NANO LETTERS

2008
Vol. 8, No. 1
227−231

Controlled Release of Chol-TEG-DNA from Nano- and Micropatterned SU-8 Surfaces by a Spreading Lipid Film

Yavuz Erkan,[†] Kiona Halma,[†] Ilja Czolkos,[†] Aldo Jesorka,[†] Paul Dommersnes,[‡] Ravindra Kumar,[§] Tom Brown,[§] and Owe Orwar*,[†]

Department of Chemical and Biological Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden, MSC, Université Paris Diderot, 10, rue Alice Domon et Léonie Duquet, F-75205 Paris, France, and School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, U.K.

Received October 1, 2007; Revised Manuscript Received November 9, 2007

ABSTRACT

We report the controlled release of immobilized cholesteryl-tetraethyleneglycol-DNA (chol-DNA) from micropatterned SU-8 surfaces by a spreading lipid film. The release of chol-DNA is rapid and on the order of the spreading rate of the lipid film $\beta = 1–3\ \mu m^2/s$ ($\sim 10^5$ molecules of DNA per second). The lipid film serves as a poor solvent for the DNA adduct, which upon contact redistributes into the aqueous phase. Thus, the release of DNA is accompanied by a change in surface hydrophobicity. The method can be used for creating arbitrary concentration profiles of DNA in solution over time or to dynamically change surface properties on demand in, for example, micro- and nanofluidic devices. Examples of DNA release from spiral, comb, meander, and triangular as well as from nanoscale SU-8 lanes are shown.

Surface properties become increasingly important at shorter length scales. In applications where length scales are on the order of micrometers to submicrometers, the chemistry can be completely dominated by surface interactions.[1] Therefore, ways of dynamically changing the surface composition is of great value. For example, adsorption, desorption, catalysis, and selective binding interactions tailored for certain species can thereby be controlled. Likewise, the solution composition within small-scale systems such as micro- and nanofluidic devices is difficult to control and manipulate, that is, to add or withdraw a component from a certain compartment within such fluidic networks might be very difficult to achieve in practice. To change the solvent composition through release of materials from surfaces on demand[2,3] is thus very attractive because it does not induce volume flow or pressure gradients, that is, the rest of the system is unbiased.

DNA as well as other polynucleotides represent important classes of compounds whose specific adsorption, desorption, and binding is of great interest to control in small-scale systems.[4,5] Controlled DNA release has been demonstrated, for example, from microelectrode surfaces by an electrochemical stimulus,[6−9] upon polymer degradation in multilayered polymer/DNA thin films,[10] cleaving of photolabile linkers in gold nanoparticle/DNA complexes,[11] by using the dual binding capability of DNA aptamers,[12] or by photothermal denaturation of DNA duplexes.[13]

Recently, we have reported two related techniques for the formation of molecularly thin films based on (i) spreading and mixing of lipid monolayers from multilamellar vesicles[14] and (ii) a one-step procedure for high-yield immobilization of cholesteryl-tetraethyleneglycol-conjugated DNA (chol-DNA). The surface coverage of immobilized chol-DNA on SU-8 surfaces is 20−95 pmol/cm^2 corresponding to a film density of 10^{13} molecules/cm^2.[15] The films were produced on planar chips with micrometer-sized hydrophobic SU-8 patterns surrounded by a hydrophilic gold layer. Both formation of lipid monolayer films and immobilization of chol-DNA take place exclusively on SU-8. Here, we demonstrate that chol-DNA can be released at will from micro-and nanopatterned SU-8 surfaces by a spreading lipid film. As the DNA is released into the solution, the surface undergoes a transition from hydrophilic to hydrophobic. Furthermore, complex patterns of DNA in solution can be created from complex geometric patterns.

Upon application of a 2−3 μM fluorescently labeled chol-DNA solution (see Table 1 for the different DNA conjugates used) onto a microfabricated device having hydrophobic SU-8 structures surrounded by hydrophilic areas of gold, chol-DNA adsorbed to the SU-8 surface, and excess of the droplet was washed away with MilliQ water.[15] After that, a single multilamellar vesicle prepared from soy bean lecithin[16] was placed on top of the chol-DNA film by a microman-

* Corresponding author. Tel: +46-31-772-3060. Fax: +46-31-772-6120. E-mail: orwar@chalmers.se.
[†] Chalmers University of Technology.
[‡] Université Paris Diderot.
[§] University of Southampton.

10.1021/nl0725087 CCC: $40.75
Published on Web 12/11/2007

Table 1. A List of Oligonucleotides Used[a]

Name	5′mod	3′mod	sequence written 5′ to 3′
DNA1	Chol-TEG	Cy3	GCTTTGACCG
DNA2	Cy3	Chol-TEG	GCCAGTTTCGTCTAAGCACG
DNA3		Chol-TEG	GCCAGTTTCG
c-DNA3		Cy3	CGAAACTGGCT
DNA4		C16-HEG	GCCAGTTTCG
DNA5	Chol-TEG	Cy3	CGAAACTGGCT

[a] Chol-TEG, C16-HEG, and c-DNAX refer to cholesteryl-tetraethyleneglycol, glyceryl bis C16-hexaethyleneglycol, and to the oligonucleotide complementary to DNAX, respectively.

ipulation technique disclosed previously.[14] Almost immediately as the multilamellar vesicle gets into contact with the chol-DNA-covered SU-8 surface, chol-DNA detaches (Figure 1a). By using confocal fluorescence microscopy, we monitored the fluorescence intensity of immobilized chol-DNA while the lipid was spreading on SU-8 lanes. The spreading velocity of a lipid film on a bare SU-8 lane is uniform and given by $v = \sqrt{\beta/t}$, where $\beta = S/2\zeta$ is the spreading coefficient.[14] The spreading power, S, represents the difference in free energy between lipids on the surface and in the reservoir (σ_{Lipid}), and ζ is the sliding friction between the lipid film and the surface. When chol-DNA is immobilized on the surface, the spreading power is reduced by the free-energy difference for chol-DNA (σ_{DNA}) in solution and on the surface. The spreading coefficients observed here for a lipid film on a chol-DNA decorated surface are close to those obtained previously for plain SU-8 surfaces,[14] and the decrease in fluorescence intensity from the substrate over time roughly follows $L = 2\sqrt{\beta t}$ where L is the distance from the multilamellar vesicle to the spreading film front, and t is time. Specifically, we obtained $\beta = 1-3$ μm^2/s for immobilized 10-mer single- (ssDNA) and double-stranded DNA (dsDNA), and $\beta = 1-5\mu$m^2/s for 20-mer ssDNA. The observed gradient in fluorescence intensity corresponds to a concentration difference in immobilized chol-DNA (Figure 1b,c). The change in fluorescence intensity is abrupt and follows almost a step function where the base levels correspond to those observed for DNA-free surfaces (Figure 1c), and from the slope it is evident that the release is rapid. Two conclusions can be drawn from these experiments: (i) on chol-TEG-DNA coated SU-8, the lipid spreads at the same velocity as on bare SU-8 surfaces, and (ii) the majority of the DNA adducts are released from the surface immediately upon contact with the lipid film. Chol-TEG-DNA leaves the surface because it dissolves much better in the aqueous phase than in the lipid film. Although the chol-DNA has a cholesterol anchor, it is less hydrophobic than the lipid film, whose lipids have aliphatic C-16 to C-20 chains. This means that chol-DNA screens the hydrophobic surface less efficiently than the lipid film and this difference in screening results in a gain in free energy when the lipid film spreads over the chol-DNA decorated surface.[17]

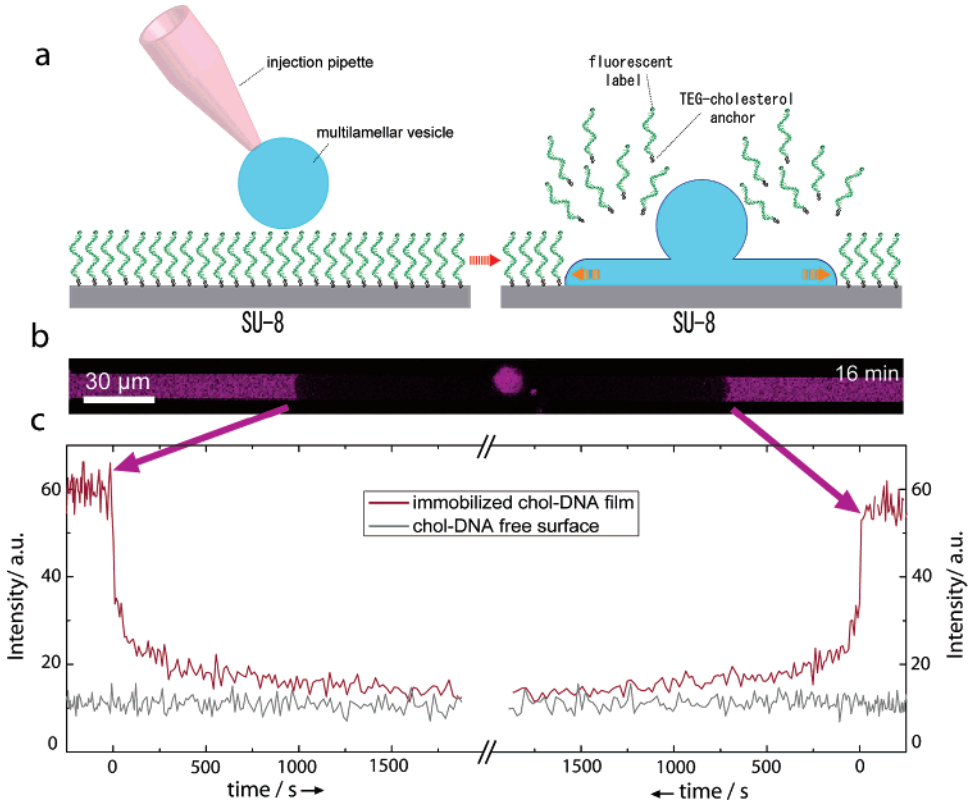

Figure 1. DNA release upon exposure of the immobilized chol-DNA to a spreading lipid film. (a) Schematic picture of multilamellar vesicle placement with a micropipette, followed by lipid spreading and release of fluorescently labeled ssDNA. (b) A fluorescence micrograph of immobilized ssDNA (DNA1), which is partly removed by the spreading lipid film. Image is artificially colored. (c) Fluorescence intensity profile of lipid spreading from the immobilized chol-DNA film and the DNA free surface over time, obtained from the spatial profile. $t = 0$ corresponds to the boundary of the lipid film as indicated by the arrows.

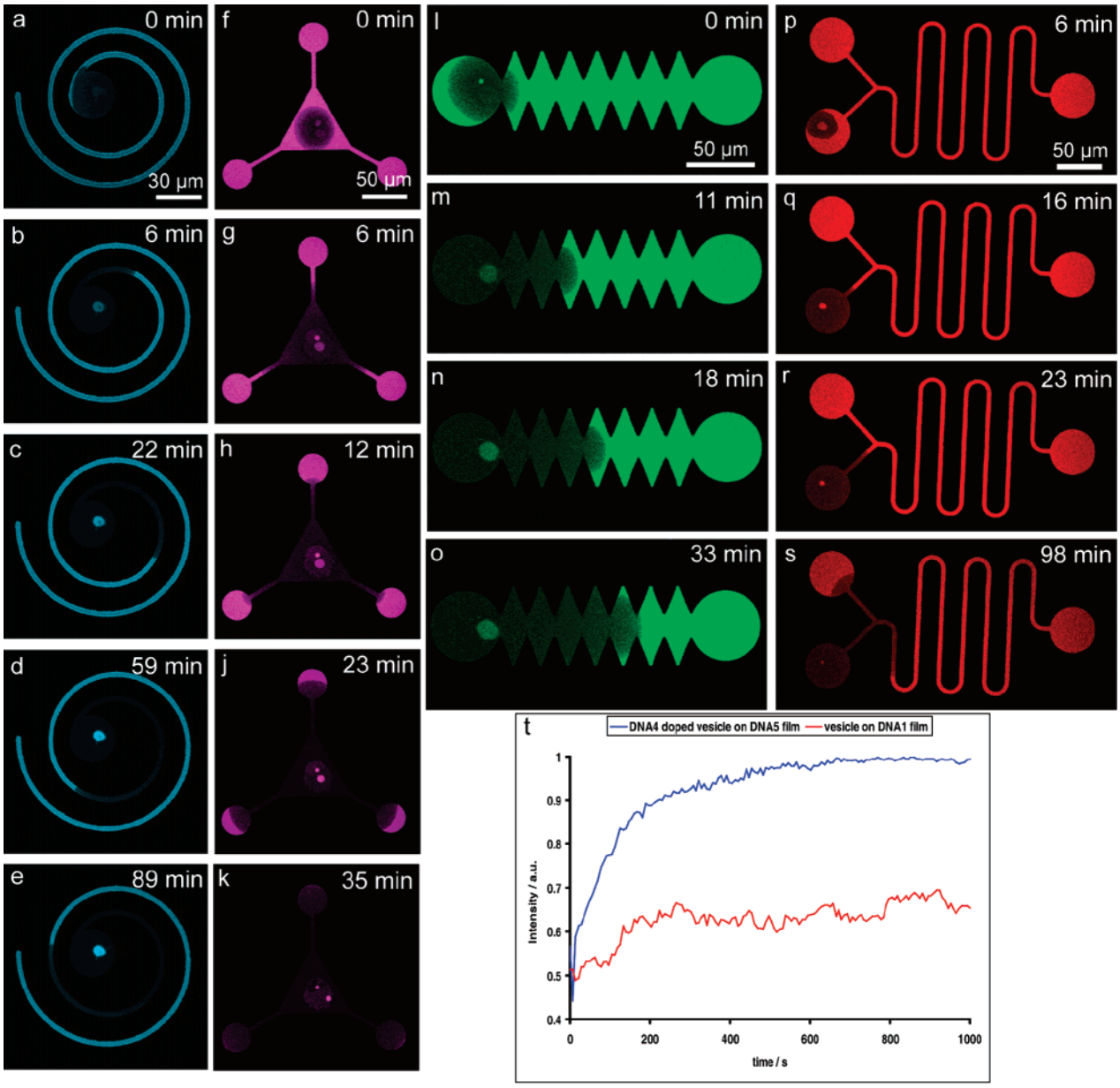

Figure 2. Time evolution of chol-DNA release from micropatterned SU-8 surfaces and their hybridization to complementary DNA strands in multilamellar vesicles. (a−o) Cy3-labeled DNA5 is immobilized on the SU-8 surface and a multilamellar vesicle doped with DNA4 is placed on the chol-DNA film. (p−s) As a control experiment, DNA1 is immobilized on the SU-8 surface and a multilamellar vesicle is placed on the chol-DNA film. Cy3 fluorescence ($\lambda_{excitation} = 543$ nm, $\lambda_{emission} = 550-650$ nm) is monitored as the lipid spreads. Images are artificially colored. (t) Fluorescence intensity profile of multilamellar vesicle as the lipid spreads. Represented data is an average of three different experiments.

We used fluorescence spectroscopy to further verify the release of chol-DNA stimulated by lipid spreading. First, Cy3-labeled DNA1 containing solutions were incubated on two separate SU-8 spots of a defined area. Following incubation and rinsing, solutions containing multilamellar vesicles and buffer only, respectively, were applied to the two spots. After 60 min, when the surface was covered by the lipid film, an emission spectrum of the supernatant to detect Cy3-labeled DNA was recorded. From calibration curves of known concentrations, we estimated the amount of chol-DNA free in solution. The concentration of released DNA was between 0.09−0.12 μM when the multilamellar vesicles were present and 0.04−0.06 μM when the spot was rehydrated with buffer alone. Because the oligonucleotides are modified with cholesterol, chol-DNA will also partition into multilamellar vesicles that have settled down on the SU-8 surface during the experiment. The experiment therefore yields a conservative estimate of DNA released from the surface.

The geometry of the hydrophobic structures that support lipid film formation can be arbitrarily controlled within the limits of micro- and nanofabrication of SU-8. This gives opportunities to create irregular and exotic surface patterns of immobilized DNA. It furthermore allows generation of complex concentration profiles of DNA in solution, as the DNA is released by a spreading lipid film. As an example

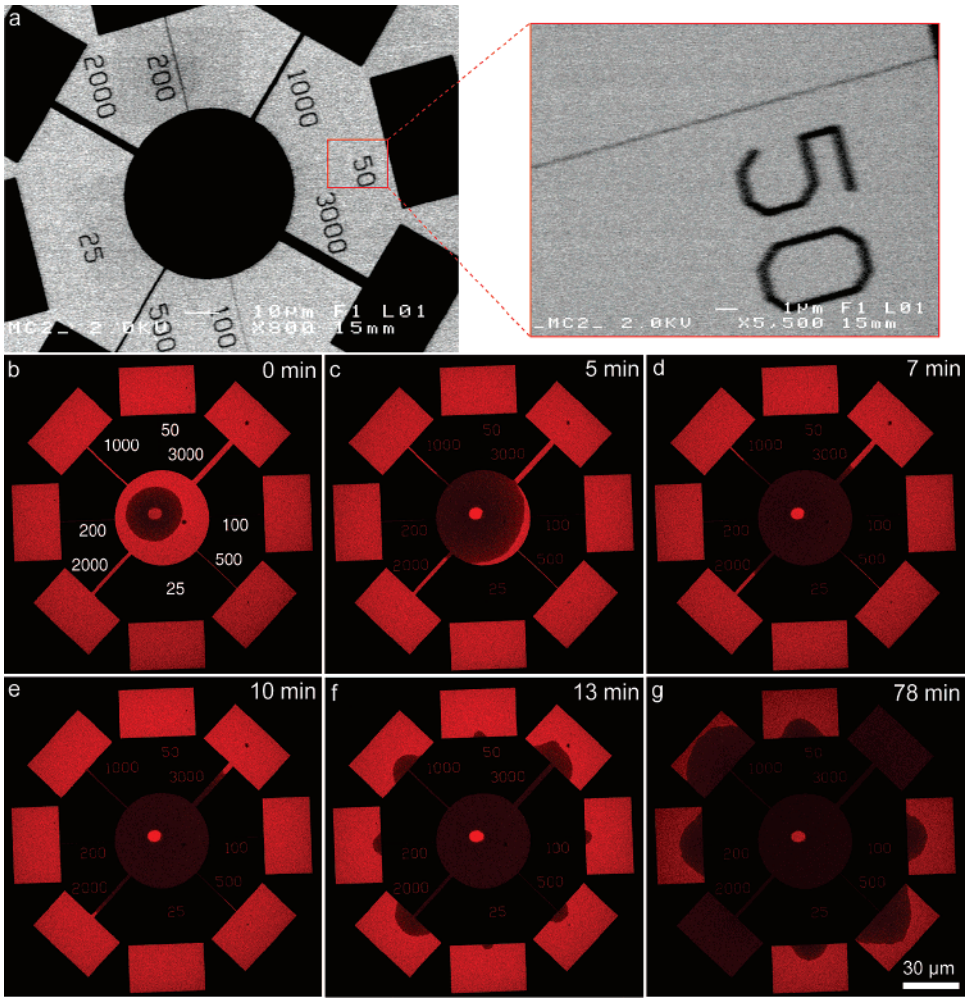

Figure 3. Chol-DNA release from 25, 50, 100, 200, 500, 1000, 2000, and 3000 nm wide SU-8 lanes. (a) A scanning electron microscope image of the SU-8 pattern. (b−g) Cy3-labeled DNA1 is immobilized on the SU-8 surface and a multilamellar vesicle is placed on the chol-DNA film in the middle of the pattern. Cy3 fluorescence ($\lambda_{excitation}$ = 543 nm, $\lambda_{emission}$ = 550−650 nm) is monitored as the lipid spreads from the center branching out via nanometer-sized lanes. (g) At a given time, the percentage of the outer rectangles that is wetted by the lipid film depends on the width of the lane connecting it to the central injection pad. Images are artificially colored.

of such designed structures, we microfabricated SU-8 patterns of spiral, triangular, comb, meander, and straight lanes where we show the spreading of a lipid monolayer and subsequent chol-DNA release into the solution (Figure 2). In these experiments, we used complementary DNA incorporated into the multilamellar vesicle. We used a soybean polar extract (SPE) lipid mixture that is doped with a lipidlike monomer "glyceryl bis C16" (see Supporting Information) modified with hexaethyleneglycol-DNA (C16-HEG-DNA) to form multilamellar vesicles. Next, a single multilamellar vesicle doped with DNA4 (n_{DNA}:n_{Lipid} = 1:1500) was placed on top of the chol-DNA film. Therefore, not only was DNA released into the solution, it was also sequestered back to the multilamellar vesicle where it hybridized with the complementary strand. Figure 2t shows the fluorescence intensity increase in the multilamellar vesicle during the time evolution of lipid spreading. As the lipid spreads, released chol-DNA molecules (DNA5) hybridize with complementary C16-HEG-DNA molecules (DNA4). In contrast, when performing the same experiment without complementary DNA in the vesicle, the increase in the fluorescence intensity is much less pronounced and shows slower kinetics.

To demonstrate the release of chol-DNA from nanometer-sized SU-8 structures, we used direct e-beam lithography[18] to create straight nanometer-wide SU-8 lanes (Figure 3a). Figure 3 shows DNA release by lipid film spreading from SU-8 lanes of different widths (25, 50, 100, 200, 500, 1000, 2000, and 3000 nm). A single multilamellar vesicle is placed in the middle of the SU-8 pattern, and the lipid spreads radially over the nanometer-sized lanes. As can be seen from the time series (see Supporting Information) narrow lanes allow the passage of less lipid material per time unit compared to wider lanes. Thus, the rate of DNA released into solution can be controlled by the width of the SU-8 lanes.

To conclude, we present a rapid and controlled way of releasing chol-DNA from SU-8 surfaces by lipid film spreading. Using a combination of nano- and micrometer-sized SU-8 patterns, DNA concentration gradients in solution can be created dynamically. Moreover, other molecules with

different hydrophobicites can likely be used such that the hydrophobicity of the molecules can be exploited as a sorting tool to alter surface properties of SU-8. It is furthermore possible to heat the surface by embedding heating coils[19] under the SU-8 substrate. Hence, by using lipids with well-defined transition temperatures, spreading and thus DNA release can be started or stopped on demand.

Acknowledgment. We thank Peter Sandin for helping with the spectroscopic measurements. This research is funded by the European Commission's sixth Framework Programme (Project reference AMNA, Contract No. 013575) and the Swedish Foundation for Strategic Research SSF (NANO-X).

Supporting Information Available: S1 is video of chol-DNA release from nanometer sized SU-8 lanes, as shown in Figure 3. S2 is detailed description of materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chiu, D. T.; Wilson, C.; Karlsson, A.; Danielsson, A.; Lundqvist, A.; Strömberg, A.; Ryttsén, F.; Davidson, M.; Nordholm, S.; Orwar, O.; Zare, R. N. *Chem. Phys.* **1999**, *247*, 133−139.
(2) Grzybowski, B.; Bishop, K. J. M.; Campbell, C. J.; Fialkowski, M.; Smuokov, S. K. *Soft Matter* **2005**, *1*, 114−128.
(3) Kastrup, C. J.; Runyon, M. K.; Shen, F.; Ismagilov, R. F. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15747−15752.
(4) Pannier, A. K.; Shea, L. D. *Mol. Ther.* **2004**, *10*, 19−26.
(5) Hook, A. L.; Voelcker, N.; Thissen, H. *Trends Biotechnol.* **2006**, *24*, 471−477.
(6) Mali, P.; Bhattacharjee, N.; Searson, P. C. *Nano Lett.* **2006**, *6*, 1250−1253.
(7) Wang, J.; Rivas, G.; Jiang, M.; Zhang, X. *Langmuir* **1999**, *19*, 6541−6545.
(8) Hook, A. L; Thissen, H.; Hayes, J. P.; Voelcker, N. H. *Biosens. Bioelectron.* **2006**, *21*, 2137−2145.
(9) Moore, E. J.; Curtin, M.; Ionita, J.; Maguire, A. R.; Ceccone, G.; Galvin, P. *Anal. Chem.* **2007**, *79*, 2050−2057.
(10) Zhang, J.; Chua, L. S.; Lynn, D. M. *Langmuir* **2004**, *20*, 8015−8021.
(11) Han, G.; You, C. C.; Kim, B. J.; Turingan, R. S.; Forbes, N. S.; Martin, C. T.; Rotello, V. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 3165−3169.
(12) Nutiu, R.; Li, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 5464−5467.
(13) Okano, K.; Yasuda, K.; Ishiwata, S. *Sens. Actuators, B* **2000**, *64*, 88−94.
(14) Czolkos, I.; Erkan, Y.; Dommersnes, P.; Jesorka, A.; Orwar, O. *Nano Lett.* **2007**, *7*, 1980−1984.
(15) Erkan, Y.; Czolkos, I.; Jesorka, A.; Wilhelmsson, L. M.; Orwar, O. *Langmuir* **2007**, *23*, 5259−5263.
(16) Karlsson, M.; Nolkrantz, K.; Davidson, M. J.; Strömberg, A.; Ryttsén, F.; Åkerman, B.; Orwar, O. *Anal. Chem.* **2000**, *72*, 5857−5862.
(17) Rädler, J.; Strey, H.; Sackmann, E. *Langmuir* **1995**, *11*, 4539−4548.
(18) Bilenberg, B.; Jacobsen, S.; Schmidt, M. S.; Skjolding, L. H. D.; Shi, P.; Bøggild, P.; Tegenfeldt, J. O.; Kristensen, A. *Microelectron. Eng.* **2006**, *83*, 1609−1612.
(19) Markström, M.; Gunnarsson, A.; Orwar, O.; Jesorka, A. *Soft Matter* **2007**, *3*, 587−595.

NL0725087