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Translating Biomolecular Recognition into Nanomechanics

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We report the specific transduction, via surface stress changes, of DNA hybridization and receptor-ligand binding into a direct nanomechanical response of microfabricated cantilevers. Cantilevers in an array were functionalized with a selection of biomolecules. The differential deflection of the cantilevers was found to provide a true molecular recognition signal despite large nonspecific responses of individual cantilevers. Hybridization of complementary oligonucleotides shows that a single base mismatch between two 12-mer oligonucleotides is clearly detectable. Similar experiments on protein A-immunoglobulin interactions demonstrate the wide-ranging applicability of nanomechanical transduction to detect biomolecular recognition.

In recent years, biomolecules and their unique ability of molecular recognition have been investigated in terms of their mechanical response to external forces. A common feature of methods such as atomic force microscopy (AFM) (1–3), optical tweezers (4), and magnetic bead experiments (5) is that the molecules are probed by applying an external force. Conversely, intermolecular forces arising from adsorption of small molecules are known to induce surface stress, directly resulting in the mechanical bending of a solid surface or a cantilever (6–9). Recently, cantilever bending by nonspecific adsorption of proteins was reported (10, 11). An analogous transduction process is found in biology, where the interaction of membrane molecules modifies the lateral tension of a lipid bilayer. The resulting curvature of the membrane is responsible for mechanically triggering membrane protein function (12).

We have taken advantage of biochemical-ly induced surface stress to directly and specifically transduce molecular recognition into nanomechanical responses in a cantilever array. This is achieved by immobilizing a monolayer of receptor molecules on one side of the cantilevers and then detecting the mechanical bending induced by ligand binding in a liquid environment. A major advantage of such a direct transduction is that it eliminates the requirement that the molecules under investigation be labeled, for example, with fluorescence or radioactive tags.

DNA hybridization is a prominent example of molecular recognition, fundamental to the biological processes of replication, transcription, and translation. Consequently, we studied the surface stress arising from Watson-Crick base pairing between unlabeled oligonucleotides and their surface-immobilized binding partners. Hybridization experiments were performed in a liquid cell containing a silicon cantilever array immersed in hybridization buffer (Fig. 1) (13). The bending of each cantilever was measured in situ, using an optical beam deflection technique (14). Synthetic 5' thio-modified oligonucleotides with different base sequences were co-

valently immobilized on the gold-covered side of the cantilevers (Fig. 2A) (15). The functionalization of one cantilever with a 12-mer oligonucleotide and the other with a 16-mer oligonucleotide was performed in parallel under identical conditions (15). The arrays were equilibrated in hybridization buffer until the differential signal became stable. Then, the complementary 16-mer oligonucleotide solution was injected into the liquid cell (Fig. 2B) followed by injection of complementary 12-mer oligonucleotide solution (Fig. 2C). The injections lead to hybridization of oligonucleotides in solution with the matching oligonucleotides immobilized on the cantilever surfaces. This results in a difference in surface stress between the functionalized gold and the nonfunctionalized Si surface, which bends the cantilever. During the entire process, the absolute deflections of individual cantilevers were recorded (Fig. 3A). Simultaneously, we extracted the differential signal (deflection of cantilever covered by the 16-mer oligonucleotide minus deflection of the cantilever covered by the 12-mer oligonucleotide) (Fig. 3B).

Signals from individual cantilevers displayed drifts of several tens of nm during equilibration (interval I in Fig. 3A). In general, injections of liquid resulted in spikes of up to 100 nm in amplitude, which are ascribable to turbulences. This was followed by an immediate increase of the signal by ~50 nm

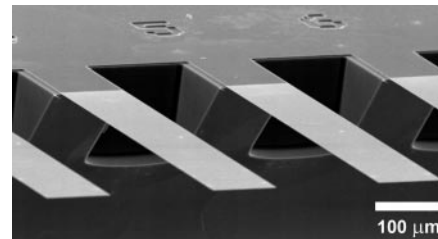


Fig. 1. Scanning electron micrograph of a section of a microfabricated silicon cantilever array (eight cantilevers, each 1 μm thick, 500 μm long, and 100 μm wide, with a pitch of 250 μm , spring constant 0.02 N m^{-1} ; Micro- and Nanomechanics Group, IBM Zurich Research Laboratory, Switzerland).

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and by a fast relaxation process (interval II in Fig. 3A). We assigned the latter to bimetallic effects arising from a slightly different temperature of the injected sample solution compared to that of the buffer in the liquid cell.

These large, unspecific signals from individual cantilevers can be removed by extracting the differential signal from two cantilevers. Because all cantilevers of an array are physically identical (16), the differential signal is sensitive only to the individual cantilevers' ability to recognize complementary oligonucleotides. Unspecific binding bends the cantilevers in parallel, leading to no overall differential signal.

The differential signal, starting from the baseline level, displayed only a slight increase with time and reached an equilibrium value of 10 nm for the hybridization of the 16-mer oligonucleotide (interval II in Fig.

3B). Subsequent injection of the complementary 12-mer oligonucleotide that matches the second cantilever decreased the differential signal by 16 nm (interval III in Fig. 3B). These observations are consistent with compressive surface stress occurring on the functionalized side of the cantilevers, where hybridization on one cantilever increases the differential signal (Fig. 2B), and subsequent hybridization on the other cantilever decreases the differential signal (Fig. 2C). The experiments demonstrate that the differential bending is clearly sequence-specific and provides an unambiguous "yes" or "no" response.

The transduction of molecular recognition into surface stress occurs from electrostatic, steric, and hydrophobic interactions whose relative contributions depend on the molecules under investigation (17). The curvature stress in lipid bilayers has been attributed principally to hydrophobic interactions modified by electrostatic interactions of molecules in the bilayer (12). During hybridization, the number of charges in the molecular layer from the sugar-phosphate backbone of the oligonucleotides and their surrounding

counterions is increased. Simultaneously, the chain packing of oligonucleotides on the surface also increases. Both interactions, electro-

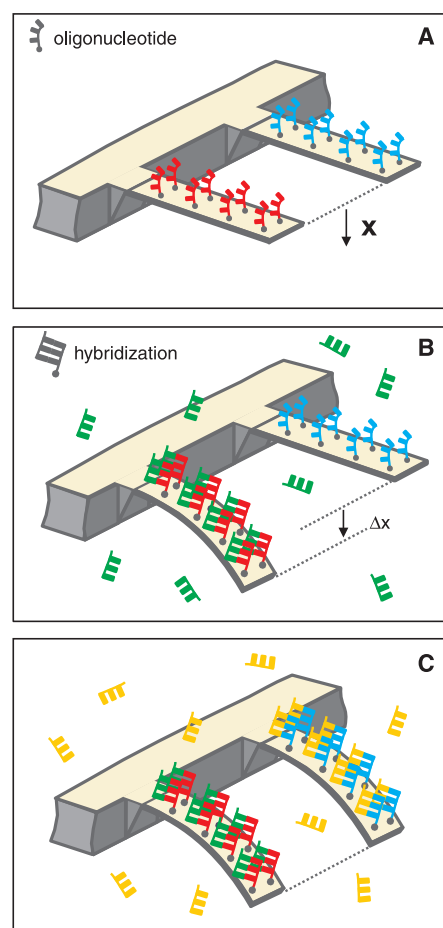


Fig. 2. Scheme illustrating the hybridization experiment. Each cantilever is functionalized on one side with a different oligonucleotide base sequence (red or blue). (A) The differential signal is set to zero. (B) After injection of the first complementary oligonucleotide (green), hybridization occurs on the cantilever that provides the matching sequence (red), increasing the differential signal Δx . (C) Injection of the second complementary oligonucleotide (yellow) causes the cantilever functionalized with the second oligonucleotide (blue) to bend.

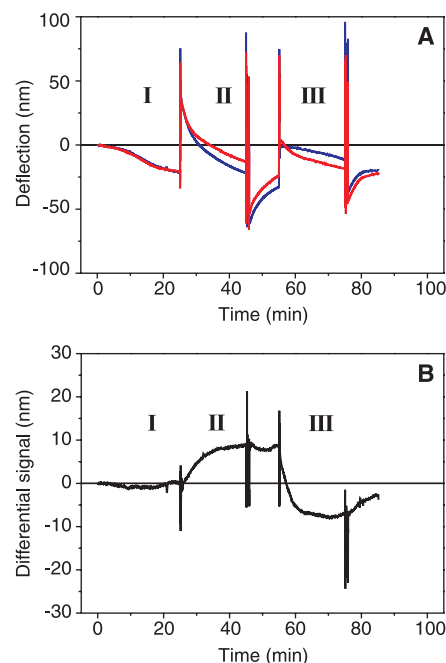


Fig. 3. Hybridization experiment using two cantilevers functionalized with the sequences 5'-TGCAGTAGACCT-3' (12-mer oligonucleotide), the complementary 16-mer oligonucleotide (1 ml, 400 nM in HB) was injected (interval II). The liquid cell was purged 20 min later with 3 ml of HB. Then, the complementary 12-mer oligonucleotide (1 ml, 400 nM in HB) was injected (interval III). The liquid cell was again purged 20 min later with 3 ml of HB. (A) Absolute deflection versus time of two individual cantilevers covered with the 16-mer (red) and the 12-mer (blue) oligonucleotide. (B) Corresponding differential signal.

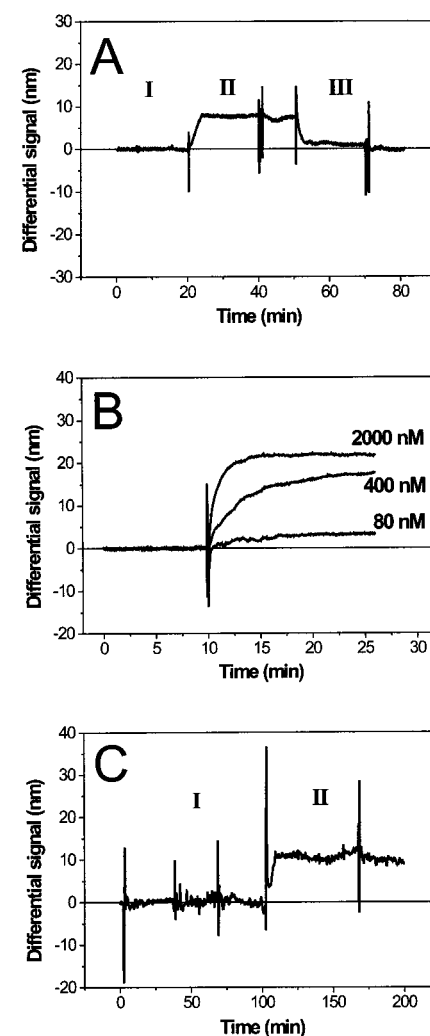


Fig. 4. (A) Differential signal of a hybridization experiment, showing detection of a single base mismatch in 12-mer oligonucleotides. Two cantilevers were functionalized with sequences differing only in one base: 5'-CTATGTCAGCAC-3' (first oligonucleotide), 5'-CTATGTAAGCAC-3' (second oligonucleotide). The injection of the first complementary oligonucleotide increases the differential signal (interval II); injection of the second complementary oligonucleotide decreases the differential signal (interval III). (B) Three successive hybridization experiments with different 12-mer oligonucleotide concentrations using one array. The concentration detection limit was calculated to be 10 nM, on the basis of a deflection noise of 0.5 nm. (C) Differential signal from a protein-protein interaction. One cantilever was functionalized with protein A and the other with BSA as a reference. First, as a negative control, buffer and then buffer containing goat IgG were injected. In both cases (interval I), no change of the differential signal was detected. In contrast, injection of rabbit IgG increased the differential signal by 12 nm within minutes (interval II). The signal persisted after the liquid cell was purged again with buffer.

static as well as steric, are repulsive and produce compressive surface stress during hybridization.

Hybridization of the 12-mer oligonucleotides produces a compressive surface stress of 5 mN m^{-1} or an actuation force of $\sim 300 \text{ pN}$. This stress is ~ 100 times smaller than that resulting from the self-assembly of alkanethiols on gold (9). The actuation force results from a collective phenomenon within the biomolecular layer and is unrelated to receptor-ligand rupture forces (1–3).

A crucial test for the analytical ability of nanomechanical transduction is whether a single base mismatch between two DNA sequences can be detected. This was investigated by using two cantilevers differing in only one base of their immobilized 12-mer oligonucleotides. Injection of the first complementary oligonucleotide increased the differential signal by 10 nm (interval II in Fig. 4A). If the hybridization and its transduction into a nanomechanical bending had not been sensitive to a single base difference, no differential signal would have been recorded. This was confirmed by injecting the oligonucleotides complementary to the sequence on the second cantilever. As expected, the differential signal was observed to return to its starting value (interval III in Fig. 4A). The experiment demonstrates the detection of a single base mismatch by a nanomechanical response, showing that the method has the intrinsic sensitivity needed to detect single nucleotide polymorphisms, and suggests the capability to determine single base mutations or to sequence DNA by hybridization.

In addition, the amplitudes of the differential signal from hybridization of 12-mer oligonucleotides depend on the similarity of the base sequences on the two cantilevers at identical concentrations (16 nm for totally noncomplementary sequences in Fig. 3B and 10 nm for sequences differing in a single base in Fig. 4A, both at 400 nM). Given that oligonucleotides with a single base mismatch hybridize to a small extent (18, 19), the cantilever with the nonperfect matching sequence bends slightly and reduces the amplitude of the differential signal.

After binding, hybridized oligonucleotides were chemically denatured by purging the liquid cell with a high concentration of urea (30% in H_2O), which is known to break the hydrogen bonds between complementary bases. This successfully reestablished the initial conditions of the experiment and enabled the same array to be reused for at least 10 successive experiments over several days. The differential signals were observed to be highly reproducible for a given concentration within $\pm 1 \text{ nm}$ for the same array and $\pm 3 \text{ nm}$ for different arrays. By varying the concentration of oligonucleotides in solution, we found that the amplitude of the differential

signal increases with increasing concentration, as expected from the equilibrium character of surface-bound hybridization (Fig. 4B) (18, 19).

In all experiments, the areal density of immobilized oligonucleotides was kept constant. We determined a surface coverage of $\sim 10^{10}$ oligonucleotides per cantilever, which is consistent with published areal densities (19–21). Optimization of buffer composition and surface immobilization could increase the differential signal. Furthermore, the use of smaller cell volumes and cantilevers (22) which accommodate 10^7 oligonucleotides is expected to greatly decrease the number of molecules needed for an experiment.

The general applicability of our detection method to biomolecular processes was demonstrated by monitoring molecular recognition between proteins. We studied the specific binding of the constant region of immunoglobulins (IgG of different species) to protein A (Fig. 4C). The experiment was performed in a manner similar to the hybridization experiments except that one cantilever was covered with protein A and the other with bovine serum albumin (BSA) as a reference (23). We observed a distinct differential signal from rabbit IgG, but not from goat IgG, thus reflecting the known specific binding properties of protein A to IgG of different mammals (24).

Our measurements demonstrate the direct translation of biomolecular recognition into nanomechanics. The method has important advantages in that it does not require labeling, optical excitation, or external probes. Furthermore, the transduction process is repeatable when denaturation or unbinding agents are used, enabling cyclic operation. The methodology is compatible with silicon technology and is suited for in situ operation. Parallelization into integrated devices, currently demonstrated with arrays of more than 1000 cantilevers (25), will allow a new generation of DNA chips and binding assays to be developed based on nanomechanics. We believe that the nanoactuation mechanism has more wide-ranging implications. The forces involved, $\sim 1 \text{ nN}$, are sufficient to operate micromechanical valves and related microfluidic devices. This would also permit the autonomous operation of micro- or nanorobotic machinery. Because the transduction eliminates the need for external control systems, in situ delivery devices could be triggered directly by signals from single cells, gene expression, or immune responses.

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13. The liquid cell has a volume of 800 μl , and the buffer solution was manually exchanged by a micropipette. All hybridization experiments were performed in saline sodium citrate hybridization buffer (HB) ($5\times$ SSC buffer, Sigma Chemicals) at room temperature ($22.0^\circ \pm 0.2^\circ\text{C}$).
14. Each cantilever is illuminated by one vertical cavity surface-emitting laser (VCSEL; wavelength 760 nm; CSEM, Zurich, Switzerland) of a linear VCSEL array (pitch 250 μm). The VCSELs are time-multiplexed with a rate of 1.3 Hz. Detection of the reflected light by a single linear position-sensitive detector (Sitek, Partille, Sweden) was used to measure the bending of each cantilever. The cantilever deflection is calculated with an accuracy of 0.1 nm [T. Miyatani and M. Fujihira, *J. Appl. Phys.* **81**, 7099 (1997)].
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