Aspects of DNA assembly: Extension, lithography and recognition

G. V. Shivashankar† and A. Libchaber

Centre for Studies for Physics and Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

In this paper we describe the micromanipulation of single genomic DNA and the lithographic representation of its sequence information on a biochip. An optical tweezer combined with force detection using light backscattering and a force cantilever is used to manipulate single molecules. Using this we probe the flexibility of a DNA template and its use as a mechanical detector to study DNA–protein interactions. We then use this approach to directly monitor the extension of a single DNA polymer beyond its contour length, due to its unwinding, induced by the polymerization of RecA protein. Finally the application of a localized light source for bio-molecular lithography is presented. We describe micropatterning DNA molecules on a solid substrate for specific bio-molecular recognition. The ability to manipulate, fabricate addressable DNA arrays and specifically recognize genomic DNA molecules opens the possibility of studying the mechanisms underlying genetic processes and biological networks.

A DNA sequence is both a code of information and a polymer. It serves as a template for information retrieval in genetic processes, while being a flexible polymer chain. The sequence of information and the mechanical properties of DNA determine the molecular recognition during DNA–protein interactions. Such interactions, at specific recognition sites, form the basis of the genetic expression[1].

In this paper we first summarize the mechanical response of the polymer using single DNA micromanipulation techniques (notes 1–4). In particular we used an optical tweezer combined with light backscattering to measure forces in the range of 0.1 to 10 picoN and an atomic force cantilever for forces greater than 10 picoN. In such experiments, a single DNA polymer is anchored at one end to a glass slide and at the other end to a latex particle, acting as a molecular handle. We then study a specific example of DNA–protein interaction: the polymerization of RecA protein on DNA. Finally we describe a novel application of a localized light source to assemble an addressable array of DNA sequences on a solid substrate, for specific bio-molecular recognition. Representation of specific DNA sequences, in addressable arrays on a substrate, form an important tool to study the complexity of gene expression[2,3].

†For correspondence. (e-mail: shiva@athena.rockefeller.edu)

Micromanipulation of a single DNA molecule

Single DNA molecule studies have become possible recently due to progress in micromanipulation technology and *in vitro* biochemical assays[4–14]. At the single molecule level one can ask specific questions about the molecular interactions which form the basis of biological functions. The elastic response and the fluctuation study of a DNA polymer are useful in studying the dynamics of a protein interacting with DNA. Such approaches, for example, provide an understanding of the linear and twist elasticity of DNA molecules, measure forces of DNA base pair interactions, measure the processive enzymatic function of RNA polymerase during transcription and the self-assembly of recombinase RecA protein filaments on DNA.

The mechanical response of a DNA molecule, to an external applied force, has two regimes: entropic and elastic. The entropic response is driven by thermal energy and the elastic response by base pair interactions. Typically the entropic forces are of the order of K_BT/nm ~4 picoN and the elastic forces are of the order of eV/nm ~160 picoN (electron volt (eV) ~ bond energies).

A schematic of the micromanipulation set-up, used in our experiments, is shown in Figure 1. A typical force vs extension study of a single double stranded DNA molecule has four distinct domains (Figure 2). For extension of up to 0.9% of the contour length of λ DNA (16.5 μm), corresponding to forces of up to 10 picoN, work is done against the entropic response of the polymer. At higher extension, the elastic response of the polymer results in an increase in force (up to 55 picoN). Extension beyond the contour length (up to 1.3% to 1.4%) results in an abrupt increase in length, at constant force. The abrupt change in length may be due to unwinding of the double helix. This is the plateau region, reported in the literature by two other groups[7,8]. For a force of ~55 picoN, DNA extends from about 16 μm to 23 μm. On further extension (up to almost twice the contour length) the increase in force may be due to shearing of the double stranded DNA molecule[13].

Fluctuation of a DNA polymer is a direct measure of its entropic stiffness. The fluctuations of a DNA tethered Brownian particle in a harmonic potential well (formed by a laser tweezer) arises due to the sum of the random fluctuations of the bead (driven by thermal

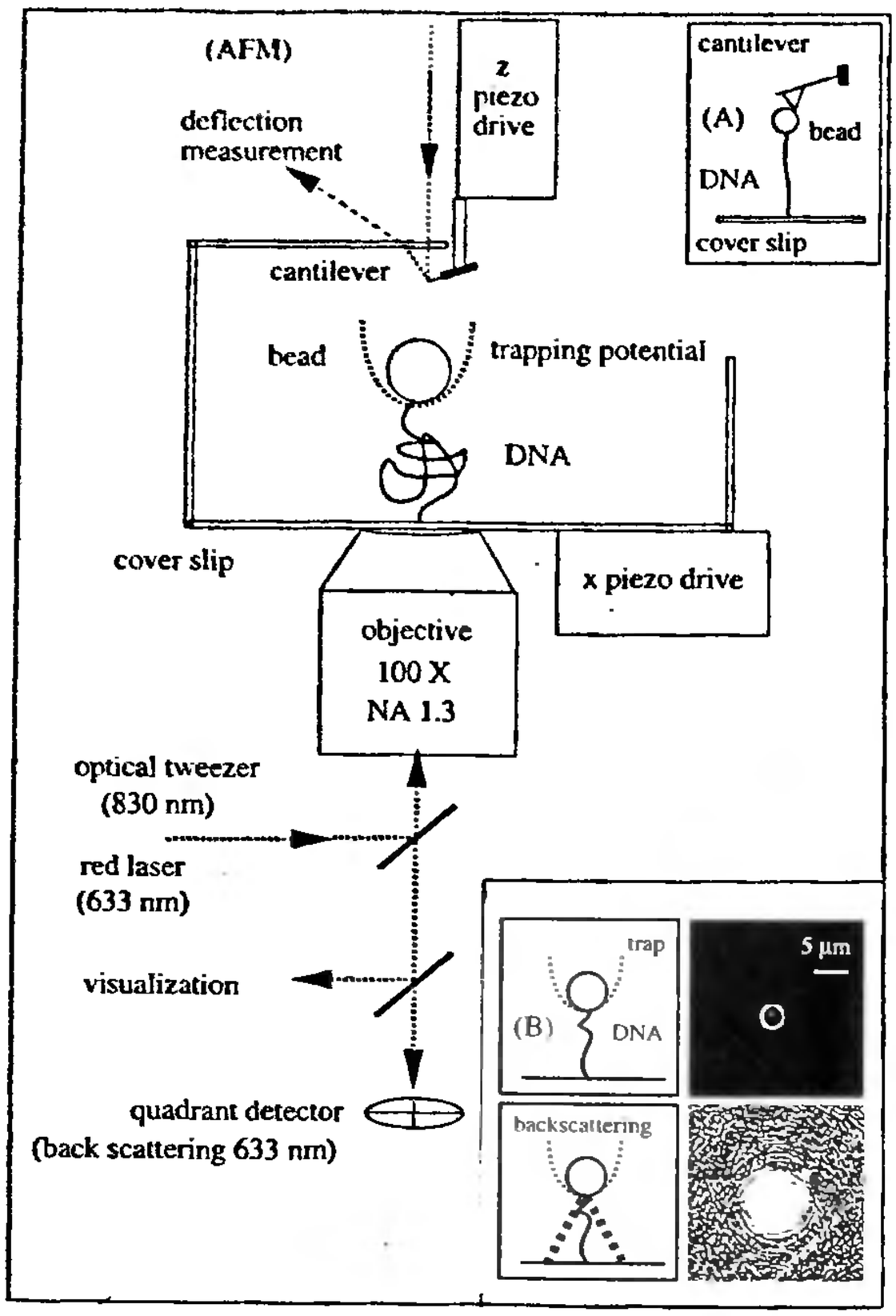

Figure 1. Schematic of the home-built set-up to manipulate a single molecule. A laser tweezer is combined with a Silicon force measurement cantilever. Upper insert: A single DNA molecule is attached to a silicon cantilever for the measurement of the mechanical response. In this case, the Z-piezo drive is vertically displaced and the corresponding cantilever deflection is monitored to measure forces greater than 10 picoN. Lower insert: Backscattering from a DNA tethered particle is used to measure displacements and forces (in the range of 0.1 to 10 picoN), within the optical trap using a quadrant detector. In this case the X-piezo drive is horizontally displaced to measure the entropic response of DNA.

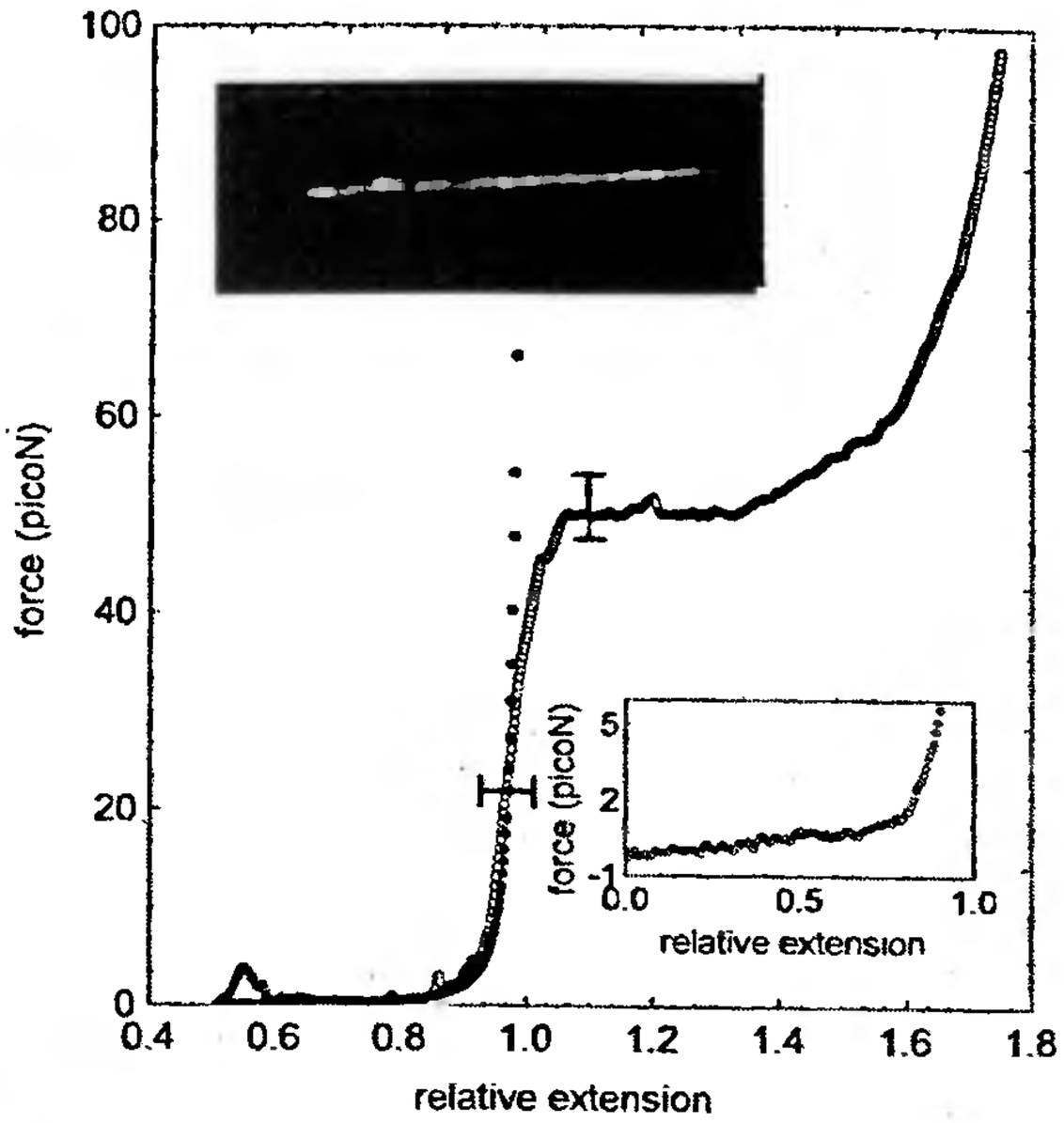

Figure 2. Force-extension curve of a double-stranded DNA molecule measured using a silicon force cantilever. The *x*-axis is plotted in terms of relative extension (z/l_o where z is the applied extension using the Z piezo drive and $l_o = 16.5$ µm, the contour length). The dotted line is a theoretical fit to the worm like chain model[15]. Lower insert: Force-extension curve of the same molecule, for low extension, measured using the optical tweezer with backscattered detection. Upper insert: Fluorescence visualization of a single λ DNA molecule (16.5 µm in length) anchored at one end to a glass slide. The same DNA molecule is anchored with a bead, at the other end, to probe its mechanical response. The fluorescent marker is used here only for visualization and is not used during micromanipulation experiments.

noise) and the fluctuations introduced by the DNA attachment. Ideally when the bead is in the equilibrium position in the trap, its mean square fluctuations along the z axis, $\langle \Delta z^2 \rangle = K_B T/\kappa_{DNA}$, where κ_{DNA} is the DNA stiffness (force/unit length). Such fluctuations can be probed using single particle backscattered light detection. By monitoring the standard deviation in the mean value of fluctuations, using a photodetector, one deduces information on the stiffness of the DNA tether. As the DNA is stretched from its equilibrium configuration, the tethered bead is displaced within the trap and its Brownian fluctuations are reduced. This information is then used to plot the entropic response of DNA (Figure 2 lower inset). From a theoretical point of view, the DNA tethered bead confined in an optical trap is described by a superposition of two potentials: one corresponding to the optical trap and the other representing the DNA polymer attachment[14].

The DNA polymer can be well described by a worm-like chain model. The radius of gyration of such a Gaussian chain, is given by $R = (2bl_o)^{0.5}$, where l_o is the chain length (number of bases × base pair distance) and b the characteristic correlation or persistence length. In the low force regime (entropic in origin), z the extension $<<l_o$, the force for stretching a Gaussian polymer chain is $f = 3KT/2b\ (z/l_o)$. At an extension approaching the contour length, the intrinsic elastic description of the polymer chain is invoked. A combined form of the force extension relation is described by the following equation, $f = (K_B T/2b)[z/l_o + 1/4(1/(1 - z/l_o)^2) - 1/4]$, where K_B is Boltzmann constant, T the temperature, b the persistence length, z the extension and l_o the contour length of the DNA[15]. The above description is invalid for $z >> 0.95\ l_o$, where cooperativity in DNA base pairing effects is important. The persistence length (the length

over which the thermal energy bends DNA) is a useful measure of the flexibility of DNA.

Single molecule study of DNA–protein (RecA) interactions

The assembly of proteins (either self-assembly or assembly catalysed by enzymes) at specific DNA sites forms the basis of the genetic expression. A ballet of well-orchestered assembly of proteins on DNA has evolved in nature to carry out specific genetic processes. Much of our understanding about DNA–protein interactions has come from careful biochemical, genetic and structural studies. A few common structural motifs, that mediate the interactions in nature, have been identified forming the basis for nucleic acid interactions[16]. However the mechanisms by which proteins find their specific sites or the process of biological assembly of proteins on specific DNA sequences are still not clear. Single molecule micromanipulation of DNA may offer a direct approach to probe DNA–protein interactions. As an example, we describe here the interaction of RecA (a recombinase protein) with a DNA polymer.

In cells, RecA is essential for homologous recombination by promoting strand exchange reaction between single and double-stranded DNA or between two double stranded DNA[17–20]. It is also responsible for catalysing DNA dependent ATP hydrolysis. RecA is a small protein found in bacteria (molecular weight 37.8 kDa) and homologs of RecA are present in all living systems. It assembles into a polymeric form (both *in vivo* and *in vitro*) in the presence or absence of DNA, under appropriate biochemical conditions. RecA requires ATP to bind to DNA and hydrolysis of ATP leads to dissociation. In the presence of the non-hydrolysable form of ATP (Atp-γs), RecA forms a stable complex on DNA.

An understanding of the mechanism of homologous recombination is based on the idea that RecA on binding to DNA (both ss and ds), locally stretches and unwinds the DNA. The only direct evidence for this has come from electron microscopic studies of RecA–DNA complex[19]. RecA polymerizes on a ds (or ss) DNA by increasing the length of the DNA. The average base pair spacing is increased from 3.4 to 5.1 Å. This process suggests a distinct structure, characterized by an overstretching transition, in the B-form or the natural form of DNA. In strand exchange reactions, RecA is believed to cooperatively bind to both homologous DNA sequences to facilitate strand exchange.

In Figure 3, we present a direct single molecule observation of the effect of RecA binding to DNA. A monomer of RecA binds to about 3 bases of DNA. The kinetics of polymerization depends on the state of the DNA molecule, whether it is in the random coil state or in a stretched form. The application of an external force reduces the kinetic barrier for RecA binding to DNA[14,21].

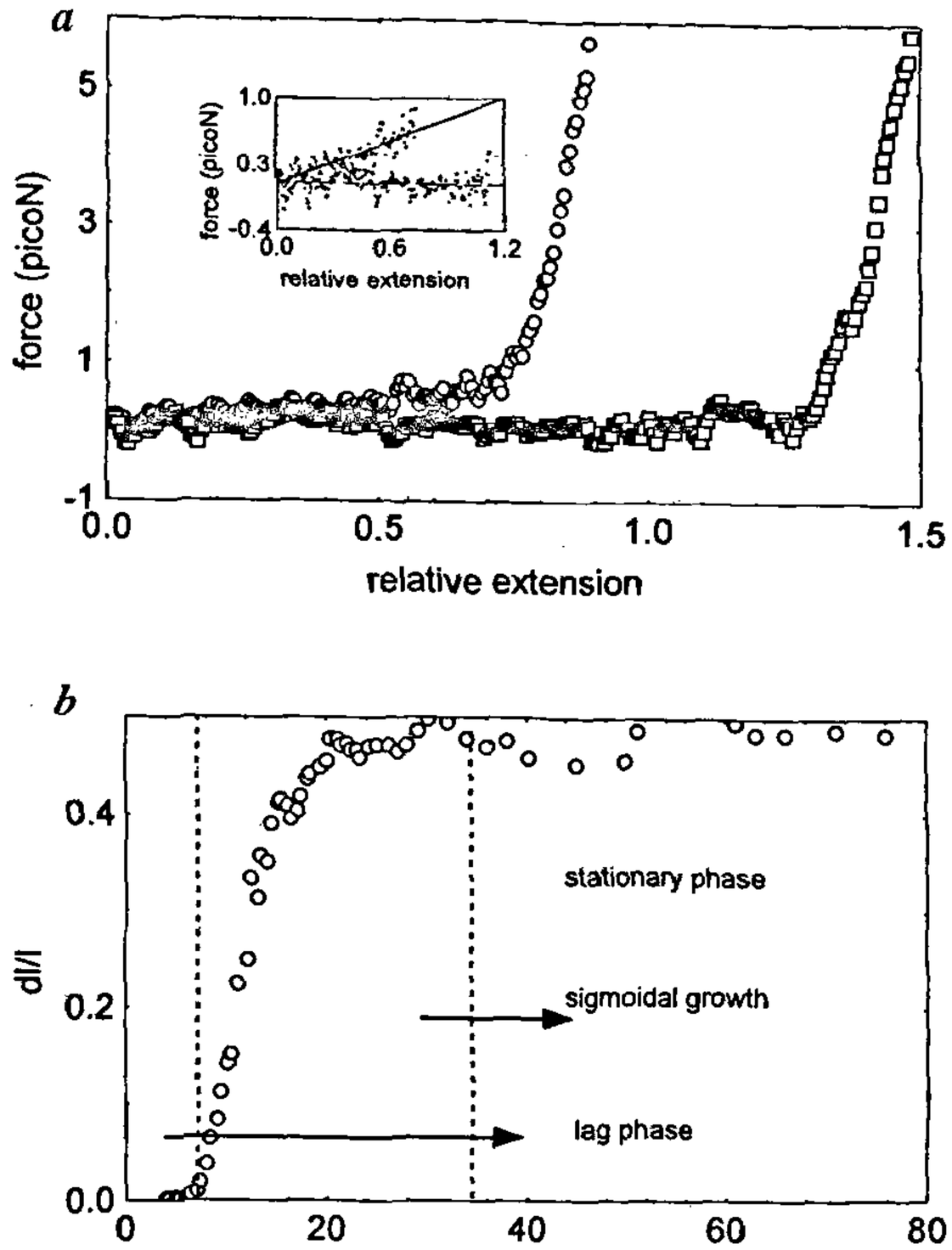

Figure 3 *a*, *b*. *a*, Force-extension curve of a naked DNA molecule and the same molecule after RecA assembly. Polymerization of RecA protein results in DNA extension, beyond its contour length, due to unwinding of the double helix. Inset: Linear regimes of the two curves to extract the change in persistence length by a factor of 4 (a lower bound value); *b*, Measurement of the change in length versus time. The kinetics (in the presence of ATP-γs) is measured by keeping the DNA molecule at a constant force of 6 picoN, during the polymerization process. A stable RecA–DNA complex is formed in about 30 min. The net rate of polymerization ~24 monomers/s.

To study the polymerization of RecA on DNA, force versus extension data are recorded for both the naked DNA molecule and on the same molecule after adding the RecA protein (in the presence of ATP-γs) into the sample cell. Polymerization of RecA in the presence of ATP-γs leads to a stable DNA–protein complex. At time $t = 0$, the naked DNA molecule extends to about 15 μm for a stretching force of 6 picoN. At $t = 30$ min, the polymerization of RecA protein results in DNA extension to about 23 μm, for the same force (Figure 3 *a*). During the polymerization reaction, the DNA molecule is held under a constant force of 6 picoN. The over extension of the DNA polymer is due to unwinding of DNA by RecA. In the inset of Figure 3 *a*, we plot the linear regimes of the force extension curves, for $t = 0$ and $t = 30$ min. From this one gets the change in the persistence length

between a naked DNA molecule and the same molecule covered with RecA. The persistence length, b, increases by a factor of 4 (0.21 μm), when covered with RecA and this is a lower bound value.

The rate of RecA protein polymerization is obtained by probing the time-dependent variation in the length of a single DNA molecule (held under a constant force of 6 picoN). The data (Figure 3 *b*) are characterized by three distinct regimes: (1) a lag or nucleation phase; (2) a sigmoidal growth phase; and (3) a stationary phase implying a stabilized complex. We find the net polymerization growth rate to be of the order of ~24 monomers/s. Lag time suggests that there is a characteristic time to form a critical nucleus to begin polymerization. A sigmoidal growth curve suggests that growth fronts emerge at multiple sites and the velocity of growing fronts reduce as they begin to collide with other growth filaments on DNA. A detailed quantitative study of the kinetics of RecA binding has been recently presented[22].

Lithographic patterning of DNA molecules: Bio-chips

Addressable arrays of DNA or proteins immobilized on a substrate provide tools for information retrieval[2,3,23–26]. In the case of DNA, the detection is by hybridization of ss DNA strand to a complementary ss strand on the substrate. For proteins or antibodies, the detection is by specific bio-molecular recognition of the immobilized ligands. Progress in the genome project, allows one to access the genomic DNA sequences of various organisms. Using the genomic sequence information, DNA probes can be immobilized at designed positions on the substrate for detecting specific genes. A collective measurement of gene expression patterns provides a physical insight into the biological networks of an organism. Furthermore patterning biomolecules on semiconductor electronic substrates, coupled with specific recognition, are essential for the realization of artificial bio-molecular networks and their integration to microelectronics.

We describe here an application of a localized light source, to create micron scale patterns of biomolecules on a substrate[27] (note 5). The essentials of the technique are straightforward and simple to realize (Figure 4). We use a near infrared laser, focused to a diffraction-limited spot, to locally heat a thin gold film substrate. Ablation of the gold leaves a permanent trace, as the laser spot is translated. Concurrently a convective flow is induced in the fluid. Particles in suspension are entrained by the flow and locally attracted by the laser, acting as an optical trap. The particles are protected from overheating by a minute gas bubble, which forms at the laser spot. As the beam is displaced, the bubble shrinks and the particles stick to the ablated surface.

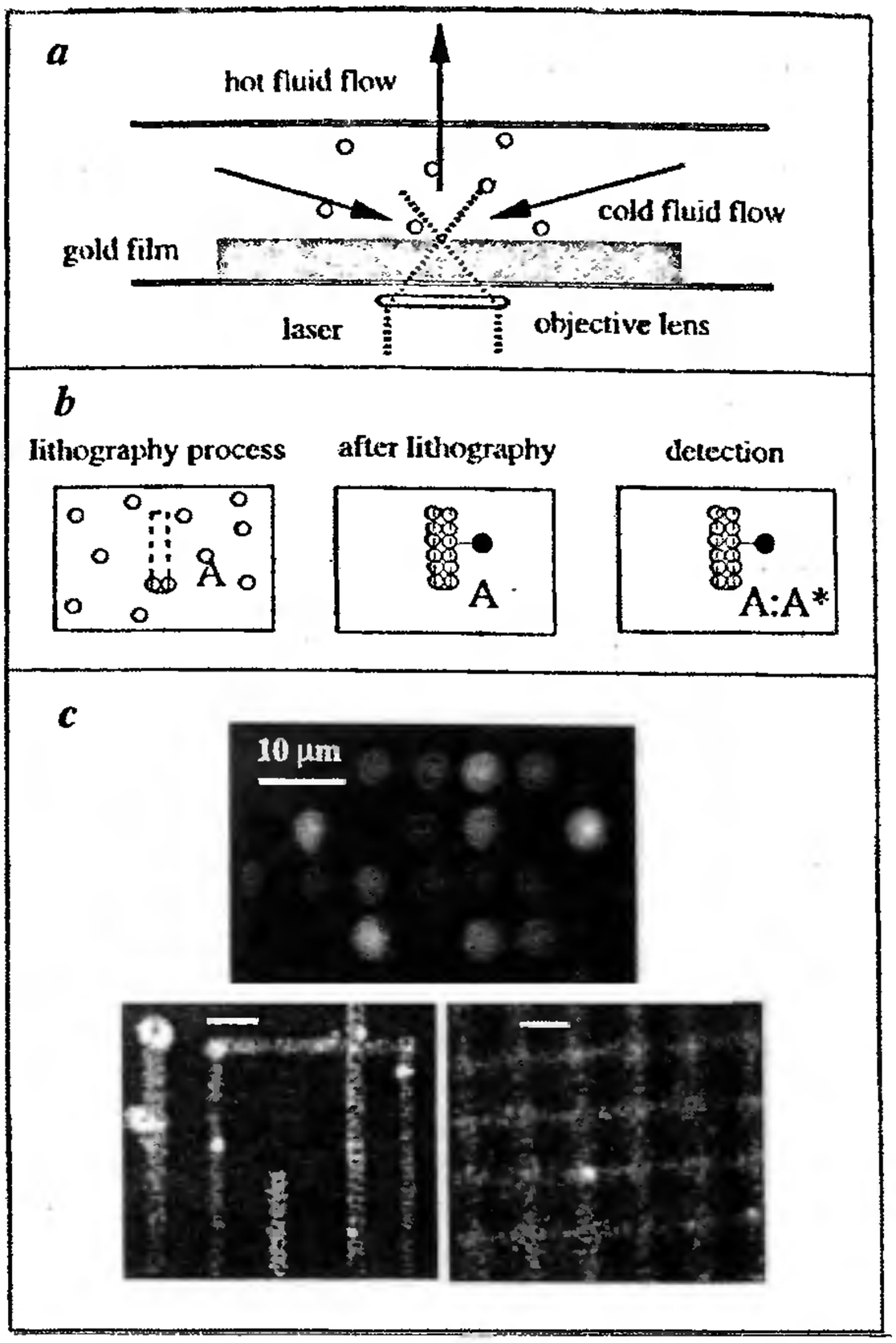

Figure 4 *a–c*. ***a***, ***b***, Schematic of laser absorption lithography. A diffraction limited laser spot is focused on to a glass slide coated with a 30 Å thin gold film. Localized heating leads to particle aggregation and immobilization at the focal plane. Any desired pattern (points, lines, arrays) can be generated by displacing the laser beam. The particles are conjugated with a known bio-molecule (A). After micro patterning, the sample cell is washed to remove free beads in solution. A fluorescent labeled complement (A*) is used for specific biomolecular recognition; ***c***, Examples of specific detection, by patterning single-stranded DNA covered polystyrene beads and their recognition by hybridization of complementary single stranded DNA sequences.

If the colloids are conjugated with biomolecules (DNA, proteins, antibodies or peptides) on their surface, a number of interesting possibilities present themselves. Different geometries, lines, points and arrays can be generated at will. A wide range of particle size can be used, from 10 nm to a few μm. The method is general and depends only on localized laser absorption on a thin film. The method has the advantage of grafting small arrays of genomic DNA or proteins on a solid substrate using simple equipment. Using these approaches we have explored the possibility of patterning single stranded DNA sequences on a substrate, with or without secondary structure, to measure the expression of a large

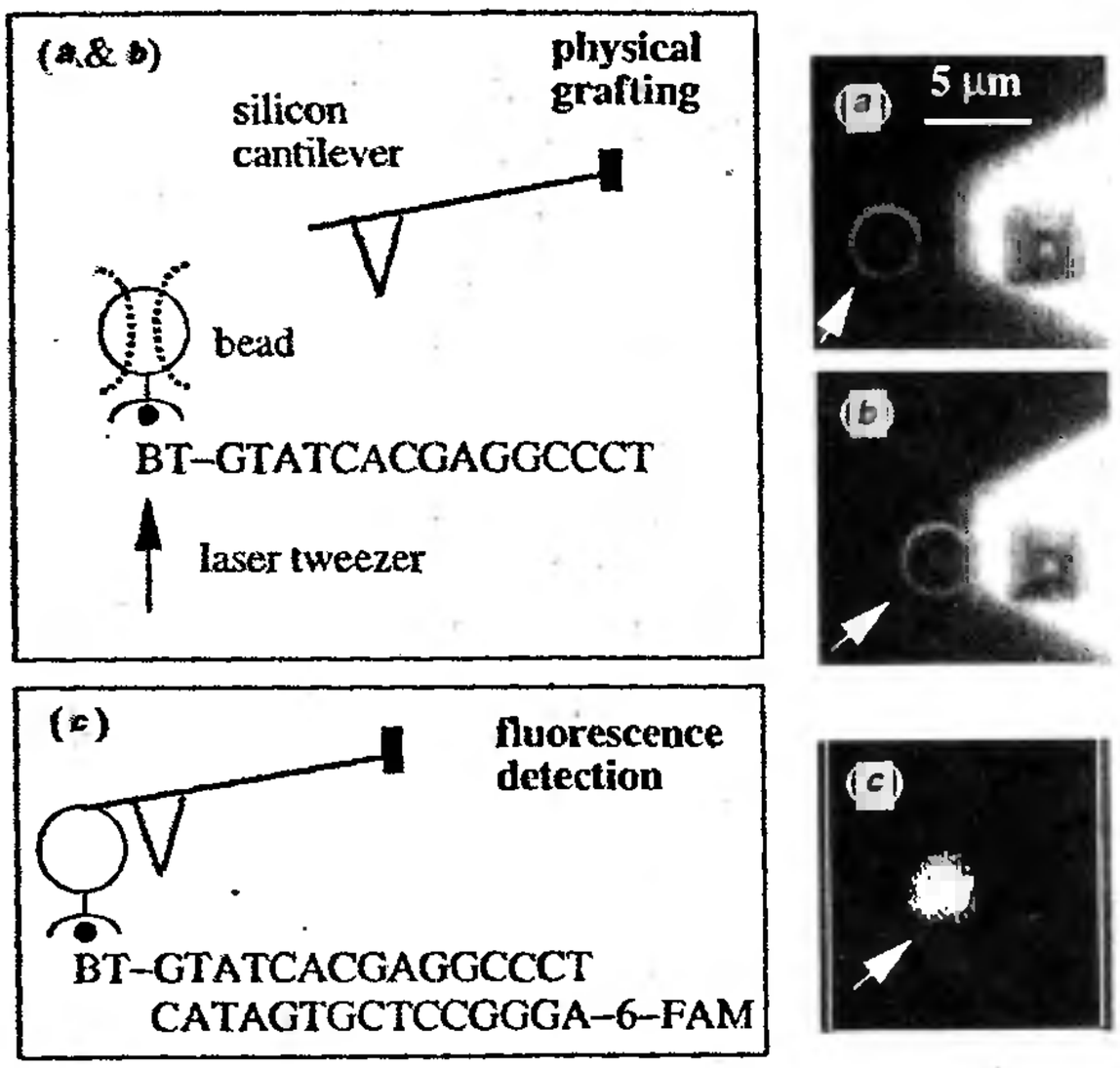

Figure 5 *a*, *b*. Grafting a micron-sized object at a designed position on a silicon substrate, using an optical tweezer. Single-stranded DNA coated bead is selected using an optical trap and grafted on to a silicon cantilever by localized laser absorption. *c*, Grafted sequences are recognized by DNA hybridization.

number of genes. In particular, we have in mind the realization of sensitive DNA oligonucleotide micro arrays for mapping gene expression patterns.

Conclusion

At the single DNA molecule level, micromanipulation and detection studies have opened the possibility to probe the molecular machinery of genetic processes. A study of the physics of such molecular machines may also give a better understanding of how to engineer self-assembled molecular scale devices.

Biomolecular recognition of molecules immobilized on an addressable substrate provide tools for mapping gene expression patterns and protein functions. The lithographic method described in this paper, using localized light, is substrate independent, has biological specificity at the micron scale, may lead to miniaturization and integration with electronic components. Micro patterning of biomolecules and quantitative measurement of time-dependent collective gene expression and function may provide new approaches to study the physics of biological networks.

Notes

1. Single DNA molecule assay

A single double stranded (ds) DNA molecule anchored at its ends, serves as a template to study DNA–protein interactions. We have used phage λ DNA (~48.5 kbases, 16.5 μm in length), purified from viral particles, for these studies and quantification of the assay. The two ends of λ DNA have 12 base single-stranded cohesive ends complementary to each other. DNA tethered polystyrene beads are biochemically prepared, by attaching one end of the λ DNA molecule to the coverslip and the other end to the bead. The biochemical attachment of λ DNA ends are quantified using fluorescence. In Figure 2 (upper inset) single λ DNA molecules, anchored at one end to the coverslip by a biotin streptavidin link, are stretched by a low velocity flow set up in the sample cell. DNA is labeled with YOYO-1 (molecular probes) in 5:1 base pair to dye ratio, for fluorescence visualization. (The dye is added here to quantify the assay and it is not used during micromanipulation experiments.) More recently we have used low pH solution (pH – 6) to anchor single DNA molecules on to beads and glass slide. RecA experiments are carried out in 150 mM PBS buffer at $T = 37°C$, pH ~ 6.8 ± 0.2, 10 μM RecA, 1 mM ATP-γs, $MgCl_2$ ~20 mM, DTT ~20 mM and Tris-HCl 60 mM concentrations.

2. Optical tweezers[28]: A force transducer

To measure the tweezer trapping force, we use the Stoke's drag method and measure the velocity of the translational stage for which a 3 μm non-tethered bead escapes from the trap ($F = 6\pi\eta a v$, where η is the water viscosity, v the velocity and a the bead radius). For 50 mW trapping power, the trapping force is ~ 3.7 picoN. The trap stiffness is calibrated using two standard methods[11,29]. In the first method, we measure the RMS fluctuations of the bead in the trap, $\langle \Delta x^2 \rangle$, and get the trapping stiffness: $\kappa_{trap} = K_B T / \langle \Delta x^2 \rangle$. In the second method, we measure the power spectrum of the fluctuations of the bead. The stiffness of the trap is given by $\kappa_{trap} = 12\pi^2 \eta a f_c$, where η is fluid viscosity, a the bead radius and f_c is the cut-off frequency of the fluctuations. From these two methods the estimate of the trapping stiffness is within 20% and is of the order of 0.05 picoN/nm, for an incident laser power of 100 mW in 0.1 M PBS buffer.

3. Selection, manipulation and grafting DNA tethered beads using optical tweezers

In the microscope field of view, a large number of beads are present. Some are directly bound to the coverslip, others tethered to it through λ DNA, with one or many attachments. Watching the Brownian motion, it is easy to discard the beads glued to the coverslip, as they do not move. To choose a bead with only one λ DNA, the laser tweezer is used. For forces of the order of 15 picoN, a single λ DNA molecule can be extended to ≥ 95% of its contour length (16.5 μm). Doing extension exercises one easily selects beads with only one polymer attached to the coverslip, as they move a longer distance, for the same trapping force, before escaping from the trap and going back to their equilibrium attachment point.

For bead grafting, the cantilever is colinearly aligned with the optical axis of the laser tweezer and brought in proximity to the DNA tethered bead. Bead grafting is done using the tweezer to heat the cantilever tip by infrared absorption, keeping the bead trapped. The polystyrene latex bead sticks to the cantilever as it is heated, presumably by polymer wetting. The heating time is very critical, both for optimized grafting and to keep the bead–DNA attachment[13]. Typically the trap is sinusoidally modulated around a mean optical power of 30 mW at low frequency (amplitude of modulation 15 mW and frequency 0.2 to 0.5 Hz). This modulation reduces the heating of the cantilever, while keeping the bead localized (for a 3 μm bead, the diffusion coefficient is $D = K_B T / 6\pi\eta a \approx 10^{-13} m^2/s$; the bead moves a distance $(Dt)^{1/2} = 0.5$ μm in one second, and thus cannot escape). The attachment time is of the order of seconds. Since the trap is modulated, the tethered bead fluctuates around a mean value. The fluctuation drops as soon as the bead is attached to the cantilever and at this stage the laser is turned off. Bead attachment in most cases is

reversible. For bead detachment, the cantilever is brought in contact with the coverslip and pressed against it.

4. Atomic force spectroscopy of single molecules

The force measurement is a standard one[30], using an optical deflection technique[31,32]. The cantilever (Park Scientific) is mounted using a magnetic holder. A light beam from a 1 mW HeNe laser diode (wavelength 632.8 nm, 3 mm beam diameter) is focused on to the cantilever. The reflected beam is detected using a quadrant position detector (UDT Sensors, quantum efficiency 0.35 A/W). The position detector is operated in the photo voltaic mode. A small change in the z deflection of the cantilever, caused by an external force, is amplified (≈ 250) at the detector level. The amplification is $(\Delta x/\Delta z) = (S/l)$, where Δx is the change in beam deflection, S the distance between the cantilever and the position detector, and l the length of the cantilever. The optical path to the quadrant detector is aligned such that, for no external force on the cantilever, the two photo detector quadrants have the same photo current. Deflection measurements are carried out using a differential preamplifier (PAR 113) connected to a A/D data acquisition board on a PC (programmed using LabView, National Instruments). The cantilever used has a specially small stiffness, of the order of 10 picoN/nm (usually for a force microscope, the cantilever stiffness is in the range of a few hundred picoN/nm).

The limiting factor in force sensitivity is due to thermal fluctuations $\langle \Delta z \rangle^2 = K_B T/k$, where Δz is the mean average displacement, k the stiffness of the cantilever, K_B the Boltzmann constant and T the temperature. In our case $\langle \Delta z \rangle \approx 0.6$ nm. In dc measurements, the cantilever deflection is measured and the force directly estimated ($F = -kz$, $k = 10 \pm 2$ picoN/nm, z measured deflection). Our force sensitivity is thus of the order of a few picoN.

5. Bio-molecular lithography

We present two examples dealing with the problem of specific recognition of a given sequence of single-stranded DNA by hybridization to the complementary strand, immobilized on a substrate using laser lithography. The first involves lithographic patterning of latex beads on a gold-coated glass substrate (Figure 4 *a* and *b*). A single-stranded DNA sequence (GTATCACGAGGCCCT), modified with a Biotin linker, is coupled to avidin covered latex beads and immobilized on the substrate. To recognize the patterned single-stranded DNA, the complementary sequences tagged with a fluorophore (FAM) at the 3′ end are added to the sample cell. The sample is incubated at room temperature for 1 h in 150 mM PBS buffer and then washed to remove the free oligomers in the solution. The resulting fluorescence microscopy picture is shown in Figure 4 *c*. The fluorescence studies are made using a Zeiss microscope equipped with a Hamamtsu intensified camera. We find that differentiation is easily detected, even for 5 μm separation between two neighbouring oligomer lines. The second involves grafting a latex bead, covered with a known DNA sequence, on to the atomic force microscope silicon cantilever (Figure 5 *a–c*).

1. Watson, J. D., Hopkins, N. H., Roberts, J. W., Steitz, J. A. and Weiner, A. M., *Molecular Biology of the Gene*, Benjamin Cummings Publications, 1987.
2. Fodor, S. P. A., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T. and Solas, D., *Science*, 1991, **251**, 767–773.
3. Southern, E. M., *Trends Genet.*, 1996, **12**, 110–116.
4. Steven, S. B., Finzi, L. and Bustamante, C., *Science*, 1992, **258**, 1122–1126.
5. Perkins, T. T., Quake, S. R., Smith, D. E. and Chu, S., *Science*, 1994, **264**, 822–826.
6. Bensimon, D., Simon, A. J., Croquette, V. and Bensimon, A., *Phys. Rev. Lett.*, 1995, **74**, 4754–4757.
7. Steven, S. B., Cui, Y. and Bustamante, C., *Science*, 1996, **271**, 795–799.
8. Cluzel, P., Lebrun, A., Heller, C., Lavery, R., Viovy, J. L., Chatenay, D. and Caron, F., *Science*, 1996, **271**, 792–794.
9. Strick, T. R., Allemand, J.-F., Bensimon, D., Bensimon, A. and Croquette, V., *Science*, 1996, **271**, 1835–1837.
10. Yin, H., Wang, M. D., Svoboda, K., Landick, R., Block, S. M. and Gelles, J., *Science*, 1995, **270**, 1653–1657.
11. Wang, M. D., Yin, H., Landick, R., Gelles, J. and Block, S. M., *Biophys. J.*, 1997, **72**, 1335–1346.
12. Essevaz-Roulet, B., Bockelmann, U. and Heslot, F., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 11935–11940.
13. Shivashankar, G. V. and Libchaber, A., *Appl. Phys. Lett.*, 1997, **71**, 3727–3729.
14. Shivashankar, G. V., Stolovitzky, G. and Libchaber, A., *Appl. Phys. Lett.*, 1998, **73**, 291–293.
15. Marko, J. and Siggia, E., *Macromolecules*, 1995, **28**, 8759–8770.
16. Travers, A. A., *DNA–Protein Interactions*, Chapman and Hall, 1993.
17. Kowalczykowski, S. C. and Eggleston, A. K., *Annu. Rev. Biochem.*, 1994, **63**, 991–1043.
18. Roca, A. I. and Cox, M. M., *Crit. Rev. Biochem. Mol. Biol.*, 1990, **25**, 415–456.
19. Howard-Flanders, P., West, S. C. and Stasiak, A., *Nature*, 1984, **309**, 215–220.
20. Honigberg, S. M. and Radding, C. M., *Cell*, 1988, **54**, 525–532.
21. Leger, J. F., Robert, J., Bourdieu, L., Chatenay, D. and Marko, J. F., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 12295–12299.
22. Shivashankar, G. V. and Libchaber, A., *Biophys. J.*, 1998, **74**, A242; Shivashankar, G. V., Feingold, M., Krichevsky, O. and Libchaber, A., *Proc. Natl. Acad. Sci. USA*, in press.
23. Goffeau, A., *Nature*, 1997, **385**, 202–203.
24. Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O. and Davis, R. W., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 10614–10619.
25. Guo, Z., Guilfoyle, R. A., Thiel, A. J., Wang, R. and Smith, L. M., *Nucleic Acids Res.*, 1994, **22**, 5456–5465.
26. Ramsay, G., *Nature Biotech.*, 1998, **16**, 40–44.
27. Shivashankar, G. V. and Libchaber, A., *Appl. Phys. Lett.*, 1998, **73**, 417–419.
28. Ashkin, A., Dziedzic, J. M., Bjorkholm, J. E. and Chu, S., *Opt. Lett.*, 1986, **11**, 288–290.
29. Simmons, R. M., Finer, J. T., Chu, S. and Spudich, J., *Bophys. J.*, 1996, **70**, 1813–1819.
30. Binnig, G., Quate, C. F. and Gerber, C., *Phys. Rev. Lett.*, 1986, **56**, 930–933.
31. Meyer, G. and Amer, N. M., *Appl. Phys. Lett.*, 1988, **53**, 1045–1047.
32. Alexander, S., Hellemans, L., Marti, O., Schneir, J., Elings, V., Hansma, P. K., Longmire, M. and Gurley, J., *J. Appl. Phys.*, 1989, **65**, 164–169.

ACKNOWLEDGEMENTS. We thank K. Adzuma, D. Chateney, M. Finegold, O. Krichevsky, S. Liu, J. F. Marko, P. Model, E. Siggia and G. Stolovitsky for useful discussions.

Received 4 December 1998; accepted 7 December 1998