Micro- and Nanocantilever Devices and Systems for Biomolecule Detection

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Abstract

Recent research trends in biosensing have been geared toward developing bioanalytical devices that are label free, small in size, and portable and that can operate in a rapid manner. The performance of these devices has been dramatically improved through the advent of new materials and micro-/nanofabrication technologies. This is especially true for micro-/nanosized cantilever sensors, which undergo a change in mechanical properties upon the specific binding of biomolecules. In this review, we introduce the basic principles of cantilever biosensors in static and dynamic modes. We also summarize a range of approaches to cantilever design, fabrication, and instrumentation according to their applications. More specifically, we describe cantilever-based detections of proteins, DNA molecules, bacteria, and viruses and discuss current challenges related to the targets' biophysical characteristics.

1. INTRODUCTION

There has been a growing interest in the development of miniaturized biosensors, which range from the size of a desktop computer to the size of a lab on a chip, that are capable of rapid, quantitative, and multiplexed detection. Over the past decade, the advent of micro- and nanotechnology has expedited the miniaturization of biosensors, resulting in devices with an analytical performance equal to or better than their larger predecessors that meet the requirements of the biological and medical community. The goal of miniaturization is a fully completed analytical and diagnostic system containing sample-loading, delivery, preparation, and rapid-detection components. Indeed, various component-based technologies and partially integrated systems have already been developed in the field of lab-on-a-chip applications (1, 2).

Current detection methods rely primarily on fluorescent labels to enhance specific signals from biological interactions. However, these methods may not be the solution for on-site and real-time monitoring, given the inherent complexity associated with multistep detection protocols. It is also challenging to multiplex targets, reduce sample volume, and quantify target molecules in subfemtomolar concentrations. Consequently, the detection unit is an important—if not the most important—component in determining the performance of current analytical systems. Micro-/nanocantilever-based detectors are a strong candidate for the ideal label-free detection system, which must be (*a*) rapid, multiplexed, sensitive, and robust and (*b*) easy to use by untrained personnel.

Surface plasmon resonance (SPR) is another powerful label-free detection method. Since its first use as a sensing tool in the early 1980s, SPR has become an important method for characterizing protein interactions in real time. For quantitative purposes, SPR's protein-detection limit is in the nanogram-per-milliliter range, which is comparable to other traditional detection methods (3). However, the fact that SPR typically requires delicate optical systems is problematic for miniaturization and multiplexing.

Nanowire sensors have been shown to perform high-sensitivity detection of oligonucleotides. Due to the high charge density along the phosphodiester backbone, silicon nanowires have a dynamic range extending from tens of femtomolars to several picomolars and a detection limit of 10^{-14} M (4). Despite these credentials, several scientific challenges remain to be resolved before nanowires can be used in a wide range of applications such as (*a*) overcoming Debye screening by physiological samples and (*b*) reproducible, high-throughput nanofabrication.

Bioparticles such as bacteria and viruses have relatively large gravimetric effects compared to DNA molecules and proteins. Quartz crystal microbalances (QCMs) are well-known mass sensors. Most commercialized quartz plates oscillate at 4–6 MHz, and their mass sensitivity is in the nanogram-per-hertz range (5). Thus, it is challenging to quantify small amounts of bioparticles even with sophisticated equipment and signal processing. A solution to this problem, as we discuss in the next section, may be microcantilevers, which can be built into various structures using different materials. Also, signal transduction in microcantilevers is very flexible and diverse. Thus, cantilever-based biosensors could be tail-designed for the detection of specific biological targets. Various cantilever biosensors have already been developed for a range of proteins, DNA, RNA, viruses, and bacteria.

During the past decade, the development of cantilever-based biosensors for biochemical studies and clinical applications has been conducted by research groups from the University of California at Berkeley, Oak Ridge National Laboratory, Cornell University, IBM Zurich Research Laboratory, and others. Since these pioneering studies were performed, a growing number of research groups have joined this field, greatly expanding the scope of microcantilever sensors. Thus, several review articles describing a diverse range of cantilever-based sensing applications have been published in

major engineering, physics, chemistry, and biology journals (6–11). A summary of the achievements of micro-/nanocantilever biosensors is given in **Table 1**.

In this review, we categorize recent achievements in this field according to their biological targets. We also discuss unique approaches to the design, material, fabrication, and signal transduction of cantilevers in relation to these targets.

2. OPERATION PRINCIPLE

Micro- and nanosized cantilevers are robust devices whose high sensitivity and selectivity allow them to detect physical, chemical, and biological components by measuring changes in cantilever bending or in resonant frequency. In this section, we briefly discuss two operational principles of cantilever-based biosensors: cantilever bending (deflection mode) and shifts in resonant frequency (resonance mode) (see **Figure 1**).

2.1. Deflection Mode

In static deflection, also known as the deflection mode, one can apply Stoney's formula,

$$R = \frac{Et^2}{6\Delta\sigma(1-\nu)}, \tag{1}$$

where R is the cantilever's radius of curvature, ν is Poisson's ratio, E is the substrate's Young modulus, t is the thickness of the cantilever, and $\Delta\sigma$ is the differential surface stress. Assuming that there is no external gravitational, magnetic, or electrostatic force, cantilever deformation depends upon the gradient in the mechanical stress generated in the devices. If one can change the stress on one side of the thin plate or beam, the entire beam structure bends according to the generated surface stress. For example, when analytes bind to only one side of the cantilever's surface, the cantilever bends up or down depending on the side to which the analytes bind. This occurs because the difference between the stresses ($\Delta\sigma$)—specifically, the difference between the surface stresses on the top and bottom surfaces of the microcantilever—acts on both sides of the cantilever, causing permanent bending.

Given that the cantilever's radius of curvature is much larger than the length of the cantilever, the tip deflection of the cantilever can be expressed as

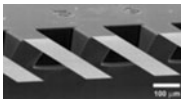
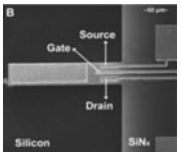
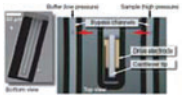
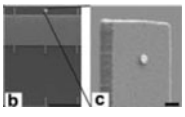
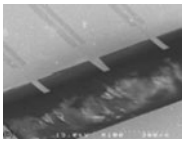
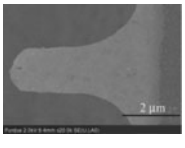
$$\Delta z = \frac{3l^2(1-\nu)}{Et^2}\Delta\sigma, \tag{2}$$

where l is the length of the cantilever, Δz is the tip deflection, and the surface stress is expressed as a function of the deflection of the cantilever Δz. Equation 2 suggests that there is a linear relation between the cantilever bending and the differential surface stress.

2.1.1. Measurement techniques. A variety of techniques have been developed for detecting the deflection of a cantilever. In this section, we introduce the sensors most commonly used for this purpose.

2.1.1.1. Optical lever technique. One of the most common means of measuring the deflection of a cantilever is the so-called optical lever technique. This technique generally requires silicon or silicon nitride cantilevers, as shown in **Figure 2**. Recently, the use of microcantilevers with a lower Young modulus, which were based on an SU-8 polymer, were shown to decrease thermal deflection noise.

Table 1 Examples of cantilever-based biomolecule detection

Affiliation	Device figure	Detection mode	Analyte	Detection limit	Medium	Reference[a]
IBM Zurich Research Laboratory		Static	DNA	10 nM	Liquid	50
Northwestern University		Static	Biotin	100 fg ml^{-1}	Liquid	13
Oakridge National Laboratory		Static	SNP[b]	n.d.	Liquid	57
University of California, Berkeley		Static	PSA	0.2 ng ml^{-1}	Liquid	30
Massachusetts Institute of Technology		Dynamic	Goat antimouse IgG	0.7 nM	Vacuum	27
Cornell		Dynamic	dsDNA	1.65 ag	Vacuum	90
Korea Institute of Science and Technology		Dynamic	PSA	10 pg ml^{-1}	Controlled air	24
Purdue		Dynamic	*Vaccinia virus* particle	10^{11} pfu ml^{-1}	Air	34

[a]Data reproduced with permission from the publishers.

[b]Abbreviations: dsDNA, double-stranded DNA; IgG, immunoglobin G; n.d., not determined; PSA, prostate-specific antigen; SNP, single nucleotide polymorphism.

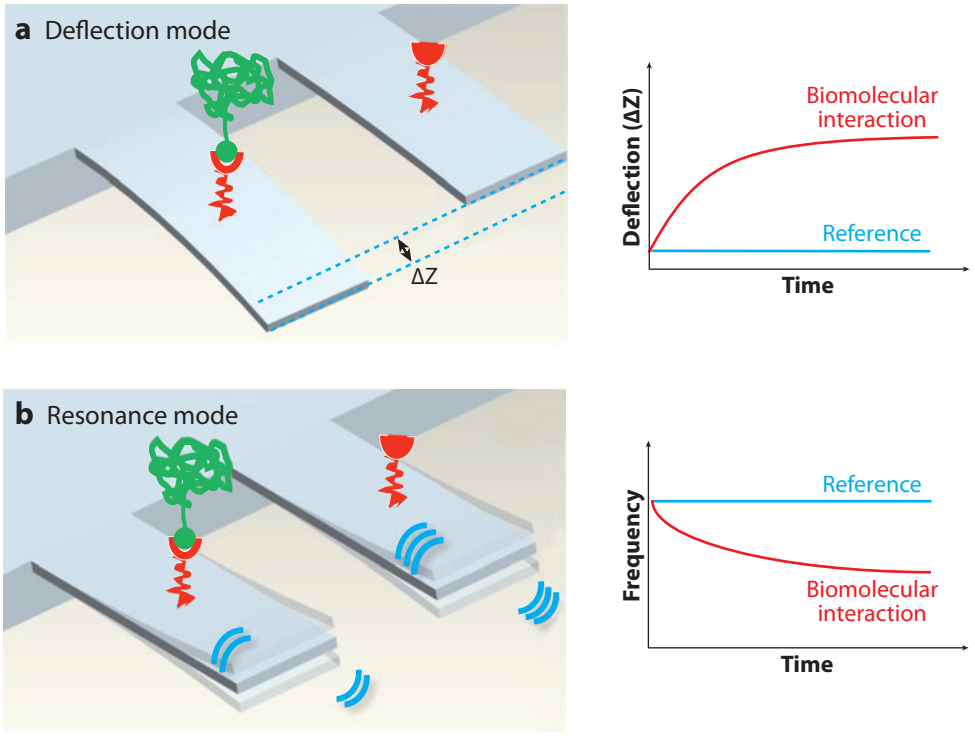


Figure 1

Two modes of cantilever-based biomolecule detection: (*a*) deflection mode and (*b*) resonance mode.

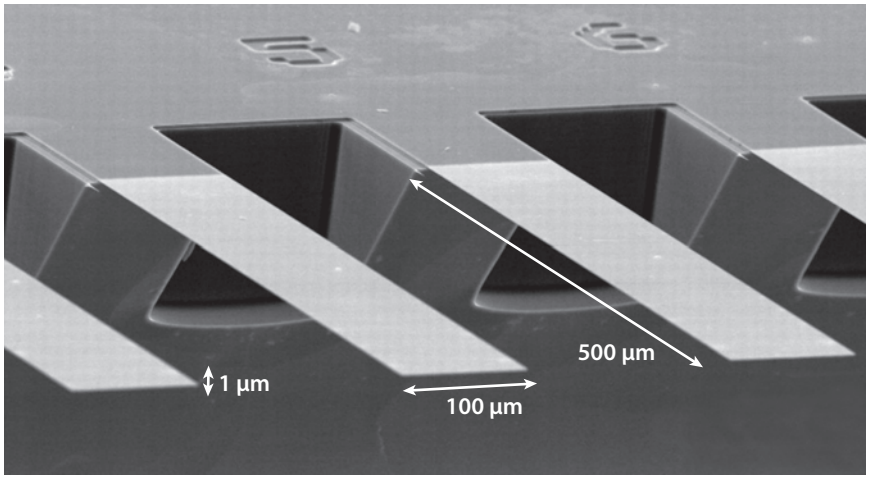


Figure 2

A scanning electron microscopy image of a microfabricated silicon cantilever array containing eight cantilevers. Reprinted with permission from Reference 50.

The merits of the optical lever method are its linear response, simplicity, and reliability. However, they require two alignment steps for physical positioning: First, a laser must be aligned to the end of the cantilever, and second, a split photodiode must be aligned with the reflected laser beam.

2.1.1.2. Interferometry. The interferometer optical-detection method, which is based on the interference between a reference laser beam and the laser beam reflected off the cantilever, provides a more sensitive technique for measuring the deflection of a cantilever. Interferometry is highly sensitive and can provide direct and absolute displacement. Rugar et al. (12) developed a deflection sensor based on the interference of light between the cleaved end of an optical fiber and the back of a cantilever. Using this technique, the authors achieved subnanometer deflection and successfully used single-spin magnetic resonance microscopy. Although interferometry is highly sensitive up to 0.1 Å, it has several disadvantages. For example, it requires tedious positioning of the fiber, it is relatively inefficient in liquids, and it works well for only small displacements.

2.1.1.3. The piezoresistive method. The piezoresistive method can measure stress changes using a Wheatstone bridge when the piezoresistive material, such as doped silicon and doped polysilicon, is strained. Generally, a detection chip that uses a piezoresistive method contains four resistors. Two of these resistors are placed on the cantilever: One is an active cantilever that reacts with the analyte in the sample, and the other is a passive cantilever that filters out signals that are identical for both piezoresistors. The deformation of the cantilever, which arises from variations in surface stress, can usually be measured from changes in the resistance of the piezoresistive materials.

The primary advantage of the piezoresistive technique is its readout system. This technique needs no optical components and can operate in nonopaque liquids. When using the piezoresistive technique, the resistor bridge can simply be balanced by changing the resistance of one of the elements. However, the deflection resolution for the piezoresistive readout system is only 1 nm, whereas the deflection resolution for an optical readout is 10 nm. Another disadvantage of this method is that heat from the working current causes a temperature fluctuation in the cantilever, which can lead to parasitic cantilever deflection and to piezoresistive changes.

2.1.1.4. The capacitance method. Another frequently used technique is the capacitance method. This approach allows one to measure the changes in capacitance that arise from the displacement of a conductor on the cantilever and a fixed conductor on the substrate. Because the cantilever is separated from the fixed conductor by a small gap, deformation of the cantilever can generate a variation in capacitance that is inversely proportional to the separation gap. The capacitance method provides high sensitivity and absolute displacement, and its performance is comparable to that of an integrated microsystem. However, the capacitance method does not work well in an electrolyte solution due to the Faradic current, corresponding to the reduction or oxidation of a chemical species that obscures a desired signal. Furthermore, detection can be adversely affected by variations in the dielectric constant of the medium.

2.1.1.5. Metal-oxide semiconductor field-effect transistor on the cantilever. Recently, researchers demonstrated another interesting approach for detecting surface stresses by embedding a metal-oxide semiconductor field-effect transistor (MOSFET) at the base of a cantilever (see **Figure 3**) (13). The gate region of the MOSFET was found to increase with surface stress, suggesting that the source of the drain current decreases with deflection. The authors of this study claim that the MOSFET technique offers low noise, high sensitivity, and direct readout.

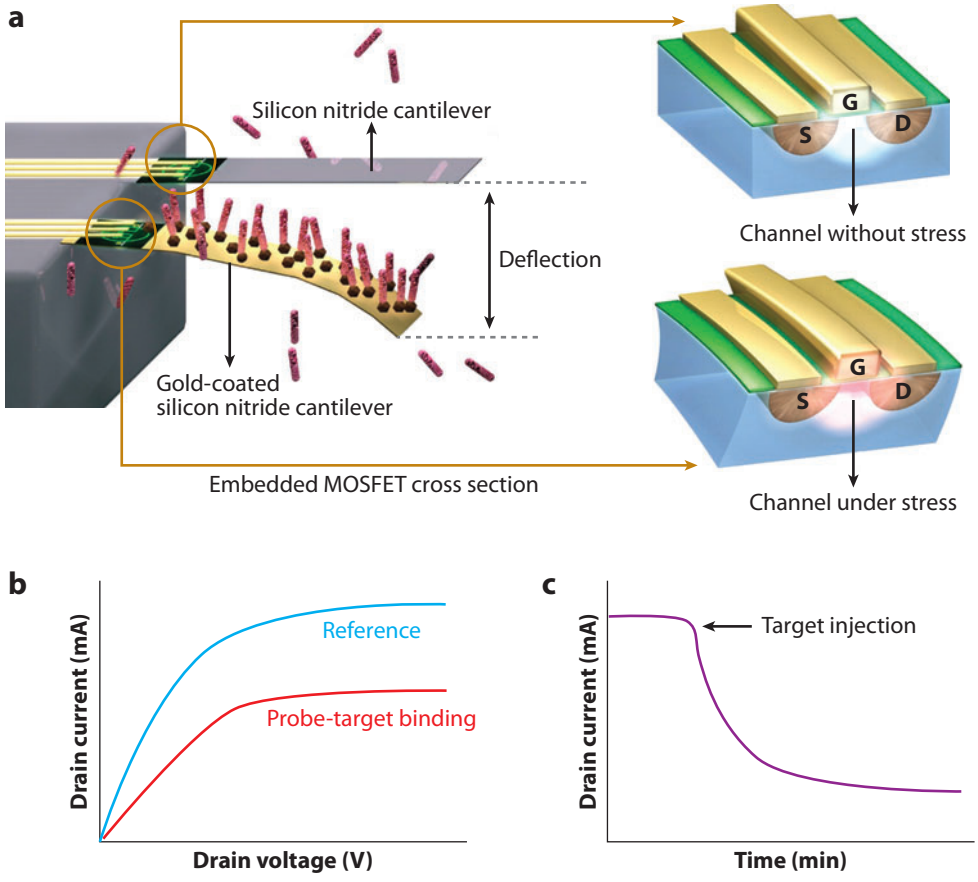


Figure 3

(*a*) Schematic of an embedded metal-oxide semiconductor field-effect transistor (MOSFET) cantilever system. (*b*) Schematic of change in a MOSFET drain current upon probe-target binding. (*c*) Change in drain current over time due to the deflection of the microcantilever. Reprinted with permission from Reference 13. G, S, and D refer to the gate, source, and drain in MOSFET, respectively.

2.2. Resonance Mode

The resonant frequency of an oscillating cantilever can be derived from the mass-spring-dashpot model,

$$m\frac{d^2x}{dt^2} + c\frac{dx}{dt} + kx = F(t), \tag{5}$$

where m, c, and k are the mass, the dashpot constant, and the spring constant of the system, respectively, and F is the external force that is exerted on the system. Assuming that there is no external force [i.e., $F(t) = 0$] or damping ($c = 0$), the natural resonant frequency of vibration (f) can be given by Equation 6,

$$f_0 = \frac{1}{2\pi}\sqrt{\frac{k}{m^*}}, \tag{6}$$

where k is the spring constant and m^* is the effective mass of the cantilever.

Based on the assumptions that k remains constant during mass loading and that the mass is uniformly distributed (i.e., where $m^* = 0.236$ m for a rectangular cantilever), the loading mass Δm can be estimated as

$$\Delta m = \frac{k}{\pi^2}\left(\frac{1}{f_2^2} - \frac{1}{f_1^2}\right), \tag{7}$$

where f_2 and f_1 are the resonance frequencies after mass loading and before mass loading, respectively.

In the resonance mode, the quality factor signifies the absolute mass sensitivity as well as the resolution. The quality factor Q can be defined as the resonance frequency divided by the full width at half maximum (FWHM),

$$Q = \frac{f_0}{\Delta f_{FWHM}}, \tag{8}$$

where f_0 is the resonant frequency, and Δf_{FWHM} is the FWHM of the resonance peak. The quality factor strongly relies on the cantilever geometry and the medium in which the cantilever operates. In Equation 9,

$$\Delta m_{\min} \propto \frac{m}{Q}, \tag{9}$$

m is the initial mass of the cantilever and $\Delta m_{\min}$ is the minimal detectable mass, which decreases when the quality factor Q increases. Thus, the quality factor dictates the limit of detection.

2.2.1. Measurement techniques. A variety of techniques, including the thermal (14–16), electrostatic (17), and magnetic excitation (18, 19) methods, have been developed for actuating resonance. We refer the reader to other review articles (10, 20) for details.

2.2.1.1. Piezoelectric integration. Rather than rely on external actuating, researchers have developed self-actuating and sensing cantilevers through the direct integration of piezoelectric material into the cantilever. The main advantage of the piezoelectric method is that it can convert a mechanical signal into a direct electrical signal with a high quality factor, thereby providing a sensitive tool for qualifying and quantifying biomolecules (22–25).

2.2.1.2. Microfluidic channel integration. An approach suggested by Burg et al. (26, 27) integrates microfluidic channels into the microcantilever (see **Figure 4**) and shows significantly enhanced sensitivity and very low limits of detection. The authors operated a cantilever in a vacuum while samples flowed through the microchannel inside the canitilever. Importantly, this allows one to prevent mechanical damping and thus to maintain in situ detection of liquid samples.

3. BIOMOLECULE DETECTION

Cantilever-based biosensors allow one to observe biomolecular events through either in situ or ex situ monitoring. Although most static cantilever sensors can detect specific targets present in an aqueous environment in real time, dynamic cantilever sensor–based protein detection is performed in mainly air or in vacuum because the quality factor of the cantilever sensors decreases in aqueous environment, degrading the resolution of the sensors. In the following three sections, we discuss various cantilever-based biomolecular-detection efforts classified according to their targets: proteins, DNA molecules, and bioparticles.

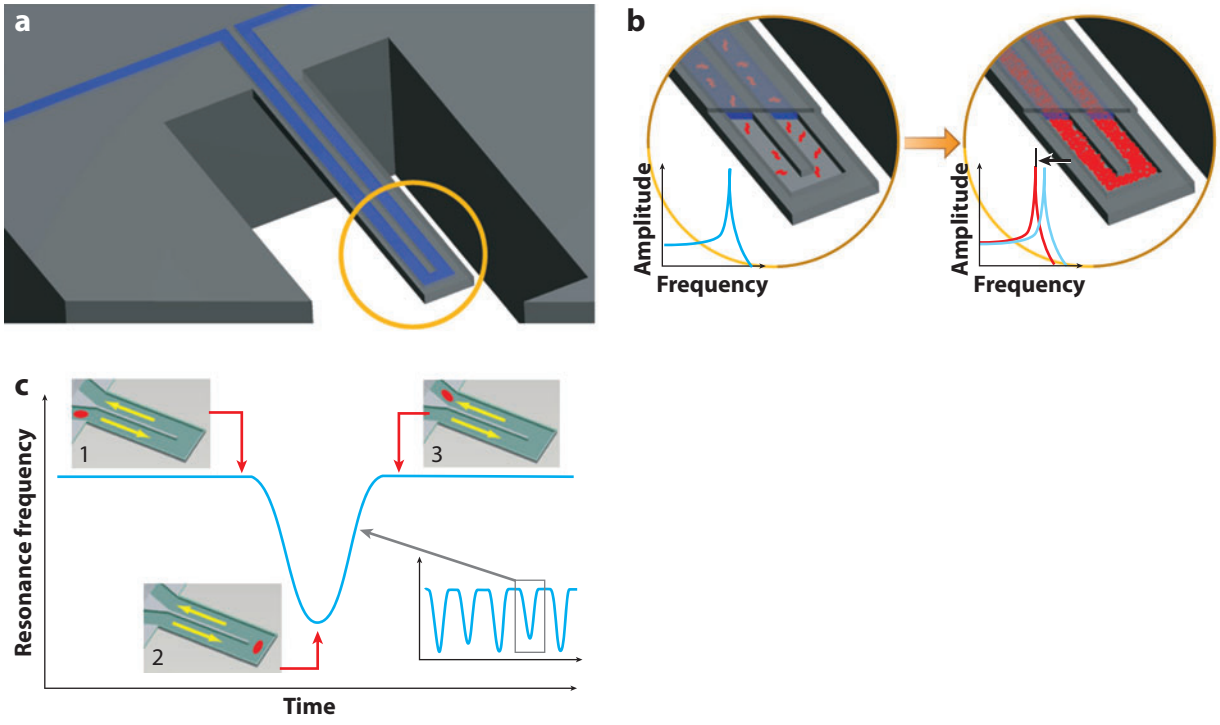


Figure 4

Illustration of microcantilever integrated with microchannels. (*a*) A suspended microchannel translates mass changes into changes in resonance frequency. Fluid continuously flows through the channel and delivers biomolecules or synthetic particles. (*b*) Although both bound and unbound molecules increase the mass of the channel, species that bind to the channel wall accumulate inside the device; as a result, their numbers can greatly exceed the numbers of free molecules in solution. This enables specific detection by way of immobilized receptors. Reprinted with permission from Reference 27.

3.1. Protein Detection

Anderson et al. (28) reported the concentration ranges of several biologically relevant biomolecules present in human blood. This study showed that numerous disease markers are present below the nanogram-per-milliliter level. Cantilever biosensors for proteins aim for a sensitivity that can quantitatively measure proteins in ultralow concentrations. In this section, we discuss cantilever-based protein detection in light of specific studies, the utility of protein-detection methods in disease diagnosis, and current efforts to improve sensing performance.

3.1.1. Current applications in disease diagnosis. Cantilever biosensors are used in marker detection for application in disease diagnosis. Here, we describe examples of such applications.

3.1.1.1. Prostate cancer detection. The prostate cancer–marker protein, prostate-specific antigen (PSA), is one of the most selective of all disease markers. PSA is also used as a marker for female breast cancer, and the clinical threshold in diagnosis of breast cancer is below the nanogram-per-milliliter level (29). For these reasons, PSA has long been a target protein for sensitivity validation of advanced biosensors.

A remarkable study of cantilever-based PSA detection was performed by Wu and coworkers (30), who were the first to perform quantitative detection of PSA. Using static-based cantilever

detection, the authors detected two types of PSA, free PSA and complex PSA, over a range of concentrations from 0.2 ng ml^{-1} to 60 μg ml^{-1} against a background of human serum albumin and human plasminogen. The concentration level of nonspecific background proteins was as high as that of real serum. Additionally, Lee et al. (24, 31) electrically detected PSA using dynamic response change (i.e., resonant frequency) with piezoelectric layer–embedded microcantilevers in aqueous (31) and humidity-/temperature-controlled-air environments (24). The authors integrated a piezoelectric material [lead zirconate titanate (PZT)] into the cantilever structure as a 0.5-μm-thick layer. PZT improved the cantilever's quality factor and enabled direct electrical transduction from mechanical oscillation. The achieved detection limit was 10 pg ml^{-1} in controlled air and 1 ng ml^{-1} in liquid.

3.1.1.2. Acute myocardial infarction detection. In addition to their advantage of high sensitivity, cantilever sensors do not require complicated assay protocols such as labeling, secondary antibody binding, and enzyme reaction. Because of its capability for rapid and simple detection, this technology is optimally suited for diagnosis of acute diseases that require immediate clinical warning. Arntz et al. (32) performed real-time detection of two markers for acute myocardial infarctions, myoglobin and creatine kinase. The authors achieved this detection by measuring the differential static deflection between an antibody-immobilized cantilever biologically passivated with bovine serum albumin (BSA), which was used as the reference cantilever, and antibodies on another immobilized cantilever. They calculated the differential deflection to avoid errors resulting from thermal drift, undesired biochemical reactions, and turbulence arising from the liquid injection. The detectable concentration achieved for myoglobin using this method was below 20 μg ml^{-1}.

In a similar study, Hwang et al. (33) developed a bioassay for myoglobin (ranging from 1 to 100 ng ml^{-1}) using two resonance modes of fabricated piezoelectric layer–embedded microcantilevers that contained immobilized antibodies. The authors theoretically and experimentally demonstrated that the surface stress generated from specific interactions dominantly influenced the resonant frequency shift of the cantilevers when bound proteins were distributed throughout the entire surface of one side of the cantilever.

3.1.2. Strategies for increasing sensor performance. We categorize newly proposed strategies for increasing sensor performance into two main approaches: developing novel sensor platforms and enhancing the signal transduction of the biomolecular interaction through surface-modification techniques.

3.1.2.1. Approaches using newly designed surface platforms. The first of the two strategies named above is based upon designing unique sensors. Biomolecular sensors using MOSFET-embedded microcantilevers have been developed, and they allow one to electrically measure cantilever deflection on the order of tens of nanometers (13). In this study, the authors detected biotin at low concentrations ranging from 100 fg ml^{-1} to 100 ng ml^{-1} by measuring the drain current change that was generated from the specific interaction between the streptavidin-coated MOSFET-embedded microcantilever surface and the biotin in the analytes. When biotin interacted with streptavidin on the cantilever surface, the surface stress generated from the intermolecular forces led to a decrease in the drain current of the buried MOSFET in the anchor of the cantilever. This approach offers many advantages over the use of traditional piezoresistive and capacitive sensors, such as low noise, high sensitivity, and direct readout.

Another approach to designing hardware to improve the performance of sensors was developed by the Bashir group (34), who fabricated cantilever sensors with submicrometer thickness for label-free protein and virus detection. In contrast to larger cantilevers, nanocantilevers show larger

changes in resonant frequency when biomolecules interact with the cantilever surface. It has been demonstrated both theoretically and experimentally that an increase or decrease in resonance frequency upon loading molecules is related to cantilever thickness.

3.1.2.2. Approaches for surface functionalization. An ideal sensor surface consists of immobilized receptors that bind only to specific target molecules with high stability against environmental changes. Savran et al. (35) reported Taq DNA polymerase detection using a microfabricated cantilever that was bent into a comblike structure and that was functionalized with DNA aptamers, which acted as receptor molecules. The use of aptamers as receptors for biosensing has several advantages over the use of traditional antibodies. Although many antibodies are vulnerable to temperature and humidity changes, aptamers have long-term stability at ambient conditions and can be recovered from denaturation (36). Moreover, due to aptamers' small sizes, receptor layers can be close-packed to increase sensitivity to biochemical interactions (37). Hwang and coworkers (38) used RNA aptamers as receptor molecules for the detection of the hepatitis C virus helicase, which was found in concentrations ranging from 100 pg ml^{-1} to 100 ng ml^{-1} using resonant frequency shift of piezoelectric layer–embedded microcantilevers. An advantage of using aptamers as receptors is that they can be easily immobilized on the functionalized surfaces of biosensors through amination or carboxylation of terminal sites. The authors immobilized the RNA aptamer on the calix crown–based surface via amine modification at the 3′ end of the aptamer. Kang and coworkers (39) quantitatively detected myoglobin using biotinylated myoglobin antibodies that were immobilized onto streptavidin-coated cantilevers. They observed enhanced signals arising from the ordered configuration of the antibody layers.

Recently, Yue et al. (40) developed an in situ antigen-antibody-binding assay using an optical-detection method to measure microcantilever deflection in an array format. In this study, antibodies were immobilized onto one side of the cantilevers using three different cross-linkers. The other side of the cantilevers was passivated by either BSA or polyethylene glycol (PEG)-silane. Successful surface modification of the microcantilever was achieved only when dithiobis-[sulfosuccinimidylpropionate] (DTSSP) or N-hydroxysuccinimide (NHS)-thiol was used for the cross-linkers and when PEG-silane was used for passivation. Backmann et al. (41) developed an immunoassay with single-chain Fv (scFv) antibody fragments by monitoring the differential deflection of the cantilevers. Using the oriented scFv fragments as receptor molecules, the authors increased the sensitivity of the microcantilevers to 1 nM.

3.2. DNA Detection

To date, nucleic acids have been the most popular target biomolecules because (*a*) they have simple repeating units (A, G, C, and T), (*b*) they are easy to modify for immobilization, and (*c*) they allow convenient length control (42–47). Based on these properties, researchers have used these attractive analytes to confirm the feasibility of cantilever-based biosensors and to develop novel cantilever-based biosensors (35, 48, 49).

3.2.1. Nucleic acid hybridization. The use of cantilever arrays for the detection of nucleic acid hybridization was first reported by Fritz and coworkers (50). In this study, the authors measured the bending of a silicon cantilever coated with a gold recognition layer via the optical beam deflection technique, and they performed in situ monitoring of the differential deflection signals between two DNA oligomers. Hybridization of a 12mer DNA at a concentration of 400 nM generated a 16-nm deflection, which corresponded to a surface stress of 5 mN m^{-1} or an actuation force of ~300 pN. In addition, the authors demonstrated the discrimination of single nucleotide

polymorphisms (SNPs). However, they did not clearly explain why the deflection of the 12mer DNA hybridization was larger than that of a slightly longer, 16mer DNA hybridization. The authors subsequently expanded this work to develop a multiple DNA assay, to quantify the target molecules, and to build upon earlier studies of nanomechanical motion (51, 52). They used differential deflection measurements between reference and functionalized cantilevers on an array of eight sensors to specifically detect unlabeled DNA targets in an 80-fold excess of a noncomplementary DNA sequence as a background; they were able to discriminate 3′ and 5′ overhangs. Based on these results, the authors suggested that nanomechanical motion originates from predominantly steric hindrance effects and that it depends on the concentration of DNA molecules in solution. They found that hybridization between two complementary 12mers generated an average compressive surface stress of 2.7 mN m^{-1}.

3.2.2. DNA transduction mechanism studies. On the basis of nucleic acid hybridization and intrinsically involved phenomena, Majumdar, Thundat, and colleagues (53–56) designed cantilever-based DNA sensors and developed their own hypothesis for the mechanical driving force related to actuating transduction. Hansen et al. (57) evaluated the capability of cantilever sensors to detect single-nucleotide mismatches (see **Figure 5**) and determined that the direction of cantilever deflection, whether tensile or compressive, depends on the number and location of mismatch sites along the double-stranded DNA pairs. Gold-coated silicon cantilevers were functionalized with thiolated 20mer or 25mer probe DNA oligonucleotides and were hybridized with target oligonucleotides of varying sequence under static and flow conditions. Although hybridization with targets containing one or two internal mismatches resulted in a net negative deflection, hybridization of 10mer complementary target oligonucleotides resulted in a net positive deflection. In addition, Wu et al. (58) showed that the absolute magnitude of the cantilever deflection during a hybridization event depends on the hybridizing environment such as buffer concentration (see **Figure 6**). By studying the nanomechanics of the cantilever as a function of DNA length, they demonstrated that the origin of this motion lies in the interplay between changes in configurational entropy and intermolecular forces induced by specific biomolecular interactions.

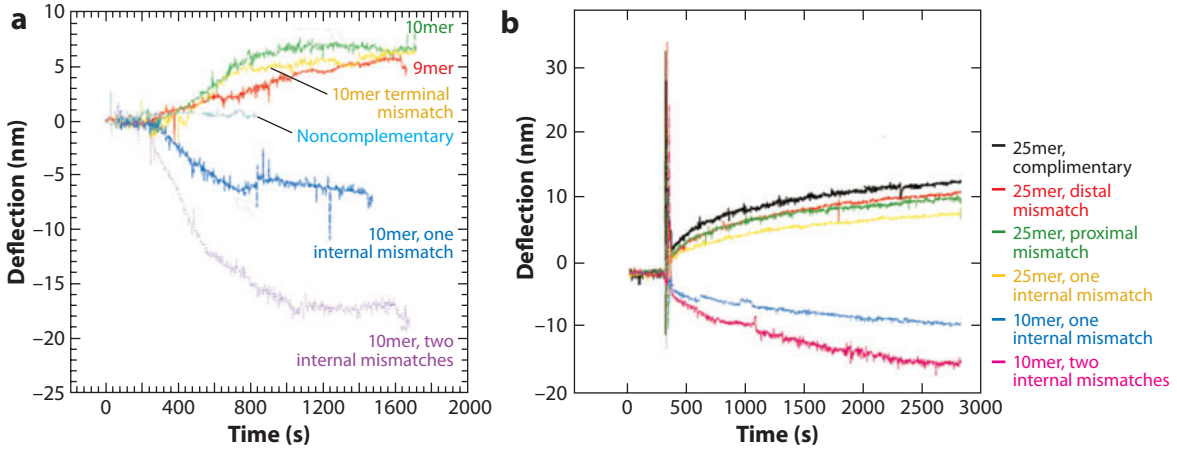


Figure 5

(*a*) Cantilever deflections of DNA hybridization. Cantilever surfaces functionalized with thiolated 20mer probe DNA and hybridized with complementary, mismatch, and noncomplementary target DNAs. (*b*) Cantilever deflection for thiolated 25mer probe DNA hybridized with complementary and mismatch target DNAs. Reprinted with permission from Reference 57.

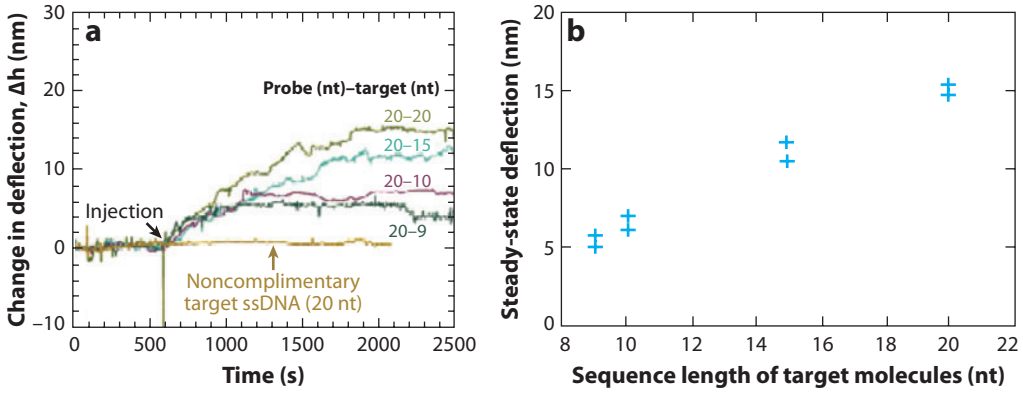


Figure 6

(*a*) Changes in Au-Si cantilever deflection caused by hybridization of a 20-nt-long probe single-stranded DNA (ssDNA) on the distal end, with changes in deflection by noncomplementary target ssDNA (sequence NC20) as a negative control and (*b*) complementary target ssDNAs of different lengths (20 nt, 15 nt, 10 nt, and 9 nt). Reprinted with permission from Reference 58.

Hagan et al. (59) developed a hypothesis to explain the phenomena observed in the cantilever deflection after probe immobilization and subsequent hybridization of DNA molecules. Using an empirical model, the authors predicted cantilever deflections after hybridization that were consistent with experimental results. They explained that hydration forces, not conformational entropy or electrostatics, are the dominant driving forces affecting deflections generated from DNA hybridization. They showed that predicted deflections before and after hybridization strongly depend on surface coverage as well as on the degree of disorder on the surface. In addition, Stachowiak et al. (60) provided experimental evidence that the surface grafting density of probe DNA on a given surface strongly depends on its persistence length and on the DNA chain length. The persistence length correlates with the ionic strength, which in turn affects the probe DNA–immobilizing density. They argued that the surface stresses produced by the hybridization between two single-stranded DNA molecules on surface and bulk solution depend on the length of the DNAs, their grafting density, and the hybridization efficiency. However, double-stranded DNA on the surface is a major driving force of microcantilever deflection.

3.2.3. Practical DNA detection. The clinical and practical detection of nucleic acids has recently been demonstrated (61–63). Zhang et al. (64) first demonstrated the use of biosensors for the differential nanomechanical analysis of multiple gene expression (see **Figure 7**). Probe oligo-DNAs specific for target-gene fragments and reference-gene fragments were individually immobilized on eight parallel cantilevers. The specific oligo-DNA-immobilized cantilever displayed a differential deflection signal (35 nm) of interferon-α-inducible 1–8-U gene against the internal control, the aldolase A gene's complementary RNA obtained from the cell extract. The limit of detection of the device was determined to be ∼10 pM, which is comparable to the low-picomolar limit of detection of the conventional gene microarray.

DNA detection in physiological fluids using large-sized dynamic cantilevers has also been performed. Rijal et al. (63) measured DNA hybridization at femtomolar concentrations in human serum using millimeter-sized piezoelectric cantilevers. They extracted signals from extremely low amounts of DNA fragments (down to 1 fM) in complex fluids without using polymerase chain reaction. Cantilever-based DNA-detection systems with circulation-available fluid collected target DNAs efficiently and lowered the limit of detection. For more sensitive DNA detection,

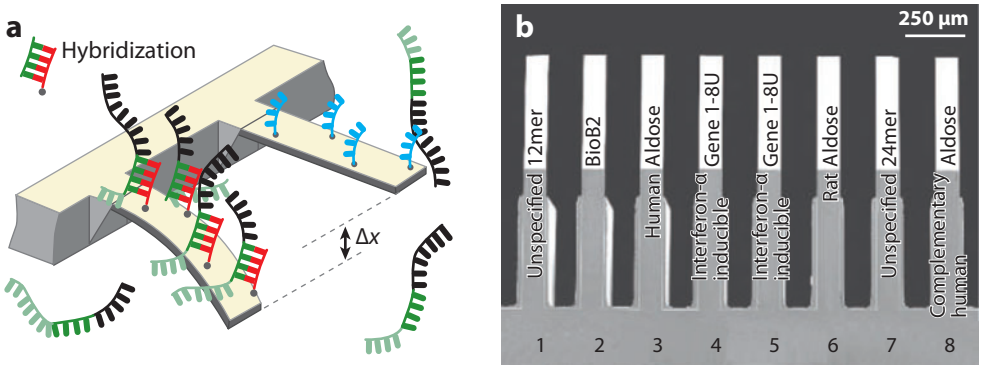


Figure 7

(*a*) DNA hybridization detection via sensor and reference cantilevers. Δ*X* refers to the difference in deflection. (*b*) DNA-immobilized cantilever microarray for multiple gene expression. Reprinted with permission from Reference 64.

Su et al. (65) used gold nanoparticle–labeled oligonucleotides, followed by a silver enhancement step, to improve the mass sensitivity of resonant frequency–based microcantilever detection of nucleic acid hybridization. Using this method, the authors detected DNA concentrations as low as 50 pM. They then compared the structural and working advantages of their cantilever-based DNA-detection systems with those of the conventional QCM systems.

3.3. Bioparticle Detection

Most infectious diseases are caused by organisms that resemble bioparticles, such as viruses and bacteria. There is a growing environmental and military need to develop methods to detect diseases, given that local outbreaks spread rapidly due to growing trade and transportation. Advances in biotechnology have enabled the design and production of highly selective and specific recognition molecules that can capture a variety of bioparticles. Cantilever biosensors equipped with components for direct capture, identification, and quantitative assessment of pathogenic organisms are a strong candidate for a rapid first-alert detection system.

Single viral particles weigh several femtograms, and a bacterium or spore weighs several hundred femtograms. Because each bioparticle contains absolute mass, most cantilever detection has been carried out in the dynamic mode, which is more sensitive to mass loading. **Table 2** lists the mass sensitivities and quality factors of resonating cantilevers with various structures.

3.3.1. Mass sensitivity improvement. When the cantilever biosensors were applied to bioparticle detection, Craighead et al. (15) measured the resonance frequency under atmospheric

Table 2 Examples of in situ virus detection schemes

Author	Size (l × w × t)	Natural frequency (Hz)	Mass sensitivity (g Hz^{-1})	Quality factor	Reference(s)
Bashir	20 × 9 × 0.2 μm^3	$5*10^5$	10^{-14}	2	70, 71
Manalis	200 × 33 × 7 μm^3	$2*10^5$	$3*10^{-13}$	15,000	27
Mutharasan	1.5 × 1 × 0.3 mm^3	10^6	$3*10^{-15}$	15	75
General quartz crystal microbalance	10 × 0.1 mm^3[a]	$\sim 10^7$	10^{-9}	~1000	5

[a]Diameter times thickness.

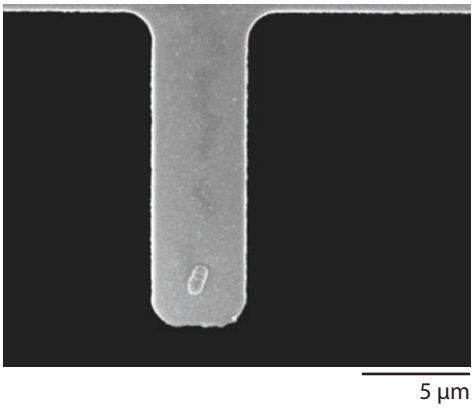


Figure 8

A scanning electron microscope image of a single *Escherichia coli* O157:H7 cell bound to an immobilized antibody layer on top of an oscillator. Reprinted with permission from Reference 66.

environment before and after binding of bacterial particles. After measuring the resonant frequency of the antibody-immobilized cantilevers, the cantilevers were dipped into a solution containing *Escherichia coli* (**Figure 8**). After incubating for 15 min, the cantilever was rinsed, and the change in resonant frequency following incubation was determined. The mass sensitivity of the cantilever was 0.2 pg Hz^{-1}. The authors suggested that a single cell could be detected in vacuum, which would improve the resolution of the frequency spectrum. Subsequently, they fabricated much smaller cantilevers and measured a signal change resulting from a single *E. coli.* particle under vacuum conditions (66).

However, viral detection is much more challenging due to the particles' smaller sizes. Craighead and colleagues (67) therefore fabricated thinner cantilevers with a mass sensitivity below the attogram-per-hertz level. They also measured viruses in a vacuum environment to avoid the viscous damping effect of air.

Bashir et al. (68) fabricated 20-30-nm-thick cantilevers with a mass sensitivity of 0.16 ag Hz^{-1} to measure the absolute mass of a *Vaccinia virus* particle, which they determined to be 9.5 fg. In contrast to its impressive mass sensitivity, the ultrathin cantilever had a quality factor of ~5 under atmospheric conditions, which limited its use as a versatile viral sensor. Due to the small size of the cantilevers, this method is not ideal for collecting target particles from low-concentration samples. To bypass these limitations, the authors designed new methods for cantilever fabrication that increased the available surface area. Mass sensitivity was reduced to $\sim 10^{-14}$ g Hz^{-1}, and surface area was increased 1000-fold. Moreover, the cantilever's quality factor improved to ~50 under atmospheric conditions (69).

Another way to increase the detection of scarce targets is to improve the resolution of the frequency spectrum. Instead of using thermal vibration, Gupta's group (70) utilized a piezoelectric ceramic combined with 200-nm-thick cantilevers. The device offered a mass sensitivity of several attograms per hertz and measured the mass of a single virus particle in air. With the same cantilevers, the authors attempted in situ detection of *Bacillus anthracis* Sterne spores in liquid (see **Figure 9**). However, the frequency spectrum deteriorated due to the viscous damping of the liquid, resulting in a quality factor of less than 3 and a minimum detectable number of 50 (71).

3.3.2. Resolution (quality factor) improvement. The two problems associated with nanometer-sized cantilevers, inefficient particle adsorption and poor quality factor in liquid, were addressed

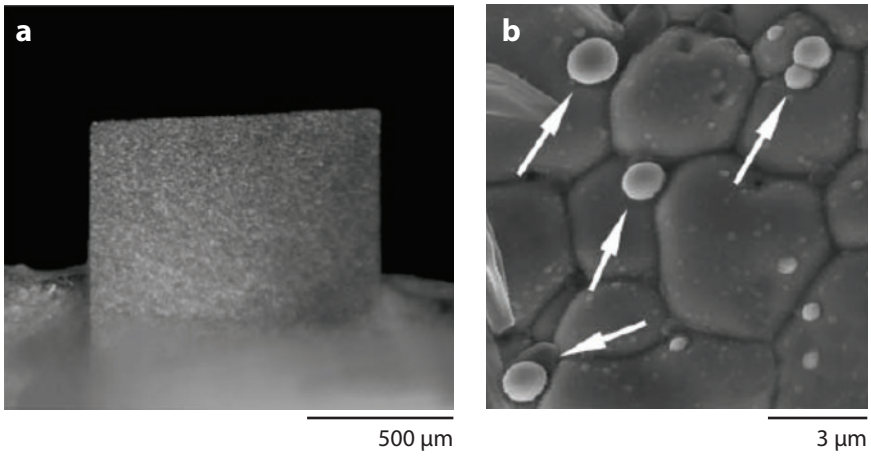


Figure 9

(*a*) Optical image of piezoelectric microcantilever sensor for bioparticle sensing. (*b*) A scanning electron microscope image of *Bacillus anthracis* spores (*indicated by arrows*) captured on the sensor surface. Reprinted with permission from Reference 78.

by two research groups at Drexel University (72–79). Millimeter-sized PZT-integrated cantilevers provided a solution for in situ detection. Mutharasan and Shih's groups (72–79) used piezoelectric films that were greater than 20 μm in thickness; these films improved the quality factor in liquids to 10 and higher. To collect bioparticles in various samples, the researchers built microfluidic circuits that circulated the sample over the cantilevers, as is done in DNA detection. Although the mass sensitivity was in the femtogram-per-hertz range, the authors still quantified tens of copies of spores and bacteria dispersed in several milliliters of liquid. However, millimeter-sized cantilevers were not produced from typical microfabrication processes, so cost effectiveness remains a concern.

Another approach for in situ detection involves integration of a microchannel into a cantilever sensor (see **Figure 4**). Manalis et al. (27, 80) dramatically raised the quality factor of cantilevers up to 15,000. The authors successfully counted bioparticles flowing through the channel while simultaneously calculating the particles' mass. The channel-embedded cantilever provided a powerful tool for the characterization of various particles and for micro-/nanofluidics. However, the requirements of vacuum environment and fluidic control may impede development of quantitative, on-site detection systems.

3.3.3. Other applications. In situ detection of specific bacterial strains has rarely been demonstrated with silicon nitride cantilevers in static mode (81). At least 25 adsorbed bacteria are required for detection (82). Severe acute respiratory syndrome–associated coronavirus has been detected through induced surface stress (83). Anthrax spore models have been detected by higher-mode resonance through the use of commercialized cantilevers (500 μm × 100 μm × 1 μm) that were mounted onto a piezoelectric actuator (84). However, the limit of detection was higher than clinical criteria require, preventing the use of this device in practical applications. In another experiment, researchers monitored the seeding and growth of microorganisms and found that the resonance frequency shifted within a few hours (as measured by dynamically operated cantilever arrays), whereas conventional techniques required several days for such a shift (85, 86).

Tamayo et al. (87, 88) analyzed the effect of bacterial adsorption onto cantilevers and claimed that the cantilevers' response depends on the stiffness of the sample as well as on its mass. The

authors also reported that a stiffer cantilever is relatively insensitive to the mechanical properties of bioparticles but that its mass sensitivity is limited. A pliant cantilever usually has better mass sensitivity, but its response may be complex (89). However, we may be able to use these techniques to obtain information about the material properties of bioparticles.

4. CONCLUSIONS AND OUTLOOK

Micro- and nanocantilever biosensors can be built into various structures ranging in size from the nanometer scale to the millimeter scale. A variety of materials have been utilized for the fabrication of cantilevers. Several types of optical and electrical signal transductions have been reported for diverse applications in vacuum, air, and liquid. Indeed, cantilever biosensors are flexible enough to be used as a common platform for high-throughput detection of proteins, DNA molecules, and bioparticles. This platform can also be customized for use in clinical diagnosis, analytical system development, and environmental monitoring.

Recent reports have demonstrated that both the static mode and the dynamic mode can be successfully used for protein detection. As a result of the high sensitivity of cantilever sensors, they have the potential to be used as label-free diagnostic devices for detection of extremely rare biomarkers. For them to find use as versatile biosensors, advances in surface science must focus on stabilizing the cantilever surface to resolve the issues of functionalization and passivation. An optimized surface should lead to an enhanced mechanical response from specific interactions to enable accurate detection of biomolecules in real blood, serum, and urine samples.

Due to their structural and chemical simplicity, DNA molecules and other oligonucleotides are the most popular models for biophysical studies. Most static cantilever sensors have demonstrated their feasibility as biosensors with target 10mer to 20mer DNA molecules. Due to their clinical importance, SNPs have also been measured. However, the mechanical signal from DNA hybridization is qualitatively different from that the signal from protein interactions. The relation of DNA hybridization's biophysical mechanism to mechanical transduction is not yet clearly understood.

Nanocantilevers have an intrinsically high mass sensitivity and thus have evolved into a powerful tool for the absolute mass detection of bioparticles. In parallel, the resolution of the frequency spectrum must be improved to quantify scarce amounts of viruses or bacteria in liquid samples. Efficient recovery of bioparticles is also important for practical applications. In addition, the integration of microfluidic control will enhance the performance of cantilever sensors.

Most cantilever sensors are label free, highly multiplexed, and capable of being miniaturized. These characteristics are ideally suited for the development of point-of-care (POC) diagnostic/monitoring systems. For this reason, researchers have expended much effort in developing highly sensitive cantilevers. However, interdisciplinary efforts are required to achieve this goal. To compete with commercially available systems, a robust, reliable, and cost-effective method to fabricate micro-/nanocantilevers is necessary. The integration of micro-/nanocantilever biosensors into microfluidic systems is another critical challenge for the realization of a POC system. If chemists and biologists can help develop suitable recognition layers for flexible mechanical biosensors, they will expedite the advent of cantilever-based POC systems and aid in expanding their areas of application.

SUMMARY POINTS

1. Micro- and nanocantilever biosensors measure mechanical response changes arising from specific interactions between biomolecules. The advantages of these biosensors include

label-free detection, high sensitivity, and small size, which will aid in the development of a portable system. A general knowledge of cantilever sensors, including their basic working principle and the technical background, will help researchers evaluate micro-/nanocantilever biosensors as an analytical tool.

2. Research trends in protein detection can be divided into two main areas: disease diagnosis and improvement of sensor performance. With regard to medical applications, micro-/nanocantilever biosensors have been used to quantitatively analyze disease-marker proteins in extremely low concentrations. Moreover, new efforts to improve detection capability have been aided by better hardware design and surface modification.
3. DNA and oligonucleotides have not only been widely used as model biomolecules, they have also contributed to the development of cantilever-based sensing due to their structural simplicity and predictability. The feasibility of using cantilevers as DNA sensors was investigated with 10mer to 20mer target DNA molecules. However, the transduction mechanism of DNA detection, especially in the deflection mode, is still not clearly understood.
4. Most bioparticle-detection experiments have been carried out using resonating cantilever sensors. The high mass sensitivity of nanocantilevers has enabled direct detection of the absolute masses of single bacterial and viral particles. Enhanced quality factor and microfluidic components improved the system's performance, but it must be further improved to be useful for practical environmental monitoring.

FUTURE ISSUES

1. What designs, materials, and fabrications will result in cantilevers with optimal robustness, reliability, and cost effectiveness?
2. For POC and biochemical-analysis systems, how are micro-/nanocantilever biosensors integrated into the total-analysis system (including sample preprocessing, preparation, and delivery)? What microfluidic system will highlight the advantages of cantilever sensors, such as their sensitivity, their ability to be miniaturized, and the fact that they are label free?
3. With recent advances in surface science, how can sensor surfaces be optimized to enhance the mechanical responses caused by specific interactions while inhibiting nonspecific adsorption?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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