we chose horseradish peroxidase—a classic enzyme about 4–6 nm in three dimensions[16]—as the building block. Considering that horseradish peroxidase contains an iron porphyrin group, the magnetostatic field was expected to enhance the effect of an alternating electric field. Thus, we employed two metal permanent mag-

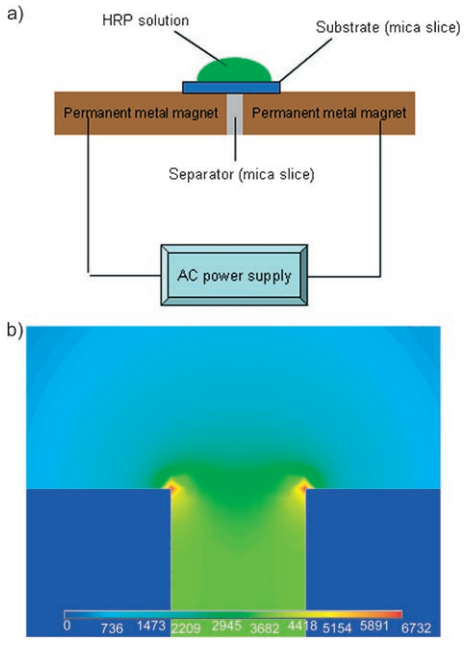

Figure 1. Experimental configuration and the generated electric field. a) Device generating simultaneous magnetostatic and electric fields. The two types of field are independent from each other. b) Simulated distribution of the electric field. Blue and red indicate minimal and maximal field intensity, respectively.

[a] *Dr. J. Sun, Dr. Y. Jin, Dr. M. Wang, Prof. Dr. N. Gu*
State Key Laboratory of Bioelectronics
Southeast University, 2 Sipailou, Nanjing 210096 (China)
Fax: 86-025-83794960
E-mail: guning@seu.edu.cn
sunzaghi@seu.edu.cn

[b] *Dr. J. Sun, Dr. Y. Jin, Dr. M. Wang, Prof. Dr. N. Gu*
Key Laboratory of Biomaterial and Device of Jiangsu Province
Southeast University, 87 Dingjiaqiao, Nanjing 210019 (China)

[c] *Dr. J. Sun, H. Zhou, Dr. Y. Jin, Dr. M. Wang, Y. Li, Prof. Dr. N. Gu*
School of Biological Science and Medical Engineering
Southeast University, 2 Sipailou, Nanjing 210096 (China)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cphc.200800237.

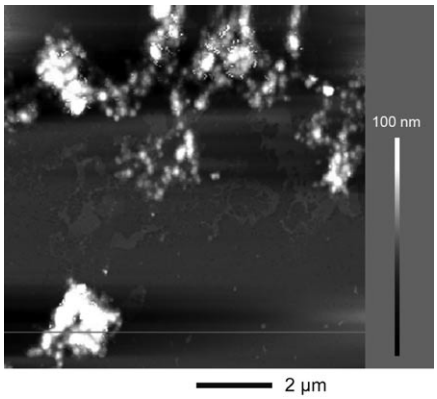

Figure 2. AFM images of HRP aggregation without any field treatment.

nets with an insulating slice to space them as electrodes (Figure 1 a). Due to the attractive interaction of heteropolar magnets, the gap between the two electrodes was equal to the thickness of the slice (400 μm in our experiments). In this case, there were both magnetostatic and electric fields in the gap and their directions were identical. The simulative distribution of the electric field is presented in Figure 1 b. Based on Equation (1), the field gradient was necessary. We experimentally placed the samples on the edge of gap, where the drain field provided the gradient for assembly (Figure 1 a).

HRP molecules aggregate into an amorphous bulk after drying naturally without any field treatment (Figure 2), which is nearly identical to what happens in the presence of either an electric or a magnetostatic field (see Figures S1 and S2 in the Supporting Information). Here, neither the dielectrophoretic force nor the magnetostatic force can overcome the thermal motion, due to the small size of the objects. However, in the presence of both the alternating electric field and the magnetostatic field, the molecules assemble into evident linear alignments that depend on the frequency. This indicates that the dielectrophoretic force plays a role (Figure 3). At a frequency of 1 kHz, the linear structures are about 50 nm wide and over 20 μm long. Based on the section analysis (See Supporting Information, Figure S3) of the green line marked in Figure 3, the assemblies are about 4–5 nm thick, approximating the size of a single HRP molecule. Thus, we conclude that the HRP aggregates are molecular monolayers.

To determine whether the magnetostatic field is involved in the assembly process, we changed the distribution of the magnetic field by introducing an additional pair of permanent magnets in orthogonal direction (for the experimental configuration, see the Supporting Information, Figure S4). With this setup, the four magnets generated a quadrapolar field with a different distribution from that of a dipolar field.[17,18] Through this alteration, the linear assembled morphology is destroyed (Figure 4). However, the aggregates remain molecular monolayers, likewise indicating that the magnetic field truly contributes to the assembly of HRP molecules.

Also, we substituted BSA (bovine serum albumin), a common protein without any metal centers, for HRP to confirm our hypothesis that the magnetostatic field, in the pres-

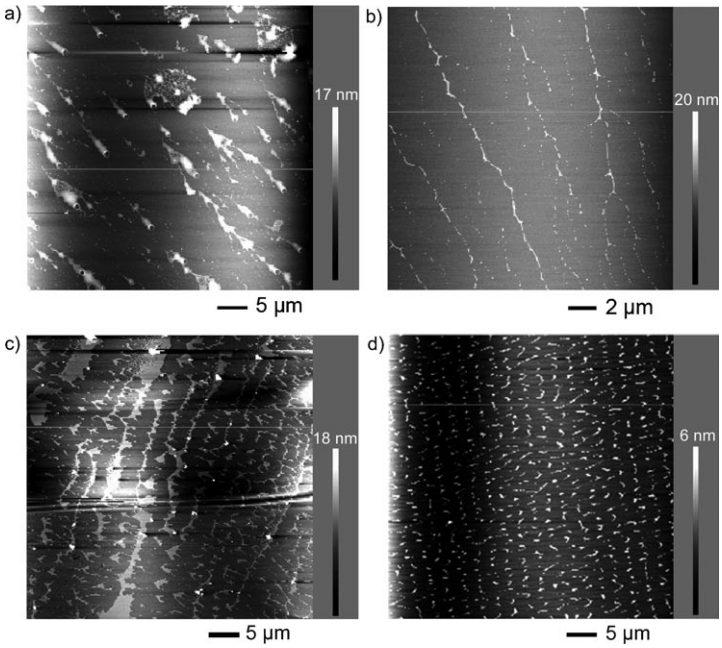

Figure 3. AFM images of HRP aggregation in the presence of both magnetostatic and alternating electric fields. a) 100 Hz, b) 1 kHz, c) 10 kHz, d) 100 kHz.

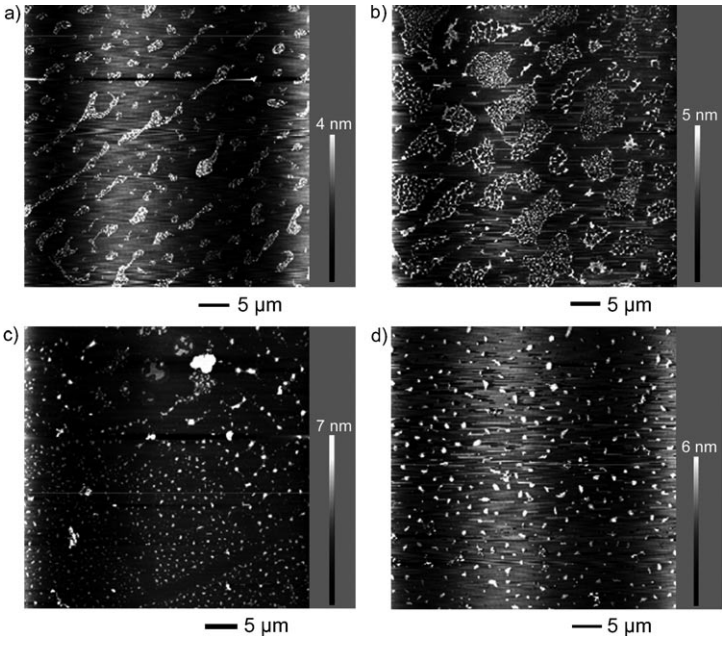

Figure 4. AFM images of HRP aggregation in the presence of quadrupolar magnetostatic and alternating electric fields. a) 100 Hz, b) 1 kHz, c) 10 kHz, d) 100 kHz.

ence of an alternating electric field, can only affect the assembly process for molecules with a high magnetic response. The BSA assembly shows little linear conformation (Figure 5), but rather resembles the amorphous bulk aggregates of HRP obtained at either an alternating electric field or a magnetostatic field. As the Fe-containing HRP molecules certainly have a high magnetic response, as opposed to metal-free BSA, this confirms our hypothesis.

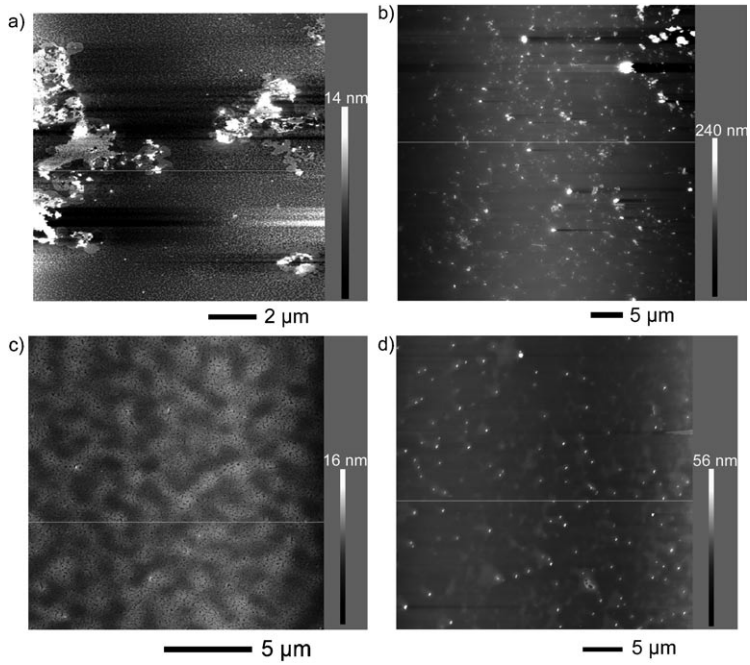

Figure 5. AFM images of BSA aggregation in the presence of both magnetostatic and alternating electric fields. a) 100 Hz, b) 1 kHz, c) 10 kHz, d) 100 kHz. The mass concentration is identical to that of the HRP experiments.

Concerning enzyme assembly, the catalytic function should be reserved. Figure 6 shows a plot of the frequency-dependent catalytic activity of the assembled HRP molecules with respect to those free of assembly. We found that the electromagnetic treatment had little influence on the HRP catalytic activity. Interestingly, the catalytic activity of assembled molecules can even be higher than that of molecules without assembly.

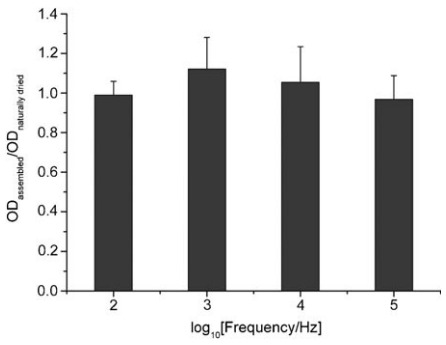

Figure 6. Frequency-dependent catalytic activity of assembled HRP versus naturally aggregated HRP. The comparison of catalytic activity is represented by the ratio of respective optical densities (OD).

The formation of the HRP molecular monolayer can account for the increase in catalytic activity of the assembled molecules. Upon formation of the monolayers, all the HRP molecules are exposed to substrate solution. In the absence of an external field, the spontaneously-formed aggregates are dispersed in the bulk, where a proportion of the molecules are screened by the outer molecules. The inner molecules can play a catalytic role only after the outermost molecules diffuse into the solution. Thus, within a given time span, the catalytic activity of electromagnetically-treated HRP molecules is higher than that of those without assembly.

In conclusion, we have realized for the first time the electromagnetically induced assembly of enzyme molecules below 10 nm in size, free of any special modifications of either the enzyme molecules or the substrate. In our experimental design, the HRP molecules are not in contact with the electrodes, thus avoiding some physical and chemical reactions and enabling the perfect preservation of biological catalytic activity. Also, the mechanism is based on field-induced moment–moment interactions, different from the aggregation–growth mechanism along electrodes that was previously proposed for the assembly of Au nanoparticles.[19] Our work provides a flexible technique for the assembly of building blocks, synergetically exploiting electric and magnetostatic fields. Due to the generality, simplicity and adaptability of the design, we believe that this work should benefit the theoretic development and technological applications in life science and bioelectronics.

Experimental Section

Molecule Assembly: The HRP molecules were assembled on a freshly cleaved mica surface. The substrate was placed onto the gap between two magnets, as depicted as Figure 1a. A drop of HRP solution of about 8 μL was spread onto the substrate. The power supply with a constant voltage output of 50 V (peak–peak value), was switched on until the solution was dried. As our equipment has four frequency ranges, we chose one typical frequency in every range. The purchased HRP (Sigma Corp.) was dispersed in ultra-pure water at a concentration of 0.05 mg mL^{-1}. All samples were characterized by AFM in tapping mode, 20 °C.

Measurement of the Catalytic Activity: The catalytic activity of HRP was measured by the classic catalytic chromogenic reaction of TMB (3,3,5,5-tetramethylbenzidene) in the presence of H_2O_2. The mica slice containing the assemblies was dipped in the 1 mL mixture of TMB and H_2O_2 for 5 min, and simultaneously another mica slice containing the naturally formed aggregates of HRP in the absence of an external field underwent the same treatment as the comparison. Then 50 μL 2 M H_2SO_4 were added into the two mixture systems as a terminator. Finally, the both optical density at 450 nm were recorded.

Acknowledgements

This work is supported by grants from the National Natural Science Foundation of China (NSFC, 60371027, 90406023, 60725101) and The National Basic Research Program of China (2006CB933206, 2006CB705600). J. Sun is also thankful for the support from The Foundation for Excellent Doctoral Candidates of Southeast University.

Keywords: biophysics · colloids · enzymes · nanostructures · self-assembly

[1] G. M. Whitesides, B. Grzybowski, *Science* **2002**, *295*, 2418–2421.
[2] S. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171–1178.
[3] J. Dutta, H. Hoffman, *Ency. Nanosci. Nanotech.* **2004**, *9*, 617–640.
[4] O. D. Velev, K. H. Bhatt, *Soft Matter* **2006**, *2*, 738–750.
[5] Y. Sahoo, M. Cheou, S. Wang, H. Luo, E. P. Furlani, P. N. Prasad, *J. Phys. Chem. B* **2004**, *108*, 3380–3383.
[6] K. D. Hermanson, S. O. Lumsdon, J. P. Williams, E. W. Kaler, O. D. Velev, *Science* **2001**, *294*, 1082–1086.
[7] J. F. Sun, Y. Zhang, Z. P. Chen, J. Zhou, N. Gu, *Angew. Chem.* **2007**, *119*, 4851–4854; *Angew. Chem. Int. Ed.* **2007**, *46*, 4767–4770.
[8] Y. J. Yuan, M. K. Andrews, B. K. Marlow, *Appl. Phys. Lett.* **2004**, *85*, 130–132.
[9] S. Lesné, M.-T. Koh, L. Kotilinek, R. Kayed, C. G. Glabe, A. Yang, M. Gallagher, K. H. Ashe, *Nature* **2006**, *440*, 352–357.
[10] M. Bucciantini, E. Giannoni, F. Chiti, F. Baroni, L. Formigli, J. Zurdo, N. Taddei, G. Ramponi, C. M. Dobson, M. Stefani, *Nature* **2002**, *416*, 507–511.
[11] Y. Levy, J. Onuchic, *Acc. Chem. Res.* **2006**, *39*, 135–142.
[12] C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144–146.
[13] S. Xu, B. Bevis, M. F. Amsdorf, *Biophys. J.* **2001**, *81*, 446–454.
[14] H. Yan, S.-H. Park, G. Finkelstein, J. H. Reif, T. H. LaBean, *Science* **2003**, *301*, 1882–1884.
[15] A. Docoslis, P. Alexandridis, *Electrophoresis* **2002**, *23*, 2174–2183.
[16] N. C. Veitch, *PhytoChem.* **2004**, *65*, 249–259.
[17] G. P. Hatch, R. E. Stelter, *J. Magn. Magn. Mater.* **2001**, *225*, 262–276.
[18] A. R. Urbach, J. C. Love, M. G. Prentiss, G. M. Whitesides, *J. Am. Chem. Soc.* **2003**, *125*, 12704–12705.
[19] K. D. Hermanson, S. O. Lumsdon, J. P. Williams, E. W. Kaler, O. D. Velev, *Science* **2001**, *294*, 1082–1086.

Received: April 18, 2008
Revised: July 15, 2008
Published online on August 14, 2008

