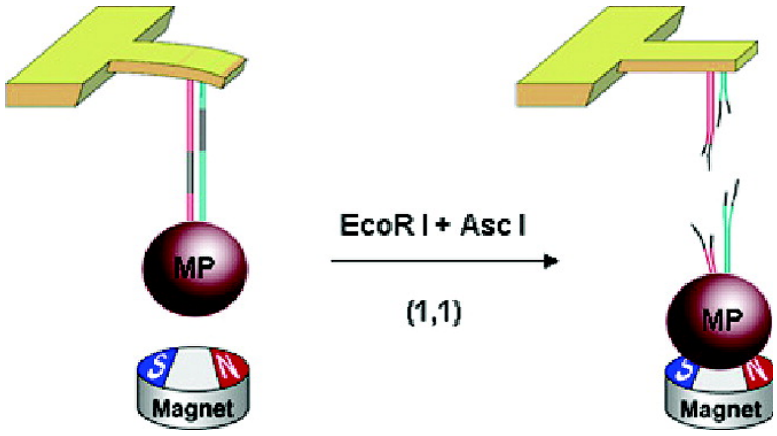
Article

Endonuclease-Based Logic Gates and Sensors Using Magnetic Force-Amplified Readout of DNA Scission on Cantilevers

Yossi Weizmann, Roey Elnathan, Oleg Lioubashevski, and Itamar Willner

J. Am. Chem. Soc., **2005**, 127 (36), 12666-12672• DOI: 10.1021/ja0533287 • Publication Date (Web): 18 August 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 16 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 08/18/2005

Endonuclease-Based Logic Gates and Sensors Using Magnetic Force-Amplified Readout of DNA Scission on Cantilevers

Yossi Weizmann, Roey Elnathan, Oleg Lioubashevski, and Itamar Willner*

Contribution from the Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received May 22, 2005; E-mail: willnea@vms.huji.ac.il

Abstract: The endonuclease scission of magnetic particles functionalized with sequence-specific DNAs, which are associated on cantilevers, is followed by the magnetic force-amplified readout of the reactions by the nano-mechanical deflection/retraction of the cantilevers. The systems are employed to develop AND or OR logic gates and to detect single base mismatch specificity of the endonucleases. The two endonucleases *Eco*RI (E_A) and *Asc*I (E_B) are used as inputs. The removal of magnetic particles linked to the cantilever by the duplexes **1/1a** and **2/2a** via the simultaneous cleavage of the DNAs by E_A and E_B leads to the retraction of the magnetically deflected cantilever and to the establishment of the "AND" gate. The removal of the magnetic particles linked to the cantilevers by the duplex **3/3a** by either E_A or E_B leads to the retraction of the magnetically deflected cantilever and to the establishment of the "OR" gate. The magnetic force-amplified readout of endonuclease activities is also employed to reveal single base mismatch specificity of the biocatalysts.

Introduction

The use of molecular[1] and biomolecular[2] systems for information storage and processing attracts substantial recent research efforts as a means to develop electronic and computing devices based on molecular-level systems. The unique electronic properties of nanoscale materials such as metal or semiconductor nanoparticles or carbon nanotubes were employed experimentally,[3] as well as theoretically,[4] for information processing and computing. Molecular,[5] supramolecular,[6] and biomolecular[7] systems were used as switching devices or as signal-gated systems, and electrical,[8] optical,[9] or chemical[10] stimuli were used to trigger the logic functions of these systems. The integration of switchable chemical assemblies with solid supports provides intelligent structures that allow the readout of the recorded stimuli and the development of input/output systems.[11−13] Among the biomaterials, proteins[14] and, specifically, DNA[15] provide unique functional structures for the design of logic gates. The possibility of encoding information in the base sequences of DNA, the feasibility of manipulating DNA by enzymes or DNAzymes, the selective intercalation of photoactive/electroactive molecular units in double-stranded DNA, and the specific tethering of fluorophores/redox-active units into duplex DNA

(1) (a) Ballardini, R.; Balzani, V.; Credi, A.; Gandolfi, M. T.; Venturi, M. *Acc. Chem. Res.* **2001**, *34*, 445−455. (b) Raymo, F. M. *Adv. Mater.* **2002**, *14*, 401−414. (c) Willner, I.; Willner, B. *J. Mater. Chem.* **1998**, *8*, 2543−2556.
(2) (a) Willner, I. *Acc. Chem. Res.* **1997**, *30*, 347−356. (b) Willner, I.; Willner, B. *Pure Appl. Chem.* **2001**, *73*, 535−542. (c) Willner, I.; Rubin, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 367−385.
(3) (a) Bachtold, A.; Hadley, P.; Nakanishi, T.; Dekker, C. *Science* **2001**, *294*, 1317−1320. (b) Huang, Y.; Duan, X.; Cui, Y.; Lanhon, L. J.; Kim, K. H.; Lieber, C. M. *Science* **2001**, *294*, 1313−1317. (c) Hunag, Y.; Duan, X. F.; Wei, Q. Q.; Lieber, C. M. *Science* **2001**, *291*, 630−633.
(4) (a) Remacle, F.; Speiser, S.; Levine, R. D. *J. Phys. Chem. B* **2001**, *105*, 5589−5591. (b) Remacle, F.; Weinkauf, R.; Steinitz, D.; Kompa, K. L.; Levine, R. D. *Chem. Phys.* **2002**, *281*, 363−372.
(5) (a) de Silva, A. P.; MeClenghan, N. D. *Chem.-Eur. J.* **2004**, *10*, 574−586. (b) Straight, S. D.; Andreasson, J.; Kodis, G.; Moore, A. L.; Moore, T. A.; Gust, D. *J. Am. Chem. Soc.* **2005**, *127*, 2717−2724. (c) Tour, J. M. *Acc. Chem. Res.* **2000**, *33*, 791−804. (d) Guo, X.; Zhang, D.; Zhu, D. *Adv. Mater.* **2004**, *16*, 125−130.
(6) (a) Collier, C. P.; Wong, E. W.; Belohradsky, M.; Raymo, F. M.; Stoddart, J. F.; Kuekes, P. J.; Williams, R. S.; Heath, J. R. *Science* **1999**, *285*, 391−394. (b) Balzani, V.; Credi, A.; Venturi, M. *ChemPhysChem* **2003**, *3*, 49−59.
(7) (a) Stojanovic, M. N.; Mitchell, T. E.; Stefanovic, D. *J. Am. Chem. Soc.* **2002**, *124*, 3555−3561. (b) Adelman, I. M. *Science* **1994**, *266*, 1021−1024. (c) Sakamoto, K.; Gonzu, H.; Komiya, K.; Kiga, D.; Yokoyama, S.; Yokomori, T.; Hagiya, M. *Science* **2000**, *288*, 1223−1226.
(8) (a) Gilat, S. L.; Kawai, S. H.; Lehn, J. M. *Chem.-Eur. J.* **1995**, *1*, 275−284. (b) Collin, J. P.; Gavina, P.; Sauvage, J.-P *Chem. Commun.* **1996**, 2005−2006. (c) Shipway, A. N.; Katz, E.; Willner, I. *Struct. Bonding* **2001**, *99*, 237−281.
(9) (a) Liddel, P. A.; Kodis, G.; Andreasson, J.; de La Ganza, L.; Bandyopadhyay, S.; Mitchell, R. H.; Moore, T. A.; Moore, A. L.; Gust, D. *J. Am. Chem. Soc.* **2004**, *126*, 4803−4811. (b) Andreasson, J.; Kodis, G.; Terazoni, Y.; Liddell, P. A.; Bandyopadhyay, S.; Mitchell, R. H.; Moore, T. A.; Moore, A. L.; Gust, D. *J. Am. Chem. Soc.* **2004**, *126*, 15926−15927. (c) Willner, I.; Marx, S.; Eichen, Y. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1243−1244. (d) Ferringa, B. L.; van Delden, R. A.; Koumbera, N.; Geertsema, E. M. *Chem. Rev.* **2000**, *100*, 1789−1816.
(10) Credi, A.; Balzani, V.; Langford, S. J.; Stoddart, J. F. *J. Am. Chem. Soc.* **1997**, *113*, 1992−1996.
(11) (a) Liu, Z. F.; Hashimoto, K.; Fujishima, A. *Nature* **1990**, *347*, 658−660. (b) Doron, A.; Portnoy, M.; Lion-Dagan, M.; Katz, E.; Willner, I. *J. Am. Chem. Soc.* **1996**, *118*, 8937−8944.
(12) (a) Willner, I.; Pardo-Yissar, V.; Katz, E.; Ranjit, K. T. *J. Electroanal. Chem.* **2001**, *497*, 172−177. (b) Willner, I.; Willner, B. *Adv. Mater.* **1997**, *9*, 351−355.
(13) Katz, E.; Lioubashevski, O.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 15520−15532.
(14) (a) Ashkenazy, G.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 11140−11141. (b) Dueber, J. E.; Yeh, B. J.; Chak, K.; Lim, W. A. *Science* **2003**, *301*, 1904−1908. (c) Sivan, S.; Tuchman, S.; Lotan, N. *BioSystems* **2003**, *70*, 21−33.
(15) (a) Benenson, Y.; Paz-Elizur, T.; Adar, R.; Keinan, E.; Livneh, Z.; Shapiro, E. *Nature* **2001**, *414*, 430−434. (b) Faulhammer, D.; Curkas, A. R.; Lipton, R. J.; Landweber, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1385−1389.

10.1021/ja0533287 CCC: $30.25

turn DNA into an attractive biomaterial for information processing and computation.[15,16]

Recently, the principles of Boolean logic gates were established at the molecular level by applying a variety of ionic,[17] photonic,[18] or DNA[19] inputs. Furthermore, the combination of these molecular gates was employed to construct half-adder or half-subtractor systems and to accomplish arithmetic operations involving two binary digits.[20] In all of these systems, tailored synthetic "gate molecules" were used, and the logic operations were performed in solution. Only a few studies demonstrated logic operations on solid supports (e.g., counters).[21]

The present study proposes a new method to assemble Boolean circuits by the application of enzymatic cleavage reactions on DNA, and to readout the processes via the deflection of cantilevers enhanced by magnetic forces between magnetic particle-functionalized DNA and an applied magnetic field. The tailored DNA duplexes are utilized as "logic strands", while two different restriction enzymes serve as inputs for the gates. The experimental outputs of the proposed systems were translated into logic outputs (i.e., "1" or "0"). We demonstrate that the proposed principle allows the construction of AND and OR gates. Furthermore, we demonstrate, in appropriate magnetic particle-functionalized DNA duplexes, the specific scission in complementary domains. Besides the demonstration of the activity of the systems as logic gates, they represent interesting sensors that follow single base mismatches by sequence-specific endonucleases.

Experimental Section

Chemicals and Materials. Amine-functionalized borosilicate-based magnetic particles (5 μm, MPG long chain alkylamine, CPG, Inc.) were employed in the study. The heterobifunctional cross-linker 3-maleimide propionic acid *N*-hydroxysuccinimide ester and the thiolated nucleic acids were purchased from Sigma.

Chemical Modification of Magnetic Particles. The magnetic particles were functionalized with the respective thiolated nucleic acids by the primary reaction of the amine-modified magnetic particles, 30 mg, with the cross-linker 3-maleimide propionic acid *N*-hydroxysuccinimide, 5 mg, in 1 mL of dimethyl sulfoxide (DMSO) for 4 h at room temperature. The resulting magnetic particles were collected by means of an external magnet and washed with DMSO and then with a 10 mM phosphate buffer solution, pH = 7.4. The modified magnetic particles were then reacted with 20−30 OD (optical density) of the respective thiolated nucleic acids in 10 mM phosphate buffer, pH = 7.4, for a time interval of 8 h. The thiolated nucleic acids were activated to their thiolated reduced state prior to the modification of the particles by reaction with dithiothreitol, followed by chromatographic separation on a Sephadex G-25 column. The resulting magnetic particles were washed with 10 mM phosphate buffer, pH = 7.4. The particles were stored in a buffer solution with 1% (w/v) sodium azide at 4 °C. The particles revealed stability for at least 6 months.

The loading of the nucleic acids associated with the magnetic particles was determined by following spectroscopically the content of DNA before and after adsorption of the particles.

OR and AND Logic Gates Construction. To construct the systems for the OR logic gates or for the systems sensing the single-base scission specificity, the respective thiolated nucleic acids were assembled on the Au-coated cantilever by interaction of the surfaces with 200 μL of phosphate buffer that included the thiolated nucleic acids, 0.5 OD, for 4 h. Hybridization of the modified cantilevers was performed in the presence of 1.5 mg of the magnetic particles functionalized with the complementary nucleic acid in 200 μL of phosphate buffer, 10 mM, and NaCl, 0.3 M, for a time interval of 4 h. For the AND gate, the functionalized magnetic particles, 1.5 mg, were hybridized at 77 °C, with the disulfide-protected complementary nucleic acid, 0.5 OD, in 200 μL of phosphate buffer 10 mM and NaCl, 0.3 M, for 2 h, followed by deprotection of the disulfide with dithiothreitol, and the magnetic collection and rinsing with phosphate buffer solution, 10 mM, that included NaCl, 0.3 M. The resulting thiolated duplex-functionalized magnetic particles were then assembled on the Au-coated cantilever. The latter protocol for assembling the AND gate system was undertaken because the two duplexes have structurally similar sequences, and thus cross-hybridization was eliminated by performing the hybridization at the melting temperature of the cross hybrids.

The system used to follow the magnetic forced-induced deflection of the cantilever and the catalytic scission of the DNA on the cantilever was described previously.[22,23]

Results and Discussion

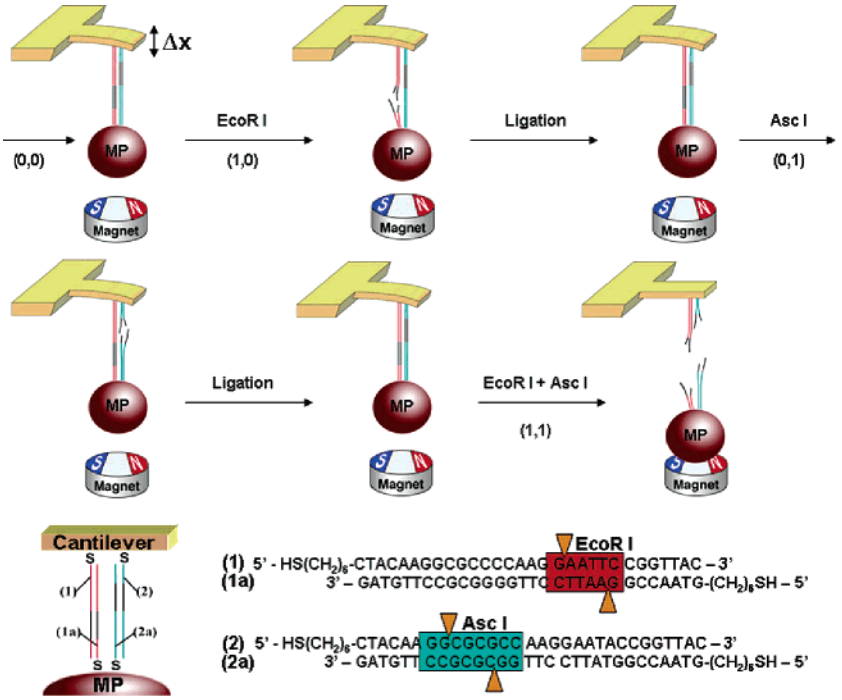
The detection of biocatalytic processes on DNA using cantilevers as mechanical probes that respond to an external magnetic field was recently reported by our laboratory.[22] Replication of double-stranded DNA (dsDNA) in the presence of dNTPs/biotin-dUTP and polymerase, or the elongation of telomeric single-stranded DNA (ssDNA) in the presence of dNTPs/biotin-dUTP and telomerase, was performed on cantilevers. These biocatalytic reactions resulted in the incorporation of biotin labels into the replicated or telomerized DNAs. Subsequently, biotin-labeled magnetic particles (MPs) were conjugated to the DNAs through avidin bridges, and the forced deflection of the modified cantilever by an external magnet, provided a mechanical signal for the biocatalytic processes. Similarly, the biocatalyzed scission of sequence-specific DNAs by the endonucleases *Apa*I or *Mse*I on cantilevers was followed by magnetic forces exerted on the cantilevers.[23] Magnetic particles were attached to the duplex DNAs as labels that led to the deflection of the cantilever under an applied external magnetic field. The specific biocatalyzed scission of the DNAs removed the magnetic particles from the cantilevers, resulting in the retraction of the cantilevers to their rest position even under an applied magnetic field.

The two inputs in our study are the endonucleases *Eco*RI and *Asc*I (E_A and E_B, respectively). The endonuclease-based AND logic gate that uses the magnetic force-amplified readout of the gate function on the cantilever is schematically depicted in Scheme 1.

The Au-coated cantilevers were modified with the magnetic particle-functionalized **1**/**1a** and **2**/**2a** duplexes, Scheme 1. The

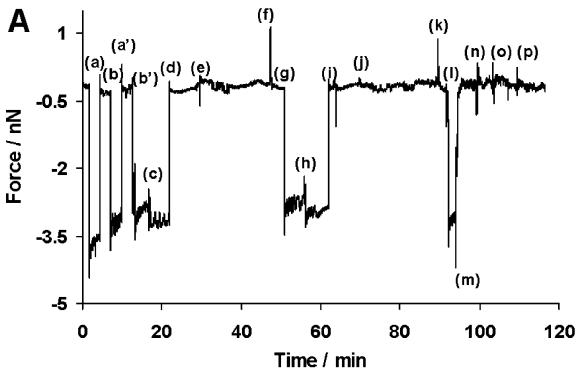
(16) (a) Liu, Q.; Wang, L.; Frutos, A. G.; Condon, A. E.; Corn, R. M.; Smith, L. M. *Nature* **2000**, *403*, 174−179. (b) Ouyang, Q.; Kaplan, P. D.; Liu, S.; Libchaber, A. *Science* **1997**, *278*, 446−449. (c) Cox, J. C.; Ellington, A. D. *Curr. Biol.* **2001**, *11*, R336−R336. (d) Stojanovic, M. N.; Stefanovic, D. *Nat. Biotechnol.* **2003**, *21*, 1069−1074. (e) Stojanovic, M. N.; Stefanovic, D. *J. Am. Chem. Soc*. **2003**, *125*, 6673−6676.
(17) (a) Raymo, F. M.; Giordani, S. *J. Am. Chem. Soc*. **2001**, *123*, 4651−4652. (b) Ji, H. F.; Debestani, R.; Brown, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 9306−9307.
(18) Guo, X.; Zhang, D.; Zhang, G.; Zhu, D. *J. Phys. Chem. B* **2004**, *108*, 11942−11945.
(19) (a) Saghatelian, A.; Volcker, N. H.; Guckian, K. M.; Lin, V. S.-Y.; Ghadiri, R. *J. Am. Chem. Soc.* **2003**, *125*, 346−347. (b) Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 9458−9463.
(20) (a) De Silva, A. P.; McCleneghan, N. D. *J. Am. Chem. Soc.* **2000**, *122*, 3965−3966. (b) Margulies, D.; Melman, G.; Felder, C. F.; Arad-Yellin, R.; Shanzer, A. *J. Am. Chem. Soc.* **2004**, *126*, 15400−15401.
(21) Remacle, F.; Willner, I.; Levine, R. D. *ChemPhysChem* **2005**, *6*, 1239−1242.
(22) Weizmann, Y.; Patolsky, F.; Lioubashevski, O.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 1073−1080.
(23) Weizmann, Y.; Elnathan, R.; Lioubashevski, O.; Willner, I. *Nano Lett.* **2005**, *5*, 741−744.

Scheme 1. Functional Activity of the AND Gate by Following the Magnetic Field-Induced Forces Exerted on a Cantilever Modified with the Magnetic Particles Functionalized with **1**/**1a** and **2**/**2a** Duplexes, and Using the Sequence-Specific Scission of the Endonucleases *Eco*RI and *Asc*I



resulting magnetic particle-labeled duplexes **1**/**1a** and **2**/**2a** associated with the cantilever include the sequence-specific domains for scission by E_A and E_B, respectively. In the presence of the external magnetic field, the cantilever is deflected due to the magnetic force applied on the particles. Scission of the respective domains by the endonucleases E_A and E_B cleaves off the magnetic particles, and the cantilever is retracted to its nondeflected position even under the applied field. Thus, by defining the deflected position of the cantilever as logic output "0", and the nondeflected state of the lever as logic output "1", the true output "1" is obtained only in the presence of both inputs E_A and E_B.

Magnetic particles (5 μm diameter) were modified with the complementary nucleic acids **1a** and **2a**. The total loading on the magnetic particles was estimated to be 50 000 oligonucleotides (see details in Experimental Section) per particle. The operation of the logic gate AND and the results corresponding to scission of the magnetic particle-functionalized duplex DNAs are shown in Scheme 1 and Figure 1A. The hybrid consisting of the magnetic particle with the duplexes **1**/**1a** and **2**/**2a** associated with the cantilever brings the lever to its rest, nondeflected position, Figure 1A, point a. By the positioning of the external magnet close to the cell, the cantilever is deflected, point b. The reversible elimination or application of the external magnetic field on the cantilever results in the bistable positioning of the cantilever in the retracted or deflected states, respectively, points a′ and b′. At point c, the enzyme *Eco*RI is injected into the cell, while the lever exists in its deflected state. The deflection of the lever is unaffected, albeit the duplexes consisting of **1**/**1a** are separated (vide infra). At point d, the external magnet is removed and the lever is retracted to its rest position. The cell is rinsed and ligase is injected into the cell to ligate the scissed strands of the duplex, **1**/**1a**, point e. At point f, the ligase is rinsed off from the system. At point g, the external magnet is applied on the cantilever that is redeflected, indicating that the magnetic particles-functionalized duplexes exist in an intact structure on the lever. At point h, the enzyme *Asc*I is injected into the cell. The lever position is, again, unaffected, indicating that although the duplex structure **2**/**2a** is scissed (vide infra) the magnetic particles are not detached from the lever. At point i, the external magnet is removed and the lever is retracted to the nondeflected state, and at point j, the cell is rinsed and the modified lever is treated with ligase, to "glue" together the scissed duplex **2**/**2a**. The cell

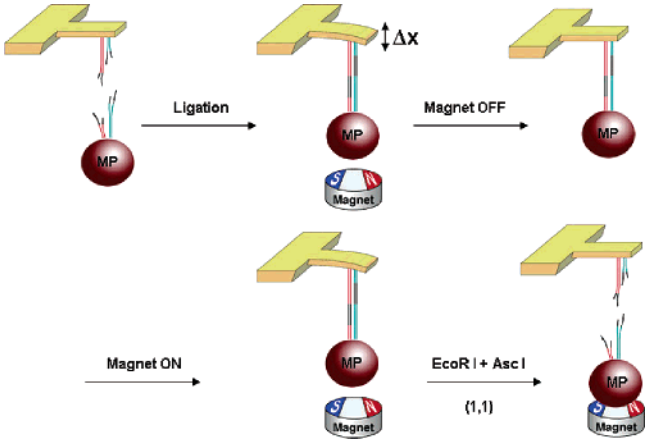


B **AND gate**

Input		Output
0	0	0
0	1	0
1	0	0
1	1	1

Figure 1. (A) Functional activity of the AND gate following the time-dependent magnetic field-induced forces exerted on a cantilever modified with the magnetic particles functionalized with **1**/**1a** and **2**/**2a** duplexes, and using the sequence-specific scission by endonucleases *Eco*RI and *Asc*I. Steps a−p are detailed in the text. (B) Truth table for the AND gate.

Scheme 2. Repeated Use of the AND Gate Using the Sequence-Specific Scission of the Endonucleases *Eco*RI and *Asc*I



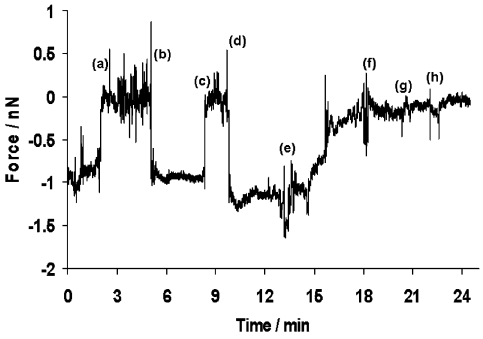
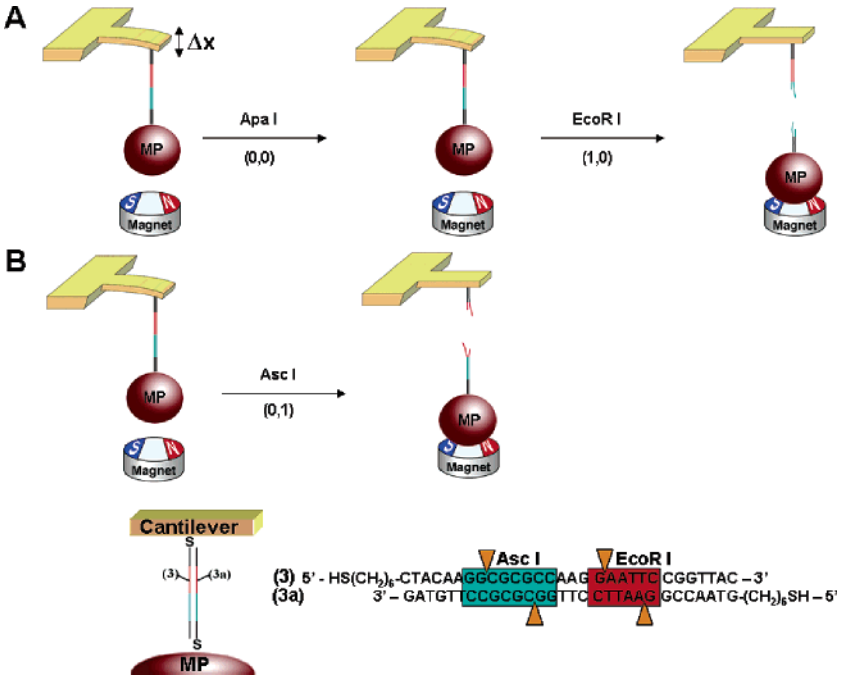


Figure 2. Repeated use of the AND gate by following the magnetic field-induced forces exerted on a cantilever modified with the magnetic particles functionalized with **1**/**1a** and **2**/**2a** duplexes, and using the sequence-specific scission by the endonucleases *Eco*RI and *Asc*I. Steps a−h are detailed in the text.

is further rinsed to remove the ligase, point k, and at point l the external magnet is applied on the cantilever, resulting in its deflection. At point m, the mixture of the two enzymes *Asc*I and *Eco*RI is injected into the system, under the conditions where the external magnet is applied on the system. The retraction of the lever to its nondeflected position is detected even though the magnet is applied on the cantilever. These results are consistent with the scission of the two duplex units **1**/**1a** and **2**/**2a** that results in the removal of the magnetic particles and the retraction of the cantilever. At points n, o, and p, the external magnet is removed, applied, and removed again, respectively. No mechanical movement is observed, consistent with the removal of the magnetic particles from the cantilever. That is, the scission of the duplexes **1**/**1a** and **2**/**2a** by the endonucleases E_A and E_B results in an AND gate. The retraction of the magnetically deflected cantilevers to the nondeflected state requires two simultaneous inputs, E_A and E_B, and corresponds to the output "True" or logic "1". The separate inputs E_A or E_B do not affect the cantilever position and correspond to "False" outputs, logic "0", Figure 1B. To demonstrate the possibility of repeated use of the logic gate, and to reveal its bistable operation, the functional cantilever was reassembled, Scheme 2.

The cantilever that included the scissed duplex structure was treated with magnetic particles that were modified with the duplex DNA fragments that were cleaved off by the mixture of two enzymes. That is, the magnetic particles are functionalized with the sticky ends that ligate to the nucleic acids on the cantilever in the presence of ligase. Figure 2, point a, shows the position of the cantilever after ligating the functionalized magnetic particles to the nucleic acids associated with the cantilever. Upon applying the external magnet, the lever is deflected, implying that the magnetic particles were reassembled on the cantilever, point b. At points c and d, the external magnet is removed and reapplied, respectively, on the cantilever, and the lever is retracted to the nondeflected and deflected states, respectively. At point e, the enzymes *Asc*I and *Eco*RI are added

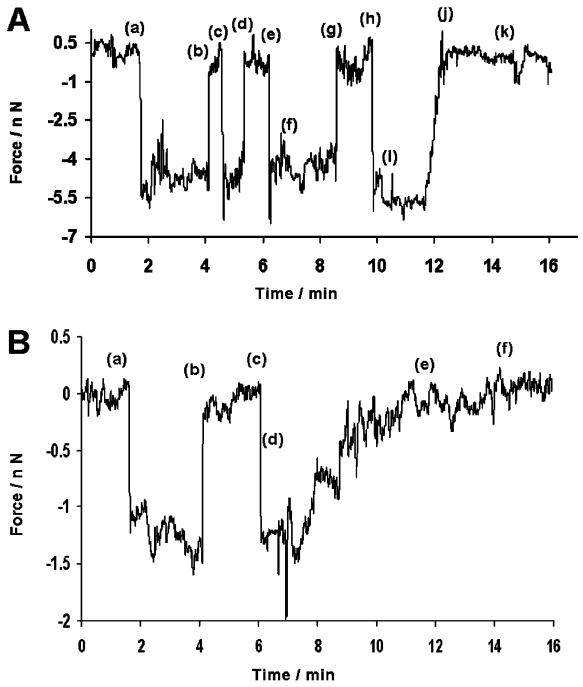
Scheme 3. Functional Activity of the OR Gate by Following the Magnetic Field-Induced Forces Exerted on a Cantilever Modified with the Magnetic Particles Functionalized with the **3**/**3a** Duplex: (A) Using the Sequence-Specific Scission of the Endonuclease *Eco*RI and (B) Using the Sequence-Specific Scission of the Endonuclease *Asc*I



to the system under the applied external magnetic field. The cantilever returns to the nondeflected position, even though it is subjected to the external magnetic field. At points f, g, and h, the external magnet is removed, reapplied on the lever, and removed again. The position of the lever is unaffected, implying that the magnetic particles were removed in the second scission cycle.

To further develop the endonuclease-based logic gates that employ the magnetic force amplified readout of the logic functions, we adapted this concept to design an OR gate, Scheme 3. For this purpose, we use a single duplex structure for the attachment of the magnetic particles onto the cantilever. The cantilever is functionalized with the nucleic acid **3**, and the surface is hybridized with the nucleic acid **3a**-functionalized magnetic particles where **3a** is complementary to **3**. The duplex DNA includes two domains that can be specifically cleaved by the endonucleases *Eco*RI (E_A) or *Asc*I (E_B).

Thus, the interaction of the magnetically deflected cantilever with either E_A or E_B as inputs will lead to the scission of the DNA and the removal of the magnetic particles from the cantilever. This results in the retraction of the cantilever and the possibility of using the mechanical relocation of the cantilever as a readout (output) signal. Figure 3A and B depicts the functional operation of the OR gate using the two enzymes as inputs. Figure 3A shows the removal of the magnetic particles by the *Eco*RI-induced scission of the duplex **3**/**3a**, and the specificity of this endonuclease toward the cleavage of the DNA structure. At points a−d, the cantilever is repeatedly subjected to the external magnetic field, followed by the removal of the external magnet. The cantilever is deflected upon the application of the external magnet, and it returns to the original state by the removal of the external magnet. The cantilever is forced to its deflected position, and at point f the foreign endonuclease, *Apa*I, was added. The cantilever retains its deflected position, and only upon the removal of the external magnet does the cantilever retract to its nondeflected configuration, point g. Reapplication of the external magnet on the cantilever, point h, results in the mechanical deflection of the cantilever. These results clearly indicate that the foreign endonuclease has no effect on the duplex DNA, **3**/**3a**, and that the magnetic particles stay intact on the cantilever. At point i, the *Eco*RI endonuclease is added, while the cantilever is deflected. The cantilever returns to its nondeflected state even though the external magnet is applied on the system. This result indicates that the magnetic particles were removed from the cantilever by the scission of the duplex DNA. Additional support that the magnetic particles were removed from the cantilever was obtained by further elimination and application of the external magnet, points j and k, respectively. The position of the cantilever is unaffected, and it rests in its nondeflected configuration. Figure 3B shows the analogous scission of the magnetic particle-functionalized duplex DNA by the endonuclease *Asc*I (E_B). This endonuclease cleaves the second sequence-specific domain in the duplex DNA **3**/**3a** and leads to the removal of the particles too. At points a−c, the external magnet is applied, removed, and reapplied on the system, respectively. The cantilever is deflected, retracted, and redeflected, consistent with the presence of the magnetic particles on the cantilever. At point d, the endonuclease *Asc*I is added to the system while the cantilever is in its deflected configuration. Addition of the biocatalyst results in the return



C OR gate

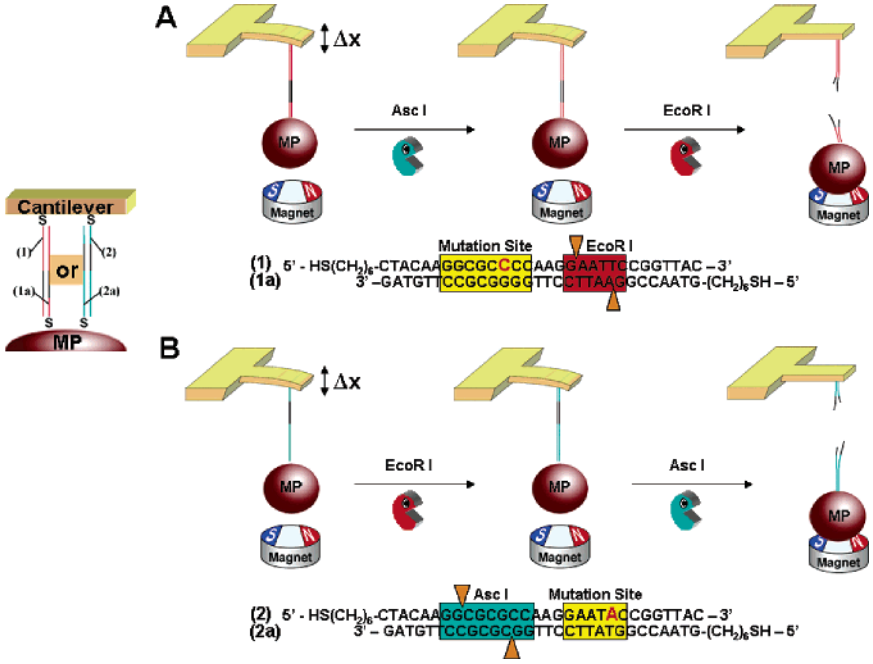
Input		Output
0	0	0
0	1	1
1	0	1
1	1	1

Figure 3. (A) Functional activity of the OR gate following the time-dependent magnetic field-induced forces exerted on a cantilever modified with the magnetic particle functionalized with **3**/**3a** duplex, and using the sequence-specific scission endonuclease *Eco*RI. Steps a−k are detailed in the text. (B) Functional activity of the OR gate following the time-dependent magnetic field-induced forces exerted on a cantilever modified with the magnetic particles functionalized with **3**/**3a** duplex, and using the sequence-specific scission endonuclease *Asc*I. Steps a−f are detailed in the text. (C) Truth table for the OR gate.

of the cantilever to its nondeflected state, although the cantilever is subjected to the external magnet. At points e and f, the external magnet is removed and reapplied on the system, and these processes have no effect on the cantilever state and it rests in its nondeflected configuration. These results are consistent with the scission of the DNA duplex **3**/**3a** by *Asc*I and the removal of the magnetic particles from the cantilever. Thus, each of the endonucleases, *Eco*RI or *Asc*I, acts as input that leads to the scission of the DNA duplex units associated with the cantilever, and the outputs are readout by the mechanical translocation of the cantilever. The schematic OR gate function and the respective "truth table" are shown in Figure 3C.

Any sensing cycle that provides Yes/No signals represents the simplest gate with a single input. Thus, the scission of a sequence-specific duplex DNA by the respective endonuclease may be viewed as a single input operation. Thus, the paradigm that was presented as the "OR" gate, where the two sequence-specific domains are selectively cleaved by the respective endonucleases, may be further extended to sense sequence-specific single base mismatches by the endonuclease-induced scission processes, as depicted in Scheme 4.

Scheme 4. Sensing Sequence Following Single Base Mismatches by the Endonuclease-Induced Scission Processes: (A) The Sequence-Specific Scission of the Duplex **1**/**1a** by *Eco*RI and (B) the Sequence-Specific Scission of the Duplex **2**/**2a** by *Asc*I



In Scheme 4A, the cantilever is modified with the nucleic acid **1**, and the interface is further hybridized with the **1a**-functionalized magnetic particles. The resulting duplex includes a fully complementary domain for scission by *Eco*RI (E_A), whereas the second domain includes a single base mismatch in the base sequence for scission by *Asc*I (E_B). Provided that the scission of the duplex DNA is sequence specific, the magnetically induced deflected cantilever will be unaffected by *Asc*I (because of the single base mismatch in the respective domain), whereas the duplex will be cleaved by *Eco*RI (E_A), which will result in the removal of the magnetic particles, and the retraction of the cantilever under the applied magnetic field. Similarly, Scheme 4B shows the magnetic force-amplified analysis of the specific scission activity *Asc*I (E_B). The magnetic particle-functionalized duplex **2**/**2a** is associated with the cantilever. It includes the fully complementary specific sequence for *Asc*I (E_B), while the second domain includes a single base mismatch for scission by E_A. Thus, provided that E_B reveals specificity, the magnetically induced deflected cantilever will not be affected by *Eco*RI (E_A), whereas the scission of the duplex by *Asc*I (E_B) results in the removal of the magnetic particles and the retraction of the cantilever. Figure 4 shows the results that demonstrate the analysis of single base mismatches, and the analysis of sequence-specific DNA regions by the endonucleases E_A and E_B. In Figure 4A, the specific scission activity of *Eco*RI is demonstrated by the magnetic force-induced deflection of the cantilever, by showing that a single base mismatch in the *Asc*I domain prohibits the cleavage process. The cantilever modified with the magnetic particles-functionalized duplex **1**/**1a** is subjected to the external magnet, which results in the deflection of the cantilever, point a. At point b, the external magnet is removed, and the cantilever is retracted to its nondeflected configuration. At points c, d, and e, the external magnet is applied on the system, removed, and reapplied, and these acts result in the deflection, retraction, and redeflection of the cantilever, respectively. These results confirm that the magnetic particles are associated with the cantilever and it responds to the external magnet, as expected. At point f, the *Asc*I is added to the system in the deflected configuration and the cantilever position is unaffected. The magnetic particles stay intact on the

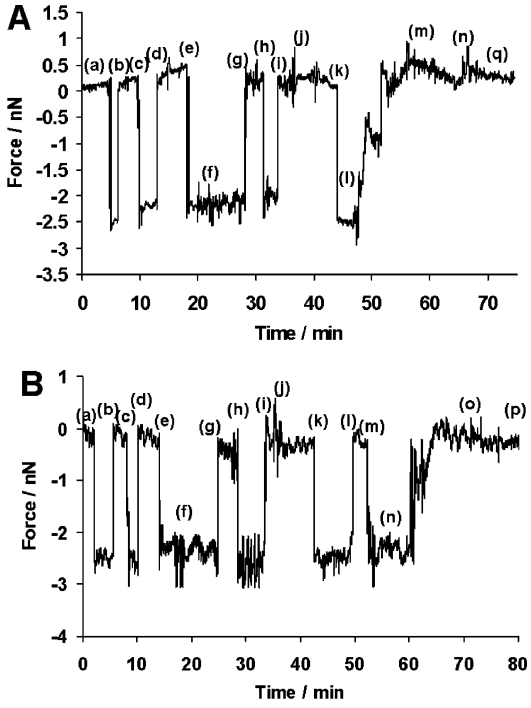


Figure 4. (A) Time-dependent magnetic field-induced forces exerted on the cantilever modified with the magnetic particle functionalized with **1**/**1a** duplex, upon the specific scission by *Eco*RI. Steps a−q are detailed in the text. (B) Time-dependent magnetic field-induced forces exerted on the cantilever modified with the magnetic particle functionalized with **2**/**2a** duplex upon specific scission by *Asc*I. At points a, c, and e, the modified cantilever is subjected to the external magnet. At points b and d, the external magnet is removed. Steps f−p are detailed in the text.

cantilever as confirmed by the fact that the further removal, application, and re-removal of the external magnet results in the retraction, deflection, and return to the nondeflected state, points g, h, and i, respectively. At point j, the cell is rinsed with the buffer solution and the cantilever is deflected by the application of the external magnet, point k. At point l, the system is subjected to *Eco*RI, while applying the external magnet on the system. A time-dependent retraction of the cantilever is observed, even though the system is subjected to the external magnet. At points m, n, and q, the external magnet is removed, applied, and re-eliminated from the system, and the position of the cantilever is not altered. These results confirm that *Eco*RI induces the cleavage of the duplex at its sequence-specific domain, and this results in the removal of the magnetic particles from the cantilever, and its retraction to the nondeflected configuration. Figure 4B shows the analogous sequence specificity scission of the duplex **2**/**2a** by *Asc*I. The different control studies are detailed in the caption of the figure. At point f, the enzyme *Eco*RI is injected into the cell, while the cantilever is forced into the deflected state by the external magnet. The position of the cantilever is unaffected, implying that the single base mismatch in the sequence domain of *Eco*RI prohibits the scission process. At point n, the cantilever exists in its deflected state, and *Asc*I is injected into the system. This results in the retraction of the cantilever, even though the external magnet is applied on the system. These results, together with the lack of the system response to the removal/application of the external magnetic field, points o and p, imply that the scission of the specific sequence by *Asc*I took place and that the magnetic particles were removed from the cantilever. Thus, the results depicted in Figure 4 indicate the specificity of the *Asc*I and *Eco*RI endonucleases. A single base mismatch in the respective double-stranded domains that are cleaved by the biocatalysts is sufficient to block the scission processes of the respective duplex structures. These results indicate that single base mismatches may be detected by following the endonuclease activities.

Conclusions

The present study has formulated a new paradigm to assemble a DNA-modified cantilever device that performs logic functions. Endonucleases are used as inputs that activate the logic gates, and the outputs of the systems are read out by following the magnetic field-induced mechanical motion of the cantilever. The present study has demonstrated the formulation of AND and OR gates. Nonetheless, by further tailoring of the DNA duplexes and the use of cantilever arrays, serial logic gates of higher complexity and computational circuits may be envisaged. It should be noted that the DNA-based logic gates operate on a time-scale of minutes, far slower than conventional computers. One should realize, however, that the integration of such DNA-based logic gates on an array of cantilevers could enable the parallel activation of DNA, thus allowing the enhancement of the computation complexity. Furthermore, the information stored in the DNA sequences, and the specific cleavage of defined nucleotide-base domains, could allow one to solve complex mathematical problems through such a biocatalytic process.

Acknowledgment. This research is supported by the EC MOLDYNLOGIC project.

JA0533287