Bioresistive identification of heat shock protein 90

Arvind Chandrasekaran, Shanmugasundaram Pakkiriswami, Jian Liang You, Ashwin Acharya, Muthukumaran Packirisamy, and Denis Maxwell

Citation: Biomicrofluidics **2**, 034102 (2008); doi: 10.1063/1.2963104
View online: http://dx.doi.org/10.1063/1.2963104
View Table of Contents: http://scitation.aip.org/content/aip/journal/bmf/2/3?ver=pdfcov
Published by the AIP Publishing

Bioresistive identification of heat shock protein 90

Arvind Chandrasekaran,[1,a)] Shanmugasundaram Pakkiriswami,[2] Jian Liang You,[1] Ashwin Acharya,[1] Muthukumaran Packirisamy,[1] and Denis Maxwell[2]
[1]*Optical Microsystems Laboratory, Department of Mechanical Engineering, Concordia University, Montreal, Quebec H3G2W1, Canada*
[2]*Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada*

(Received 23 April 2008; accepted 27 June 2008; published online 10 July 2008)

90 kDa heat shock protein (HSP90) is a ubiquitous molecular chaperone and is one of the abundant proteins present in a cell under normal and stressed conditions. The adenosine triphosphate (ATP) binding region of HSP90 is currently under a great degree of study because of the interest of its role in cancer and protein maintenance; the binding of ATP to HSP90 induces a large conformational change in the protein as a result of the activity of different types of stressors within the cells. In the present paper, a simple microfluidic biosensor is proposed for the characterization of ATP-HSP90 interactions through the principle of bioresistive variation. The experimental results prove that the present biosensor system is highly suitable for the detection of heat shock proteins present in a real-time biological sample, which is very useful for *in-situ* biomedical applications and rapid pathogenic detections. © *2008 American Institute of Physics.* [DOI: 10.1063/1.2963104]

I. INTRODUCTION

The study of protein level interactions is of significant importance in early medical diagnoses used for preventive medicine. This creates a need to develop a reliable point-of-care (POC) testing facility, which calls for the synthesis of a miniaturized, portable, and disposable biosensor device with increased sensitivity and the capacity for high throughput diagnosis. Biophysical label-free methods and microelectromechanical systems (MEMS) based biosensors offer a lot of potential and interesting possibilities for the detection of various biomolecular interactions, which have been utilized for DNA-DNA hybridization, protein-protein/ligand interaction, and antigen-antibody binding.[1] While structural level biological studies are limited, cellular and molecular level research reveals better insight into the microscopic behavior and biological phenomena associated with biomolecules. In the present study, a bioresistive sensing system is employed to detect the structural/conformational change produced by heat shock protein 90 (HSP90) when it binds to adenosine triphosphate (ATP).[2]

HSP90, along with its other members of the family, serve as central integrators of protein homeostasis within the cell. It is a ubiquitous molecular chaperone and exists in cells under both normal and stress conditions.[3] The proteins are critical to maintaining the normal protein-folding environment and their increased expression enhances cell survival in tissues damaged by a variety of stressors, including heat, heavy metals, and acidosis.[4] The latter conditions are also common within the tumors and the increased expression of chaperone proteins in several types of tumors reflects the ability of cancerous cells to maintain homeostasis in a hostile environment.[5] Thus, the conformational changes induced in the protein acts as an indicator of the stress level of the cell. The HSP90 consists of three domains, namely ATP-binding *N*-terminal domain (*N*), a middle

[a)]Author to whom correspondence should be addressed. Telephone: (514) 848-2424 (ext. 7098). Electronic address: achandra@alcor.concordia.ca.

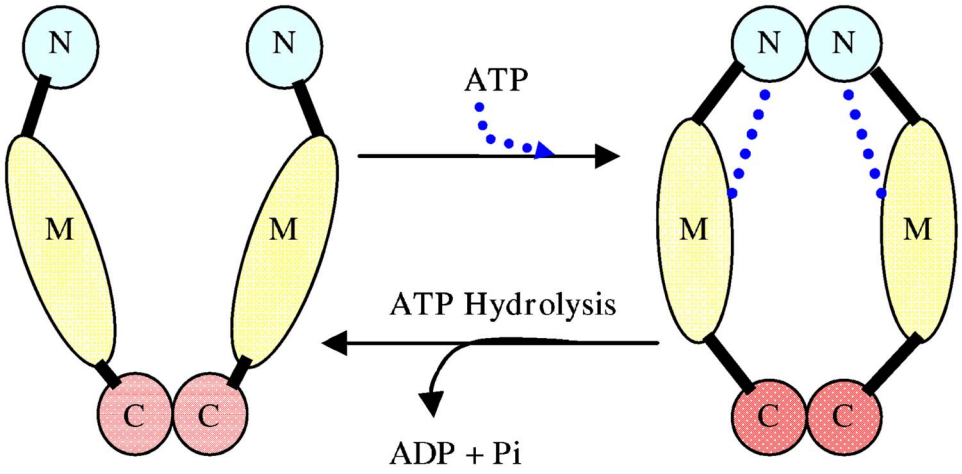

FIG. 1. Schematic of the current model for the conformational changes that accompany binding and hydrolysis of ATP by HSP90.

domain (*M*), and a *C*-terminal dimerization domain (*C*).[6] Figure 1 shows the structural variations that accompany the binding and hydrolysis of ATP with HSP90, wherein the binding of ATP stabilizes a "tense" conformational and functional state of HSP90, and subsequent hydrolysis to ATP destabilizes that conformation and allows the HSP90 dimer to regain its original shape.[7]

HSP90 utilizes the conformational change introduced by the ATP binding to assist in protein folding, inhibition of improper protein aggregation, assembly and disassembly of protein complexes, intraorganellar protein transport and proteolytic turnover of many key regulators of cell growth, differentiation, and survival.[7] Considerable research over the years developed a greater understanding of the HSP90-ATP interaction and is potentially used to develop HSP90 inhibitors for cancer chemotherapy.[8] Therefore the HSP90-ATP interaction would serve as an excellent model to study a protein-ligand interaction and, moreover, the interaction is very specific and can be well characterized for the purpose. By studying the structural amendment of the protein, one could decipher the stress variation undergone by the cell, which would be an indicator of the activity of the stressors. Hence, it is essential to devise suitable techniques by which one could study the ATP-induced conformational variation in the heat shock protein.

Herein, a simple and cost effective microfluidic biosensor system is proposed for the detection and characterization of the bioresistivity changes induced by the structural variations in the protein due to HSP90-ATP interactions. The following sections of this paper give a detailed description of the biosensing scheme, the experimental procedures employed, and the results of the characterization of protein-ATP interactions using the proposed device.

II. BIOSENSING SCHEME

The main principle of the proposed biosensing system is the measurement of the bioresistance of HSP90 and the stress-induced resistance variation due to the interaction of these proteins with ATP. The schematic for the proposed microfluidic biosensor system for the characterization of the HSP90-ATP interaction is as shown in Fig. 2. An electrode is deposited and patterned on a bottom glass substrate which forms the microfluidic channels. A transparent cover is used to seal the channel from the top; tubes and interconnections are integrated with the top cover, which acts as fluidic inlet and outlet ports for the chip.[9] The fabrication process flow is schematically depicted in Fig. 2(a) and the chip design is as schematically shown in Fig. 2(b).

However, fully packaged microfluidic chips have some inherent disadvantages due to the limitations of fluidic control and handling within the microfluidic channels, and also the difficulty of qualitative study of protein-folding kinetics using scanning electron microscopy (SEM). Therefore, in order to overcome the above-mentioned difficulties, an open-top microfluidic channel[10]

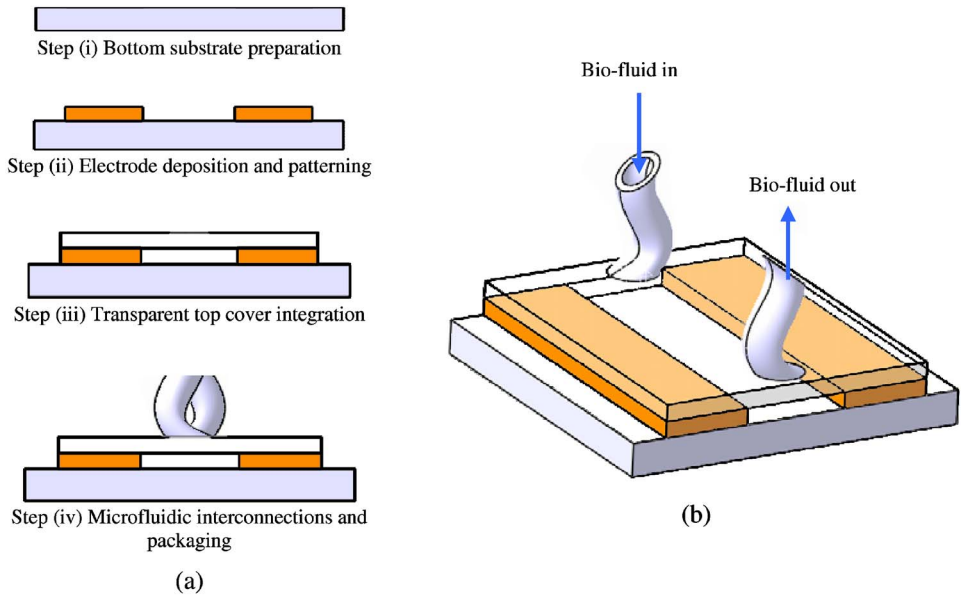

FIG. 2. Schematic representation of the (a) microfluidic chip fabrication flow and (b) fully packaged microfluidic chip.

setup was used for the present work, so as to enable more precise sample volume handling and also to carry out SEM analysis of the structural amendments in the HSP, ATP, and the interaction mixture subsequently. For the present application, both open and closed microfluidic channels are expected to give similar results. The effect of fluid evaporation from the open-top microfluidic channels has also been taken into consideration, which is subsequently discussed in Sec. IV.

The fabrication procedure employed for the open-top microfluidic channel is the same as schematically represented in Fig. 2(a) without the necessity to include steps (iii) and (iv) in the process. The present biosensing setup consists of an open-top microfluidic channel on glass substrate, 80 μm wide (δ) and 100 μm deep (h), with copper electrodes acting as its side walls, as shown in Fig. 3.

Herein, the biological specimen forms the dielectric medium in the electrode gap across the fluidic channel, as schematically depicted in Fig. 3. In order to measure the voltage variation across the electrodes, a data acquisition (DAQ) system was used. During the interaction of heat shock proteins with ATP, a structural transformation, as described before, is induced in the pro-

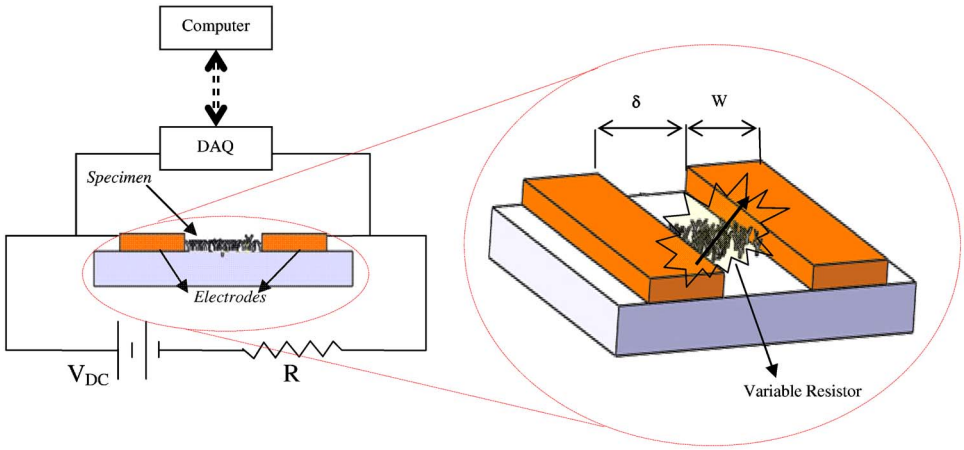

FIG. 3. Schematic representation of the proposed biosensing system.

TABLE I. Specifications of electrodes.

Parameter	Specification
δ	80 μm
h	100 μm
W	3 mm
V_{dc}	0.5 V
R	2 MΩ

teins. When placed between two electrodes, this phenomenon results in the change of electrical resistance offered by the biological specimen, or the bioresistance, in the circuit. Therefore, the HSP-ATP pair acts as a variable resistor during the course of their interaction. If this change in the bioresistance can be measured by studying the voltage variation across the electrodes, it is possible to characterize the stress variations undergone by the protein during the interaction. Thus, using this method, it is possible to quantify and measure the interaction of HSP with the ATP through bioresistive variation.

Apart from the bioresistivity of the protein, the factors on which the voltage output is dependent upon are the width of the microfluidic channel between the electrodes, the geometry of the electrodes, and the type of electrodes used. The above-mentioned parameters were characterized by repeated experiments in order to obtain clear consistent signals and were selected accordingly. The specifications of the biosensor system used in the present work are as given in Table I.

III. EXPERIMENT

The HSP90 and other chemicals used in the experiments were obtained from Sigma. The initial concentrated stock of HSP90 (2 μg/μl) was made with the 50 mM HEPES buffer *p*H 7.4. Different dilutions of HSP90 were prepared using the buffer to perform the experiments and the corresponding concentration is given in Table II. The working concentration of 0.5 mM of ATP and 10 mM $MgCl_2$ was maintained throughout the experiments.

The experimental setup is shown in Fig. 4. A function generator was used to provide dc power output, and the measuring circuits composed of an array of electrodes across the microchannel, resistors, and a DAQ board with a USB cable for I/O communication with the host computer. The time-varying electrical potential through bioresistance variations in the microchannel across the electrodes is transmitted to the computer in real time, which can thus be clearly monitored and recorded.

Calibration of the circuit was carried before the experiments. Since the variable range of the electrical potential within the microchannel is in the order of millivoltage, it was required to set the whole potential in the range of hundreds of millivolts using the circuits seen in Fig. 4. Again, in order to avoid the disturbance of small electrical noise from the utilities, the total voltage applied on the circuits was made to drop in the range of hundreds of millivoltages. Connection of a DAQ board to the electrode array was performed according to the requirements. By switching on

TABLE II. Concentration of HSP in terms of its dilution with buffer.

Ratio of HSP dilution	HSP concentration, β (μg/μl)
1:1	2
1:2	0.66
1:3	0.5
1:5	0.33
1:10	0.182
1:20	0.095

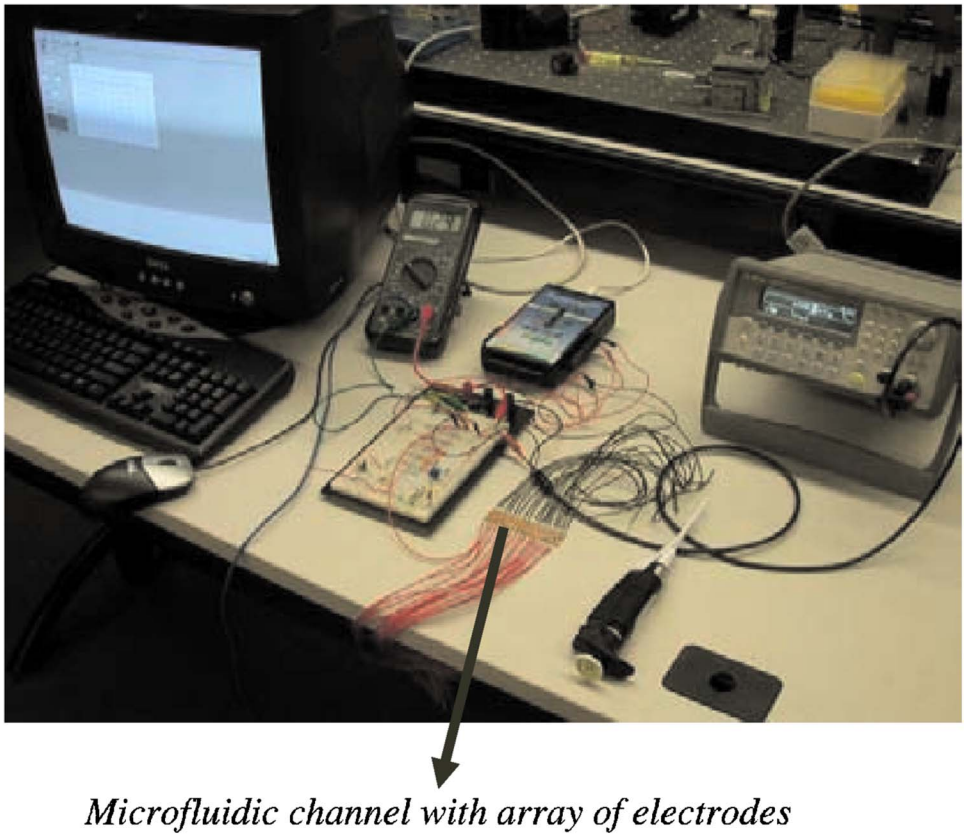

FIG. 4. Experimental setup for bioresistive detection of a protein-ATP interaction.

the right channel for a certain pair of electrodes, the process was started and the measurements were recorded. Precise volumes of HSP90 and ATP were taken in a micropipette and introduced into the microchannels across the electrodes. Once the readings were recorded, the biological specimens were rinsed with deionized water. This procedure was repeated for obtaining consistent results.

IV. RESULTS

In the initial experiment, 1 μl of ATP was added in the microfluidic channel separately and the voltage variations were recorded. The channels were cleaned thoroughly after the measurements were recorded. The procedure was repeated for HSP90 and the results are as shown in Fig. 5. It can be seen that there is absolutely no influence of ATP to cause the voltage variations across the electrodes, as seen in Fig. 5(a). However, variation in the voltage can be observed due to the presence of HSP only, caused purely by the conformational transition of the protein under the applied voltage.

In order to study the effect of interaction of the protein and the substrate, in the next experiment, 0.5 μl ATP was passed in the microfluidic channel, 0.5 μl HSP was then added to the substrate, and the effect of biointeraction on the voltage variation between the electrodes was observed. The experiment was repeated for different concentrations of the protein.

Figure 6 shows the plot of a time-dependent variation of the potential drop owing to the bioresistive nature of the conformational changes in HSP90 due to its interaction with ATP under different concentrations of the protein. For clarity, the output voltage is normalized with respect to the input. The initial voltage drops are due to the addition of ATP and HSP90, while the gradual drop in voltage after the interaction of ATP and HSP90 is due to the gradual conformational

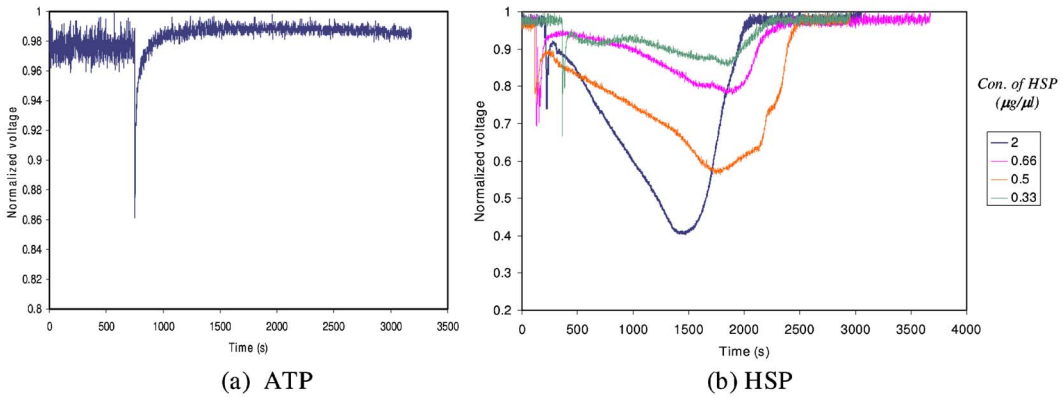

FIG. 5. Plot of a time-dependent variation of normalized voltage due to (a) ATP and (b) HSP only.

changes in the HSP90 due to the interaction. Further increase in voltage is due to the evaporation of the samples. The trend of variation obtained from the interaction study is schematically represented in Fig. 7 for discussion.

The variation in voltage measurements begin at I (as indicated in Fig. 7) when ATP is passed between the electrodes. This sudden decrease in the voltage is due to the random disturbance of the dielectric layer between the electrodes caused by the addition of a biological element. However, after stabilization, the voltage increases and remains constant for some time, with δ_1 being the drop-in voltage due to the bioresistance of ATP. The addition of HSP90 at II causes a decrease in the voltage again. But once the voltage value is stabilized, δ_2 is the total drop in voltage after the addition of HSP90. Thereafter, the further drop in voltage to δ_3 is due to the change in resistance between the electrodes as a result of ATP-HSP90 interactions. The time indicated as τ_i in Fig. 7 is the measure of the time period of interaction between the ATP and the protein for the given sensor configuration. After τ_i, the voltage increases back to the initial value in time τ_e due to the evaporation of the specimens from the dielectric region between the electrodes. Thus, the initial part of the total interaction time influenced mostly by the conformational change is the

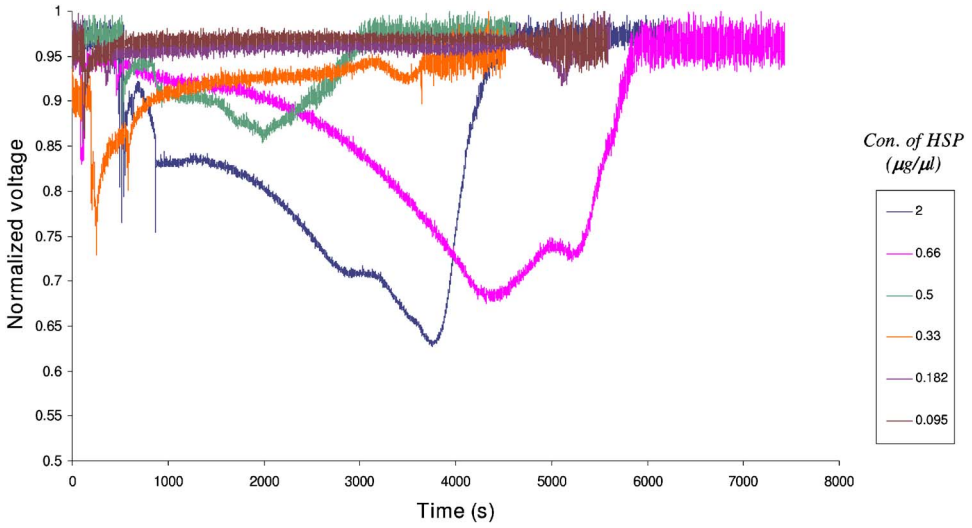

FIG. 6. Plot of a time-dependent variation of normalized voltage due to protein-ATP interaction

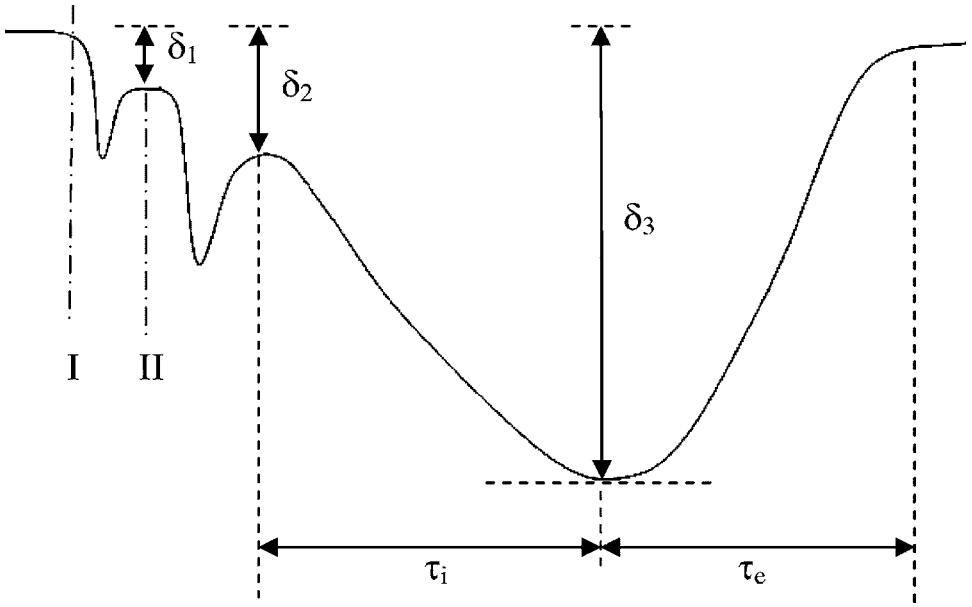

FIG. 7. Schematic variation of normalized voltage due to protein-ATP interaction.

protein structure due to the ATP interaction, while the latter part of the interaction time, denoted as evaporation time τ_e, is due to samples evaporation. The total interaction time $(\tau_i+\tau_e)$, would thus be dependent upon the concentration and the amount of samples used.

This variation in voltage due to the HSP-ATP interaction, as seen in Fig. 6, is of a similar trend to the voltage variation observed from the previous experiment due to HSP alone, shown in Fig. 5(b). However, there exists a crucial difference between the protein behavior under a particular applied voltage, in the presence of ATP and otherwise. The characteristics of the protein interaction with ATP were analyzed with respect to the time of interaction between the HSP and ATP. In order to identify only the influence of conformational changes on bioresistive property, a normalized interaction time (τ_n) is defined as

$$\tau_n = \tau_i/(\tau_i + \tau_e). \tag{1}$$

The results of this analysis have been plotted in Fig. 8, wherein the normalized interaction time is plotted with respect to the concentration of HSP (β) for the interaction of the protein with ATP and without the substrate, respectively.

In the case of protein interaction with ATP, it can be observed that at a lower concentration of the HSP, the normalized interaction time (τ_n) is lower, which indicates that the time of interaction is much less than the time of evaporation of the fluids, thereby indicating little reaction. As the concentration of the HSP is increased, the relative time of interaction increases, thereby proving that a definite interaction between HSP and ATP occurs, and that the HSP90-ATP interaction is strongly dependent upon the concentration of the individual species. However, in the case of voltage measurements using only proteins without the ATP, there is very little variation in the normalized interaction time due to the change in the protein concentration, which indicates the absence of the structural transformation of the protein that would have been caused by ATP interaction. τ_n is zero for ATP taken alone. Thus, one could quantify the interaction between HSP90 and ATP, and hence the conformational changes through the normalized interaction time τ_n.

From the results of the biointeraction based studies, δ_3 (with reference to Fig. 7), which is the maximum voltage variation during the interaction, was plotted for different concentrations of HSP in ATP, as shown in Fig. 9. This indicates clearly that the reaction between the HSP90 and ATP depends upon the concentration of HSP present in the sample. Thus, the proposed biosensor

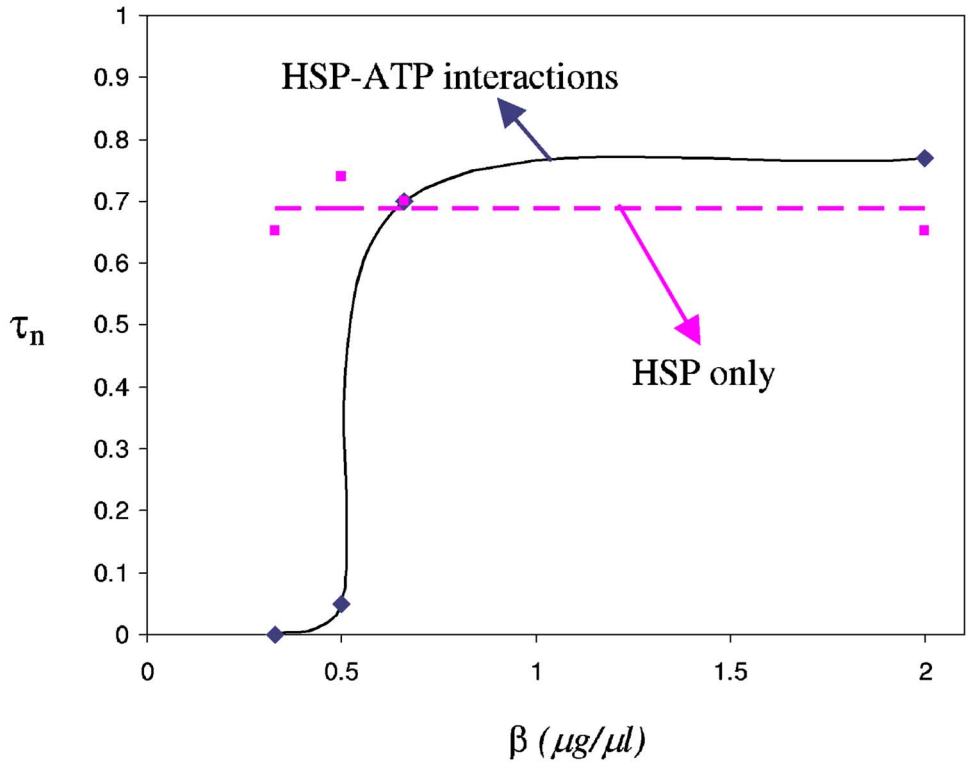

FIG. 8. Plot of the normalized interaction time of HSP in the dielectric zone with ATP and without ATP.

responds very well to the reaction and the response is maximum when the concentration of HSP90 in a sample volume is high, which is very essential for all practical applications of this biosensor.

In order to qualitatively study the structure of HSP before and after the interaction, the specimens were observed under a scanning electron microscope (SEM). The SEM graphs of the protein before and after interactions are given in Fig. 10. The size of the ATP molecules, which is smaller than the electrode gap across the microfluidic channel, could result in forming no microbridge when added in between the electrodes. But, the structure of the protein mixture is quite different before and after its interaction with ATP, as shown in Figs. 10(b) and 10(c), which demonstrates the difference in the variation of τ_n as observed in Fig. 8.

It is assumed that HSP90 forms microbridges between the electrodes, as seen in Fig. 10(b). When the protein undergoes structural changes due to its interaction with ATP, the electrical properties of the bridges vary leading to the variation in potential drop across the electrodes. The conformational change is a time-dependent phenomenon, also dependent of the HSP90 concentration, and the number of microbridges formed between the electrodes would increase with the concentration of HSP90. Hence, one could anticipate a greater magnitude of drop-in output voltage with an increase in the HSP concentration, as indicated by δ_3 in Fig. 9. But, the interaction time, as indicated by τ_i in Fig. 7, which is the time taken for the maximum change in output voltage, will only be related to the time required for the conformational changes to occur. At lower concentrations of HSP, the interaction between the protein and the ATP is minimal, thereby causing no change in the measured output due to the interaction-induced bioresistance variations. For higher concentration of the protein in the buffer, it is therefore evident that the measured output voltage is purely due to the conformational changes in the structure of the protein caused by its interaction with ATP.

Thus, the performance of the proposed biosensor system has been demonstrated through bioresistive measurements offered by the conformational variation produced in the heat shock protein 90 upon their interaction with ATP. The qualitative study of the protein-ATP interaction,

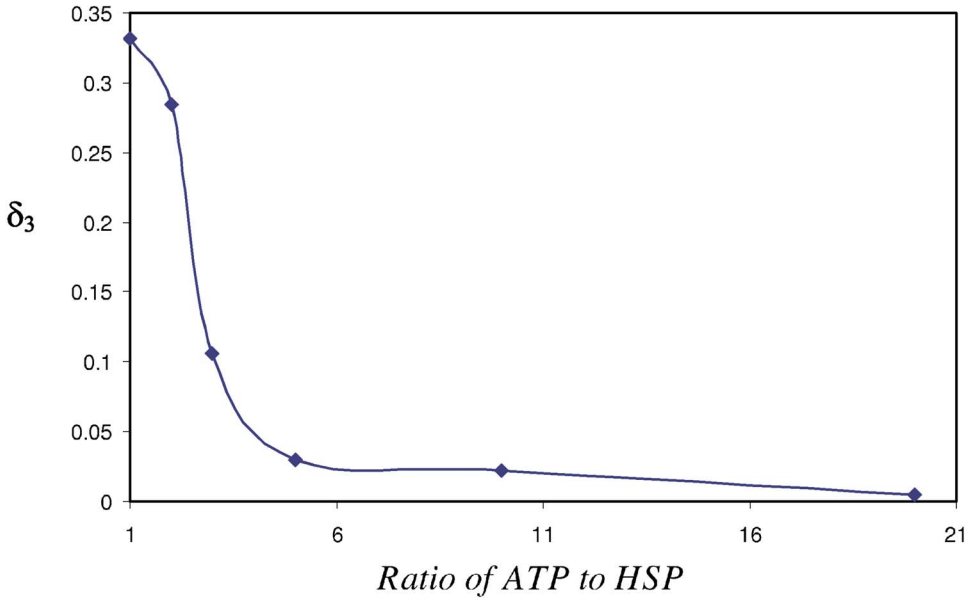

FIG. 9. Plot of variation of δ_3 with a volumetric ATP:HSP ratio.

carried out using the scanning electron micrograph method, corroborates with the concept of bioresistive variation which was quantitatively measured through the normalized interaction period (τ_n).

V. CONCLUSION

Understanding the behavior of heat shock proteins is very essential in order to study the stressor level present in a cell, through the conformational variations undergone by the protein due to its interaction with ATP. In the present work, a simple and cost-effective method has been proposed for the detection and characterization of HSP90 in a real-time biological sample. The main objective of the above experiments is to carry out a qualitative analysis of the conformational changes induced in the protein due to its interaction with the ATP. The proposed variable called the normalized interaction time shows a promise for quantifying the conformational changes due to the interaction of the protein and ATP. Though the present system provides a qualitative analysis and a proof of concept to the study of bioresistive variations, this biosensor system now serves as a platform for further extension of this work, which would include the development of a complete lab-on-a-chip–type biosensor system for carrying out biosensing in a more comprehensive and quantitative manner.

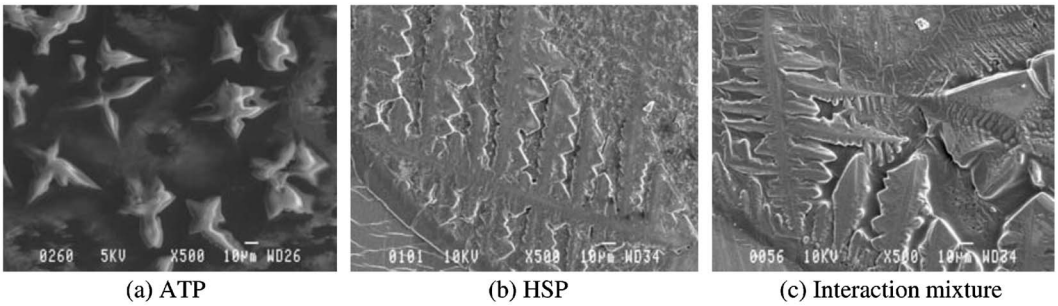

FIG. 10. SEM of (a) ATP, (b) HSP90, and (c) the HSP90-ATP interaction mixture over the electrode gap.

ACKNOWLEDGMENTS

The authors would sincerely like to thank Eric Duchesne of Ecole Polytechnique de Montreal for his assistance with scanning electron microscopy.

[1] J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. J. Güntherodt, C. Gerber, and J. K. Gimzewski, Science **288**, 316 (2000).
[2] V. M. Jakob, Columbia Sci. Rev. **3**, 20 (2006).
[3] G. Nardai, T. Schneider, C. Soti, M. Ryan, P. Hoj, J. Somogyi, and P. Csermeley, J. Biosci. **21**, 179 (1996).
[4] R. Bagatell and L. Whitesell, Mol. Cancer Ther. **3**, 1021 (2004).
[5] P. Workman, Cancer Lett. **206**, 149 (2004).
[6] K. Terasawa, M. Minami, and Y. Minami, J. Biochem. **137**, 443 (2005).
[7] L. H. Pearl and C. Prodromou, Annu. Rev. Biochem. **75**, 271 (2006).
[8] Y. Miyata, Folia Pharmacologica Japonica **121**, 33 (2003).
[9] A. Chandrasekaran, A. Acharya, J. L. You, K. Y. Soo, M. Packirisamy, I. Stiharu, and A. Darveau, Sensors **7**, 1901 (2007).
[10] Y. C. Lo, C. S. Huang, W. Hsu, and C. Wang, in *Proceedings of the International Conference on MEMS, NANO and Smart Systems (ICMENS'04)* (IEEE Computer Society, Washington, D.C., 2004), pp. 671–674.