Sensors and Actuators B 134 (2008) 613–617

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Specific detection of proteins using nanomechanical resonators

L.M. Fischer[a,c], V.A. Wright[b,c], C. Guthy[a,c], N. Yang[b,c], M.T. McDermott[b,c], J.M. Buriak[b,c], S. Evoy[a,c,*]

[a] Department of Electrical and Computer Engineering, University of Alberta, Edmonton, Alberta T6G 2V4, Canada
[b] Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada
[c] National Institute for Nanotechnology, Edmonton, Alberta T6G 2M9, Canada

ARTICLE INFO

Article history:
Received 20 February 2008
Received in revised form 2 June 2008
Accepted 3 June 2008
Available online 12 June 2008

Keywords:
Sensor
Chemical sensor
Protein
MEMS
NEMS

ABSTRACT

Rapid, sensitive and inexpensive analysis of biological molecules is vital to many fundamental problems in molecular biology. Nanomechanical resonators are of great interest for such applications. The use of these devices for the analysis of protein mixtures would however require the immobilization of probes onto their surfaces in order to enable the specificity of the detection. Such nanoresonator-based specific detection of proteins is here reported using streptavidin as target system, and immobilized biotin as probe. Nanomechanical resonators resistant to stiction were first realized from silicon carbonitride using a novel fabrication method. Vapor-phase deposition of mercaptopropyl trimethoxysilane was performed, and an added mass of $2.22 \pm 0.07\,fg/\mu m^2$ was measured. This linker molecule was used to attach biotin onto the devices, enabling the specific detection of streptavidin. A mass of $3.6\,fg/\mu m^2$ was attributed to the added streptavidin, corresponding to one molecule per $27\,nm^2$. The specificity of this recognition was confirmed by exposing the devices to a solution of streptavidin that was already saturated with biotin. An additional negative control was also performed by also exposing bare devices to streptavidin in absence of the attached biotin. No resonance frequency shift was observed in either case, confirming the specificity of the detection.

1. Introduction

Rapid, sensitive and inexpensive identification and analysis of biological molecules is vital to disease detection and monitoring, drug discovery, and to many fundamental problems in molecular biology. For instance, the detection, identification, and analysis of proteins such as biological markers of diseases such as inborn metabolic disorders, multiple sclerosis, cancer, or microbial infections have gained considerable attention over the recent years. The identification and detection of such proteins at very low concentrations would greatly facilitate disease diagnostic and help predict disease progression. Current analytic technologies such as DNA microarray, mass spectrometry and nuclear magnetic resonance spectroscopy (for genomic, proteomic and metabolomic determinations) are too expensive and technically challenging to envision potential clinical applications. Development of low-cost and highly sensitive transducers able to detect and analyze such molecular systems has therefore been a subject of extensive research efforts over the recent years.

Micromechanical resonators have specifically been demonstrated as highly sensitive transducers for the detection of molecular systems. In the mid-1990s, Thundat et al. reported the detection of humidity [1] and mercury vapor [2] by measuring both the shift in resonance frequency of a cantilever due to the added mass, as well as the static deflection due to surface adsorption. Recently, Ilic et al. have used the frequency shift of resonant microcantilevers to detect the attachment of viruses [3] and DNA molecules [4]. Similarly, Hwang et al. demonstrated the *in-situ* dynamic sensing of a prostate-specific antigen using a self-actuating cantilever incorporating a lead zirconium titanate (PZT) layer [5].

The sensitivity of mechanical resonators scales favorably as their dimensions are reduced, offering a compelling path for the development of sensors offering exceptional mass sensitivities. While the single-Dalton range ($\sim 10^{-24}$ g) has been proposed as ultimate limit of nanoresonator-based detection [6], zeptogram-levels (10^{-18} g) detection of masses has recently been demonstrated [7]. In addition, nanomechanical resonators can operate at frequencies now reaching the gigahertz range [8]. Such higher frequencies offer additional advantages for mass sensing by providing a wider

* Corresponding author at: Department of Electrical and Computer Engineering, University of Alberta, W2-085 ECERF, 9107 - 116th St., Edmonton, Alberta T6G 2V4, Canada. Tel.: +1 780 492 5866; fax: +1 780 492 1811.
E-mail address: evoy@ece.ualberta.ca (S. Evoy).

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doi:10.1016/j.snb.2008.06.003

dynamic range and faster response times. Finally, the inherently small dimension of these devices is amenable to their implementation into large arrays, enabling the realization of multiplexed binding assays that could quantify complex mixtures with high throughput.

Recently, Li et al. demonstrated room temperature, real-time detection of chemisorbed species on nanometer scale VHF resonators with attogram resolution [9]. However, such reports of the use of nanomechanical resonators as mass-sensors have mostly involved the non-specific attachment and detection of species onto the surface [7,10]. The deployment of such devices for the detection and analysis of protein mixtures would require the immobilization of probes that will enable such specificity. We here report such specific nanoresonator-based detection of proteins using streptavidin as target system, and biotin as the recognition probe.

We previously reported the synthesis and optimization of SiCN for the fabrication of nanomechanical resonators [11]. Post-deposition thermal anneal at $T = 400$–$700\,^{\circ}C$ was found to drive out incorporated hydrogen, allowing control over stress from the compressive to the tensile range. As reported by Verbridge et al. [12,13], inclusion of tensile stress in the resonators significantly increased their resonant qualities. The work presented here leverages this tensile stress for the optimization of sensitivity, as well as for the fabrication of resonators that are resistant to stiction, a key feature for applications requiring repeated immersion in liquids.

We first report a nanofabrication method combining electron beam lithography and bulk micromachining to produce nanomechanical resonators able to withstand subsequent wet chemistry and drying. These devices were then exposed to vapor mercaptopropyl trimethoxysilane (MPTMS) for 10 h. The added mass of this linker molecule was measured by observing the induced shift of resonant frequency. This linker molecule was then used to attach biotin onto the device surface, enabling the specific detection of streptavidin. The specificity of this recognition was then confirmed by exposing the biotinylated devices to streptavidin that was already saturated with biotin. An additional negative control was also performed by exposing bare devices to streptavidin in absence of the attached biotin.

2. Experimental

A 35 nm layer of SiCN was deposited via plasma-enhanced chemical vapor deposition (PECVD) at a gas ratio of 4:1 NH_3:DES onto piranha cleaned (3:1 H_2SO_4:H_2O_2) single-crystal (1 0 0) silicon wafers (500 μm thick and 100 mm diameter). The SiCN-coated wafers were then annealed in a MiniBrute 3-zone tube furnace at 500 C for 8 h to drive off hydrogen and maximize tensile stress. Additional details on the synthesis of this material are found in Ref. [11]. The wafers were then treated with another piranha clean, and a buffered oxide etch bath (BOE, 10:1 HF:NH_4F) in preparation for resist application. These wafers were cleaved into 1.5 cm × 1.5 cm squares, and spin-coated with a bilayer of polymethyl methacrylate (PMMA) of molecular weights of 950 and 495, respectively. Patterning of the substrates was performed at the University of Alberta Micromachining and Nanofabrication Facility using a Raith 150 electron-beam lithography system using a 20-μm aperture, an accelerating voltage of 10 kV, and an areal dose of 125 μC/cm^2. Following development of the PMMA in 1:3 MIBK:IPA, an electron-beam evaporator was used to deposit a 30 nm chromium masking layer. This chrome layer was lifted off by immersing the samples in acetone for 5 min under ultrasonic agitation. A Trion Technology Phantom II reactive ion etcher (RIE) was used to anisotropically etch the Cr-patterned SiCN for 30 s, using a 4:1 SF_6:O_2 plasma recipe adapted from literature [14]. Isopropyl alcohol saturated with potassium hydroxide (35%) was then used to etch the bulk silicon and release the nanostructures. Following a 5–10 min immersion in KOH, the samples were rinsed in DI water and dried under a gentle nitrogen flow. The devices did not require supercritical drying or other post-release treatments to prevent stiction. Finally, the resonators were subjected to a final piranha-cleaning step to populate the SiCN surface with silanol groups. Electron microscopy of the fabricated devices was performed in a Hitachi S-4800 Cold Field Emission Scanning Electron Microscope.

A previously reported interferometeric technique was used to assay the resonant frequencies of the devices [15]. For this purpose, the samples were affixed to a piezoelectric stage mounted inside a vacuum chamber which was pumped to $\sim 10^{-3}$ Torr. The piezoelectric element is then actuated by the tracking output of a Hewlett Packard ESA-L1500A spectrum analyzer. A He-Ne laser ($\lambda = 633$ nm) is focused to a beam spot of ~5 μm diameter using a 0.35 NA microscope objective. When actuated at resonance, relative motion of the structure with respect to the underlying substrate modulates the reflected signal through interferometric effects [15]. The modulated signal is reflected back through the microscope objective. A beam splitter is then employed to divert the reflected signal towards a New Focus 1601 AC coupled photodetector, whose output is fed to the input of the spectrum analyzer.

One set of resonators was subjected to resonance assaying, then placed inside a vacuum desiccator with an open vial containing 100 uL of MPTMS (95% pure, Sigma–Aldrich, Inc.). The chamber was pumped to 200 mTorr and left sealed for 10 h. The resonators were measured again following this silanization to observe the frequency shift resulting from the added mass of the MPTMS.

A second set of resonators was initially measured, and subjected to the silanization procedure described above for 10 h. The sample was subsequently placed in a 0.5 mL solution of 2 mg/mL biotin-HPDP mixed with dimethyl sulfoxide, then mixed with 0.5 mL of phosphate buffered saline, incubated for 30 min, and then rinsed again. Following attachment of the biotin, the sample was then placed in a 10 μM solution of streptavidin for 30 min, and rinsed a final time. A detailed description of this protocol is outlined by Janshoff et al. [16]. The resonators were measured again to assess the frequency shift associated to the binding of streptavidin.

Two negative control experiments were performed to confirm the specificity of the detection. A first set of bare resonators (i.e. without the MTPMS linker or biotin) was placed in the streptavidin solution for 60 min at $T = 37\,^{\circ}C$ to assess any non-specific binding of the protein. A second set of biotinylated resonators was also exposed to a solution of 10 μM streptavidin (0.5 mL) mixed with a 1 mM biotin-HPDP solution (0.5 mL). Such mixture provides 100 times excess of biotin for the saturation of the four binding sites of streptavidin. Such saturation was expected to block the specific attachment of the streptavidin onto the surface-immobilized biotin. Both sets of samples were then rinsed with toluene, trichloromethane, and ethanol, gently blown dry with nitrogen, and measured again to assess any shift of resonant frequency.

3. Results and discussion

The resonators produced in our previous report were fabricated using a sacrificial oxide surface nanomachining technique [11]. These devices required critical point drying to prevent their stiction upon drying. However, we desire to avoid critical point drying, as it is known to substantially contaminate surfaces. Increasing the thickness of sacrificial oxide would help raising the gap between the substrate and the device, thus helping preventing stiction. Such approach would however increase the extent of the undercut and