

## **ACOUSTIC WAVE SENSOR FOR INVESTIGATING THE INFLUENCE OF PLANETARY CONDITIONS ON LIFE PROCESSES SUCH AS GENE TRANSCRIPTION**

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### **ABSTRACT**

In considering the scientific and technical aspects of Mars exploration and settlement, it is necessary to develop efficient experimental techniques to study the effect of environmental conditions on fundamental biological processes such as gene transcription, expression and cell differentiation. We present the development of an on-line sensor based on the thickness-shear mode (TSM) acoustic wave device for monitoring transcription initiation in order to study the physical and chemical nature of the transcription machinery. In transcription, the enzyme RNA polymerase must not only bind specifically to its promoter DNA sequence, but must also initiate the catalytic process of template dependent RNA synthesis. Environmental cues such as gravity, light and radiation can control transcription of certain genes and transcription factors at the initiation stage. The network analysis of impedance measurements allows for characterization of TSM sensors upon binding of RNA polymerase to template DNA immobilized onto a gold electrode surface. The TSM device offers several advantages in being able to detect a series of events such as adsorption, hybridization and binding on the same sensor surface. Since signaling is direct for the TSM device, no labeling agents are required. In addition, the TSM sensor is capable of supplying multi-dimensional information. Another application of this sensor technology in the context of Mars exploration could be to perform preliminary experiments on samples from Mars or polar regions on Earth that are most 'Mars like'.

### **INTRODUCTION**

In "Entering space: creating a space faring civilization", author Dr. Robert Zubrin writes that, "the most important question about the Red Planet is not "Was there life on Mars" or even "Is there life on Mars?", but "Will there be life on Mars?" Can humans go and establish a new branch of human civilization on a new world and become a multi-planet species? This, Mars' most important question can only be answered by human pioneers"<sup>1</sup>. This challenging endeavor will require pioneers to Mars, as well as scientists and engineers working with state of the art technology on Earth.

An important consideration for long term space travel and exploration will be how different planetary conditions affect fundamental life processes. These considerations are also important for us to understand the possible life forms that may exist in other worlds such as

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Mars. It is therefore necessary to develop efficient experimental techniques to study the effect of environmental conditions on fundamental biological processes such as gene transcription, expression and cell differentiation. Environmental cues such as gravity, light and radiation have been known to control the transcription of certain genes at the initiation stage.

Pioneering experiments have been carried out to detect the effects of radiation, acceleration, vibration and weightlessness on living cells in the context of space exploration. A wide range of cell types has been subjected to space flight where the cells have exhibited altered behavior compared to ground based samples<sup>2</sup>. Other than a changing gravitational field (high hypergravity at launch and re-entry; microgravity at other times), strong vibration, acoustic levels and high-energy radiation can also affect biological mechanisms during space flight. Consequently, we have to conduct adequate on-board and ground based control experiments in order to understand experiments designed to investigate the effects of, for instance, gravity.

Gravity dependent responses have been identified in a wide range of cell types. Specific genes may be switched off at 0xg, enabling the identification of the sequence of events leading to cell differentiation or to signal transduction. Furthermore, microgravity experiments at the molecular level are useful in medical diagnostics where cellular systems can be used to assess the status of certain physiological conditions of humans in space<sup>2</sup>.

If transition from 1xg on Earth to 0xg in space causes alteration of cellular behaviour, the transition from 1xg to hypergravity in a centrifuge should induce deviations as well. Following this rationale, cell functions have been studied under hypergravity conditions as these inexpensive and simple experiments in centrifuges could allow us to speculate whether changes may occur in an environment with a different gravitational field. For instance, incubation of E.coli. in an ultracentrifuge at 110,000xg for 24 hours increased lag phases and prolonged generation times for DNA, RNA or protein synthesis<sup>3</sup>. The data obtained in hypergravity indicates that changes in the gravitational environment have an important effect on cell functions.

Biochemical signals are usually relayed by recognition and binding of ligands to specific receptors in the cell. Therefore, experiments concerning the binding of ligands in micro- and altered gravity are of significant interest.

Many of the physiological changes seen in multicellular organisms during space flight may arise from dysfunction of basic biological mechanisms caused by microgravity. As microgravity appears to influence prokaryotic and eukaryotic cell function at a molecular level, research in space provides an opportunity to learn more about basic life mechanisms. Moreover, these results can yield valuable information on important life processes on Earth. For example, aging in humans shares many of the symptoms experienced by astronauts during space flight, such as reduced immune response and loss of bone. Thus, research in microgravity and aging can be synergistic; both fields can develop more rapidly than either one in isolation<sup>2</sup>.

Based on experimental results and theoretical considerations, several models have been proposed which relate to the effects of gravity at the cellular level. Such effects may result from important changes in metabolism and/or molecular organization within the cell which allow it to

adapt to a new gravitational environment. For example, the non-equilibrium thermodynamics (bifurcation theory) presents an interesting view of a direct action of gravity on single cells<sup>4</sup>. Mesland developed this theory by applying non-linear non-equilibrium thermodynamics to living cells under changing gravity conditions.

Biochemical reaction chains catalyzed by enzymes and controlled by complex feedback mechanisms are nonlinear and far from equilibrium. Under such conditions, a cell may behave differently within a given latitude referred to as chaotic. At the point of such a crossroad, the decision as to which way the reaction will develop depends on minuscule differences in the reaction condition. The system is extremely sensitive to changes in the environmental conditions at this crossroad or point of bifurcation<sup>2</sup>.

Extended exposure of humans, other animals and plants to space flight could affect important mechanisms at the molecular level including gene transcription. Altered gravitational acceleration induces changes in basic cellular processes such as cell differentiation, rate of aging and energy metabolism. Various forms of gravitational stress can influence cellular functions by altering transcription activity in the expression of genes<sup>5</sup>.

Another environmental factor different in Mars is the percent and range of frequency of sun light on the planet. Light plays a crucial role in the regulation of certain transcription factors and DNA sequences. For instance, plants use a set of sensory photoreceptors to monitor the environment for informational light signals. An unknown mechanism transduces light signals perceived by the phytochrome family of sensory photoreceptors, to genes that are photoresponsive. Phytochromes may function as integral light switchable components of transcriptional regulator complexes, allowing instantaneous and continuous sensing of changes in this environmental signal directly at target gene promoters<sup>6</sup>.

The phytochrome family comprises five members that track the red (R) and far red (FR) light wavelengths; they are capable of photoinduced, reversible switching between two conformers, the R-absorbing biologically inactive form (Pr) and the FR absorbing biologically active form (Pfr). Light driven Pfr formation induces changes in the expression of numerous genes responsible for various aspects of plant photomorphogenesis<sup>6</sup>.

It will be important to consider the chemistry and physics behind complicated processes, such as gene transcription, using technology capable of providing dynamic information in real time. Once we understand the terrestrial features of gene transcription to a greater extent, we can design experiments to probe the effects of environmental differences and inter-planetary long term travel, exploration, and terraforming on this fundamental and ubiquitous life mechanism. The thickness shear mode (TSM) acoustic wave biosensor is presented here as a potentially effective technique to further our understanding of the mechanistic details of gene transcription. In addition, the use of the TSM sensor in investigating other aspects of Mars exploration is discussed.

## **THE GENERAL TRANSCRIPTION MACHINERY OF RNA POLYMERASE**

The recognition and binding of the RNA polymerase (RNAP) to a specific DNA sequence called promoter initiates the transcription process (**Figure 1**). This is an important step at which transcription and gene expression are regulated. The sequence of the promoter DNA is a primary factor that determines the strength of the promoter and efficiency of initiation. However, the relationship between promoter DNA sequence and transcriptional efficiency is not well understood at the mechanistic level. For example, the binding of an RNA polymerase to the promoter DNA sequence immediately upstream of the gene(s) under its control is necessary for the activation of a gene in *Escherichia coli* (*E.coli*). Substantial conformational changes in both the protein and the DNA follow the initial binding of the polymerase to a promoter region, depicting a transition from a 'closed' to an 'open' promoter complex<sup>7</sup>.

The RNAP begins transcription of the DNA coding following the formation of a stable open complex. Major structural changes of the transcription complex (**Figure 2**) allow the complex to become very stable upon entering the elongation mode. The RNAP moves rapidly along the DNA template powered with free energy liberated by nucleotide polymerization and RNA folding reactions (chemical to mechanical energy conversion) when transcribing in chain elongation<sup>7, 8</sup>.

Differential gene expression in response to changes in physiological, spatial or temporal signals is a central feature of all living cells that allow the development of highly specialized functions and life styles<sup>9</sup>. A series of natural biosensors called protein transcription factors (TF) govern this differential expression. TF's transduce the environmental signals into different levels of transcription initiation at defined promoters. This control can be achieved by a series of coupled molecular recognition events that involve the TF binding to a particular ligand, an effector or mediator, whose concentration is dependent upon environmental cues. Thus, the affinity of this complex for a specific DNA sequence is modified, resulting in differential access of RNAP molecules to the promoter. This differential access in turn results in varying levels of messenger RNA synthesis.

The phage enzymes serve as model systems to study the process of transcription initiation, elongation and termination because they do not require accessory proteins and are among the simplest RNA polymerases. We present the use of the acoustic wave biosensor to monitor the binding of phage enzyme T7 RNAP to T7 promoter DNA sequences in transcription initiation. As well, we have been able to monitor the binding of partially double stranded (ds) DNA promoters containing a single-stranded template region from -5 onwards to imitate open promoter DNAs.

## **ACOUSTIC WAVE SENSORS**

The acoustic wave sensor exploits the use of piezoelectric material, which was introduced at the turn of the century by the Curies with the term 'quartz crystal microbalance'. A mechanical stress will give rise to a charge across the electrodes while an applied electric field produces a deformation in piezoelectric material<sup>10</sup>. By using both of these features, the acoustic wave sensor offers high sensitivity and good linearity for a biosensor. Electromagnetic energy is converted to acoustic energy by reversible electric polarization produced by mechanical strain in

piezoelectric crystals. Consequently, acoustic waves can be set off in materials at ultrasonic frequencies of the order of one to several hundred megahertz (MHz).

The most widely used acoustic wave sensor is the thickness-shear mode (TSM) device, which generates bulk waves with particle displacement parallel to the surface of the sensor. That the particle motion at the electrode surface is parallel to the plane of the added chemically selective layer is a particularly useful feature that allows for investigations of interfacial molecular species at the sensor-liquid junction<sup>11</sup>. Viscous forces attenuate the bulk transverse waves as they propagate into the liquid. This device is employed in the resonance condition where the standing waves reflected from the two surfaces of the disc are in constructive interference.

The piezoelectric acoustic wave device functions as a microgravimetric sensor in the gas phase where the resonant frequency decreases when material is deposited on the crystal face. Sauerbrey derived a relationship between frequency change and added mass, which predicts a linear frequency decrease with added mass per unit area<sup>12</sup>. In the liquid phase, however, a number of factors at the device-medium interface may drive the operation of the device. Yang and Thompson have examined the contribution of viscosity, density, and dielectric constant of a liquid on the response of a TSM<sup>13</sup>. With the capability for generating multidimensional chemical information, acoustic wave technology provides highly sensitive measurements of biochemical systems. Furthermore, no tagging agents are required since signaling is direct for the TSM device.

## EXPERIMENTAL

AT-cut quartz piezoelectric crystals (9-MHz) with gold electrodes (**Figure 3**) were purchased from International Crystal Manufacturing, Co., Oklahoma City, USA. These crystals were rinsed with acetone, ethanol and double distilled water, and dried in a stream of N<sub>2</sub> prior to use.

A HP4195A network/ spectrum analyzer (Hewlett-Packard, Palo Alto, CA) was used to characterize TSM devices in liquid. From measured data, the values of the equivalent circuit parameters of the quartz crystal are calculated internally by the analyzer.

A flow-injection analysis (FIA) instrument was used to obtain laminar flow over the crystal surface in order to obtain real time on-line data from the TSM device. The FIA system consists of a four-channel EVA Model 1000 peristaltic pump connected to TFE Teflon<sup>®</sup> tubing of 0.8 mm inner diameter. The flow cell containing the crystal was exposed to liquid under flow conditions on one surface sensor face, while the other side was kept under a steady stream of N<sub>2</sub> flow.

### Reagents:

- Tris buffer (10 mM tris, 70 mM NaCl, 0.2 mM EDTA, pH 7.5) was used in the preparation of the neutravidin, DNA and T7RNAP solutions as well as for washing the crystal surface by flow through during experiments.
- 0.5 M and 0.07 M NaCl solutions were prepared with double distilled water from a 5 M molecular biology grade stock solution purchased from Sigma Chemical Co., St. Louis, MO, USA.
- Neutravidin was purchased from Pierce Chemical Co. (Rockford, IL), and dissolved in Tris buffer (pH 7.5) to a concentration of 1mg / ml.
- T7 RNA polymerase was purchased from Gibco BRL Life Technologies. The enzyme was dissolved in Tris buffer to obtain a concentration of 5 units / microliter.
- DNA oligonucleotides were ordered from The Centre for Applied Genomics, Toronto, ON where automated DNA synthesis (Beckman Instruments Inc) was carried out using conventional phosphoramidite chemistry and purified using the oligonucleotide purification cartridge method. For each double stranded or partially double stranded DNA, one of the strands was biotinylated and the other strand was hybridized on-line to the biotinylated strand immobilized on the crystal surface via neutravidin-biotin interaction.

The sequences<sup>14</sup> of DNAs containing the T7 promoters under consideration are as follows: The template strand of the promoter is found in the lower row. Mimics of open promoters or partially double stranded (ds) have 17 bases in their nontemplate strand.

Non-promoter:

GCCTCAATACCAGAGTTCGTTAGAGT  
CGGAGTTATGGTCTCAAGCAATCTCA

Strong promoter:

AAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC  
T T TAATTATGCTGAGTGATATCCCTCTGGTGTGCCAAAG

Strong open promoter:

AAATTAATACGACTCAC  
TT TAATTATGCTGAGTGATATCCCTCTGGTGTGCCAAAG

## Procedure:

### I. T7RNAP and DNA promoter interaction during transcription initiation:

Prior to sample introduction, buffer was flowed through over the cleaned piezoelectric quartz crystal incorporated in the flow-through cell at a rate of 0.06 ml / min to achieve a steady laminar flow and to acquire a stable resonance frequency signal from which changes could be easily monitored. 500  $\mu$ l of 1mg / ml neutravidin in tris buffer was then passed through the sensor in a similar manner in order to coat the crystal. After allowing a residence time of about 1 hr for the neutravidin to settle completely on the gold surface and signal was stable, the surface was washed with buffer. About 400  $\mu$ l of the biotinylated DNA strand was then flowed through at a concentration of 0.7 mg / ml (approximately 300  $\mu$ l of this DNA solution was usually sufficient to achieve the maximum drop in resonance frequency). The signal was allowed to

stabilize over a few minutes, after which the surface was washed with buffer to remove non-specific adsorption. Similarly, the complementary DNA strand was then introduced to the system. In this way, hybridization of the DNA strands was monitored on line prior to T7RNAP binding. Following hybridization to generate double stranded DNA containing T7 promoters with varying affinity for the T7RNAP, 500  $\mu$ l the T7RNAP (5 units /  $\mu$ l) was introduced into the system and its interaction with the DNA and sensor surface was monitored. These experiments were performed at room temperature.

## II. Response of the TSM device in a solution containing an electrolyte:

Double distilled water (ddH<sub>2</sub>O) was flowed through over the cleaned piezoelectric quartz crystal incorporated in the flow-through cell at a rate of 0.06 ml / min to achieve a steady laminar flow. 0.07 M NaCl solution was then flowed through until a steady resonance frequency (fs) signal was achieved, after which ddH<sub>2</sub>O was flowed through in order to wash the ions off of the crystal surface. Once a steady fs signal was obtained, a 0.5 M NaCl solution was flowed through the system as before. The runs with the 0.5 M NaCl and 0.07 M NaCl solutions were repeated in the same experiment. Following these runs with NaCl solutions, tris buffer solution (containing 0.07 M NaCl) was flowed through and subsequently washed off with ddH<sub>2</sub>O in a similar manner.

## RESULTS AND DISCUSSION

### I. T7RNAP and DNA promoter interaction during transcription initiation:

A common feature in all of the plots involving T7RNAP interaction is that the increase in motional resistance is more prominent than the decrease in resonance frequency. However, for the simple adsorption process of neutravidin, the frequency drop in response to the mass deposited on the crystal surface is dominant compared to the change in motional resistance. Furthermore, an instantaneous strong interaction is evident from the spike that consistently appears on the left shoulder of the motional resistance rise upon introducing T7RNAP into the system. The relatively large change in motional resistance with T7RNAP introduction suggests a high degree of energy transfer and bulk viscosity effects associated with T7RNAP interactions. The T7RNAP interaction with DNA promoter may include conformational changes as well. This situation is most likely in the interaction of T7RNAP with the strong open promoter, where the motional resistance appears to be sensitive to changes not shown by the resonance frequency when the crystal surface is washed off with buffer following T7RNAP interaction.

In the plot showing non-promoter interaction with T7RNAP, the instant rise in motional resistance resulting from introducing a viscoelastic protein layer returns to the baseline upon washing off non-specific interaction with buffer (**Figure 4a**). Also, the net decrease in frequency is about 50 Hz. Compare these results with the strong promoter, where the net decrease in resonance frequency is around 140 Hz, while the net increase in motional resistance is 3 ohms (**Figure 4b**). The results from strong promoter - T7RNAP interaction indicates specific interaction and energy dissipation. From these changes, we can clearly distinguish the non-promoter from the promoter sequence in binding to T7RNAP.

Now, compare the interaction between the strong (closed - fully double stranded DNA) promoter and T7RNAP with the strong - open (partially double stranded DNA) promoter and T7RNAP; the stronger binding affinity for the strong-open promoter is obvious (**Figure 4c**). The strong-open promoter binds to T7RNAP with a net increase of about 12 ohms in motional resistance, and a net decrease of over 200 Hz in resonance frequency. It is thus evident that the T7RNAP binds more strongly to the open-promoter compared to the closed one.

## II. Response of the TSM device in a solution containing an electrolyte:

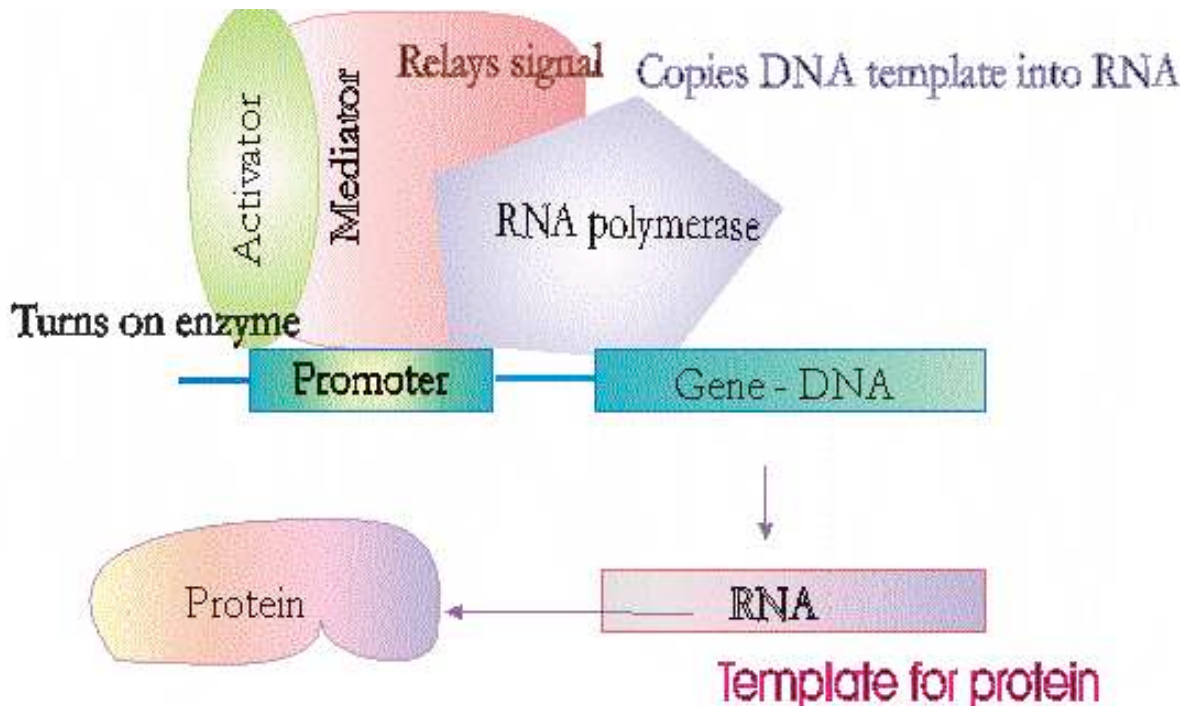
This data is presented in order to illustrate how the TSM sensor responds to solutions containing electrolytes of different concentrations. The electrical properties of the liquid layers in the vicinity of the sensor electrode allows for the use of network analysis to monitor the resonance frequencies and motional resistance for a variety of electrolytes. Unlike the case of viscous-loading of the sensor, a non-linear and periodic dependence was identified between the series resonance frequency and the motional resistance parameter of the device with respect to the concentration of the electrolyte<sup>15</sup>. It is suggested that change in resonance frequency vs. concentration profiles can be used to describe the differences between the 0.07 M and 0.5 M NaCl solutions in the resonance frequency vs. time plot<sup>14</sup> (**Figure 5**). One obvious difference is the longer time requirement to remove ions from the crystal surface in the case of the stronger electrolyte concentration. The dip in resonance frequency prior to signal stabilization upon introduction of the electrolytic solutions can also be predicted from the resonance frequency vs. concentration profiles. Consequently, we can exploit the unique characteristics of electrolyte concentration compared to viscous loading at the sensor interface to investigate the chemical and physical properties of liquid samples, such as ground water from the lakes of the Polar Regions.

## CONCLUSION

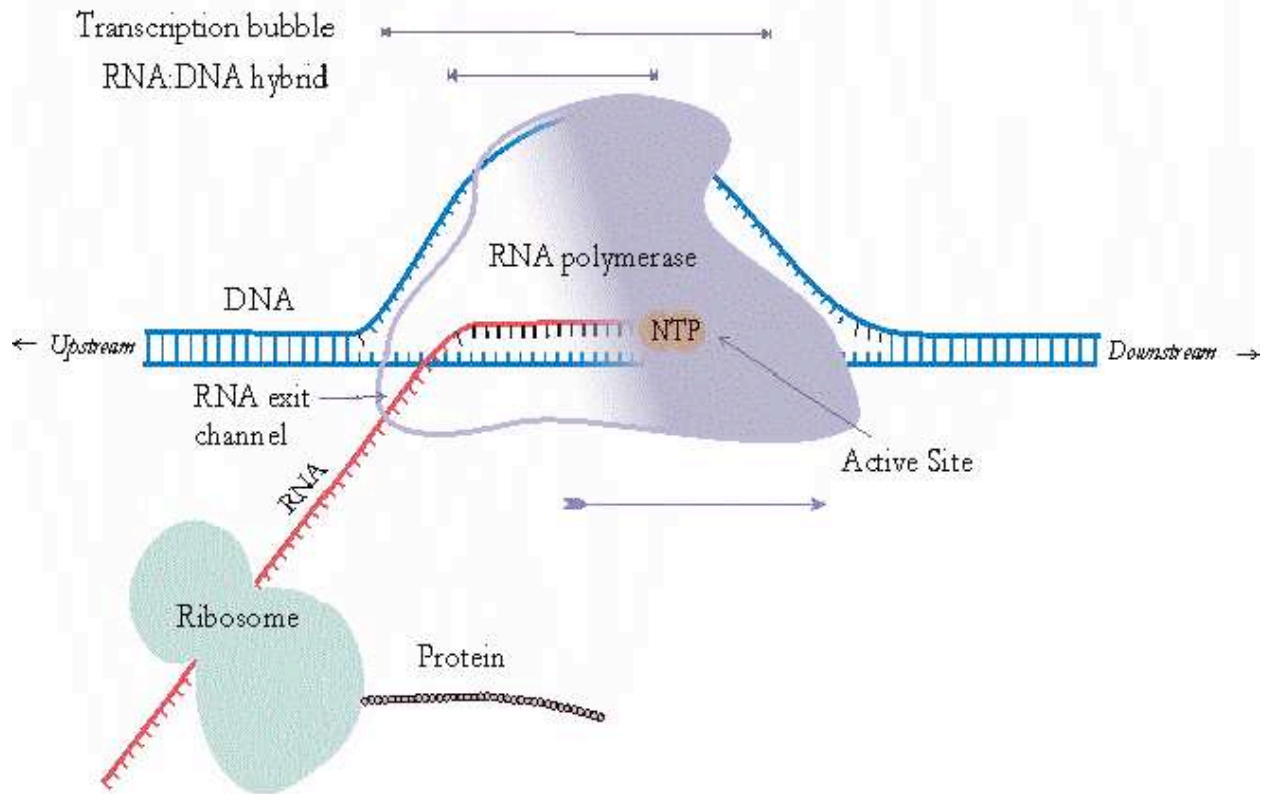
The TSM acoustic-wave biosensor is capable of supplying multi-dimensional information concerning vital cellular processes and how they may be influenced by environmental conditions. An important step in transcription initiation is the binding of the RNA polymerase to the promoter sequence of the template DNA that determines the level of gene expression in subsequent steps. External conditions such as gravity and light can control transcription at this stage depending on the template DNA sequence, or via transcription factors. As well, drug molecules can inhibit the transcription process at the initiation stage. The data we have obtained indicate that the TSM acoustic wave device is an effective biosensor for detecting binding of the RNA polymerase enzyme to the promoter DNA in transcription initiation. In addition, we propose that the TSM device can be used to monitor a wide range of samples including liquids containing electrolytes cells and gels. An important field study associated with Mars exploration is directed towards understanding the nature of the shape of diatoms in arctic lake sediments. Some of the work carried out in our lab with blood platelet cells has demonstrated that the TSM device can provide information about structural features of cells including shape<sup>16</sup>. As well, structural changes induced by changes in pH and ionic strength can be monitored. Another curiosity concerning the robust microbial life forms found under extreme environmental conditions on Earth are colonies of little balls of algae and bacteria which form a bio-film of gel

for protection against ultraviolet light. The TSM sensor could be used to study certain properties of this type of gel, including the gelling phenomena<sup>17</sup> and physical properties.

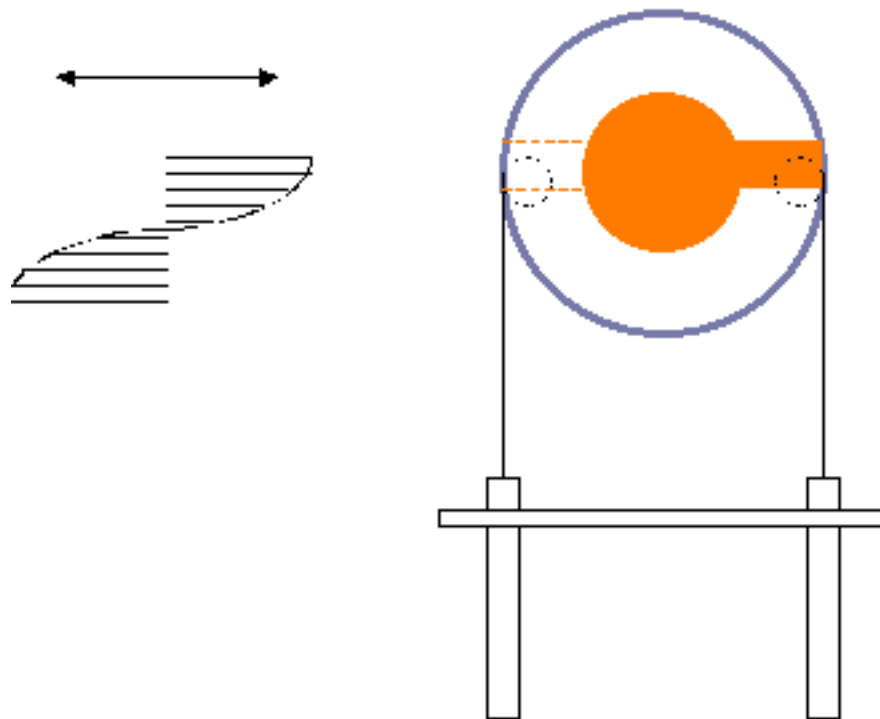
## FIGURES



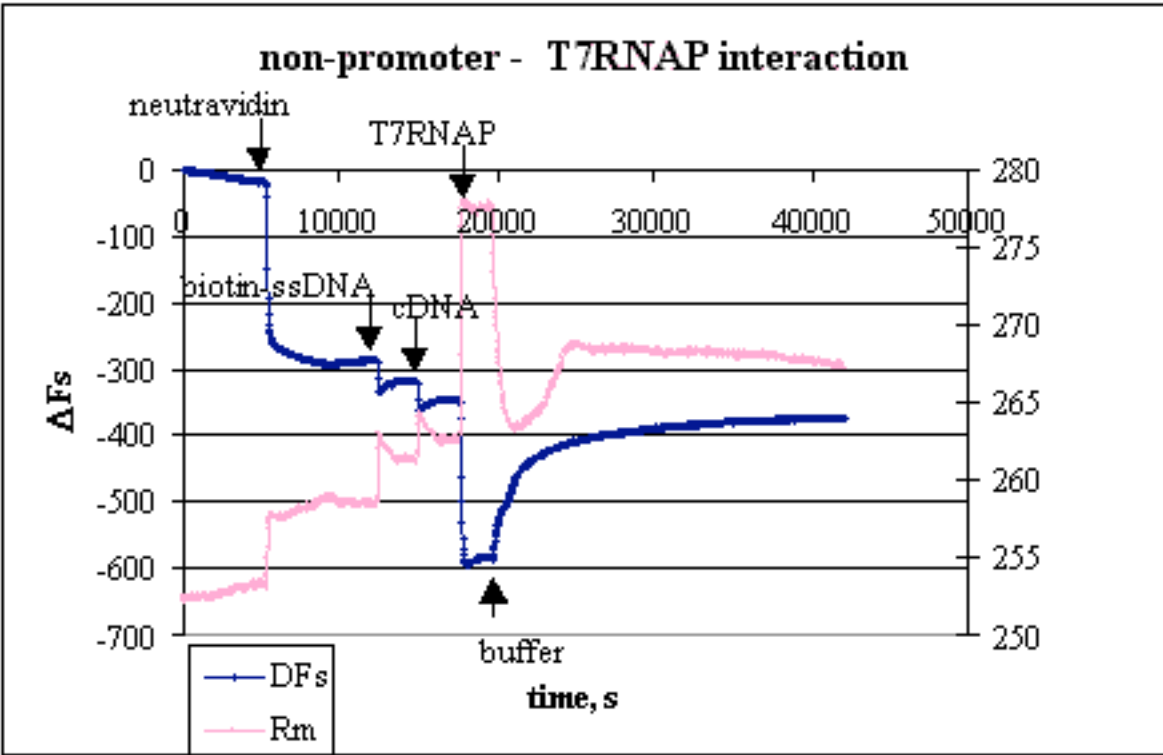
**Figure 1.** The basics of transcription are illustrated here. An activator protein binds to the promoter region of DNA upstream of the gene that is to be transcribed and turns on the RNA polymerase (RNAP) enzyme. The activating signal may be relayed through a mediating complex composed of many different protein subunits. The RNA product of transcription becomes a template for protein synthesis.



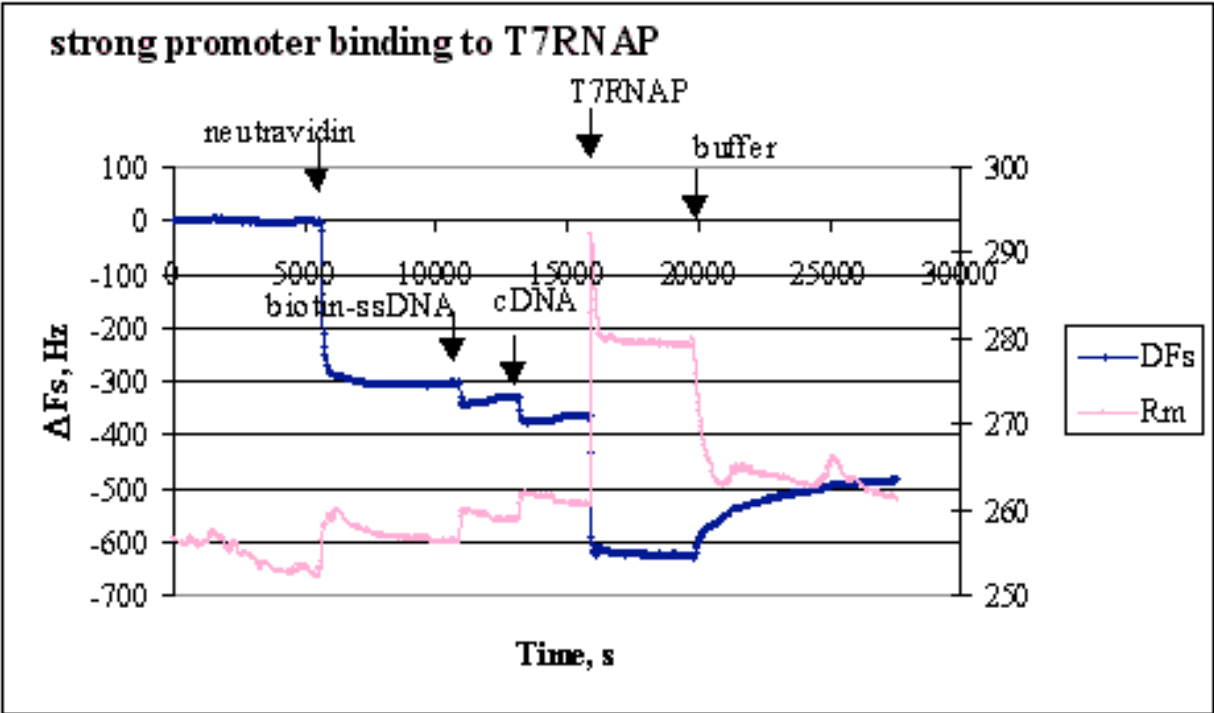
**Figure 2.** The RNA polymerase moves along the DNA during transcription, synthesizing messenger RNA (mRNA). The ribosome, in turn, copies information from mRNA by synthesizing protein.



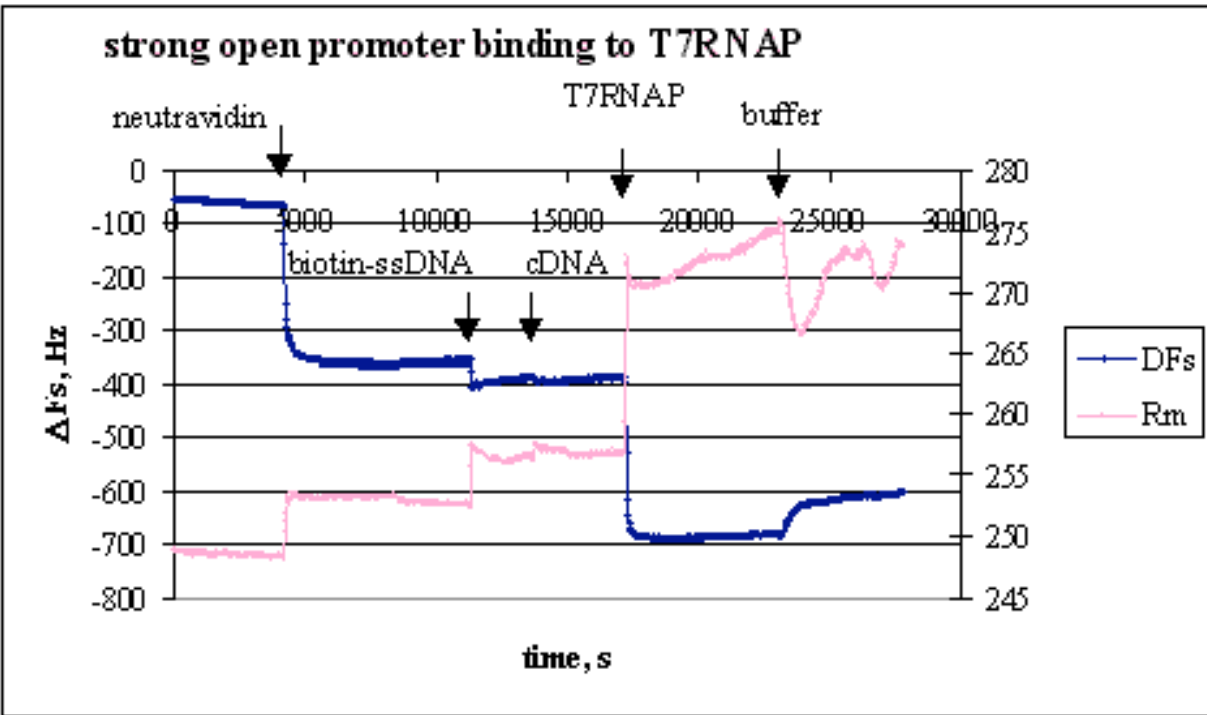
**Figure 3.** A representation of the top view of a TSM consisting of a quartz disc and metal electrodes. The quartz disc is 0.178 mm thick and has a diameter of 13.7 mm. The smaller figure to the left illustrates the wave motion and the direction of particle displacement.



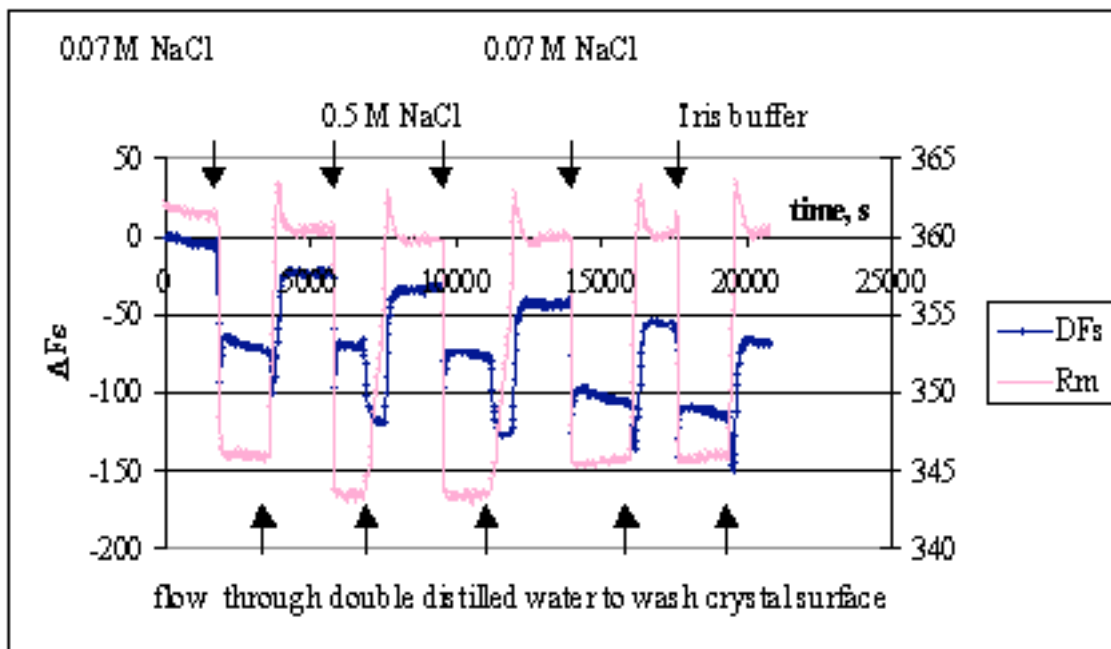
**Figure 4a.** No binding is detected in the interaction between T7RNAP and the non-promoter DNA sequence. The initial rise in motional resistance (Rm) returns to the baseline. There is a 50 Hz decrease in resonance frequency (Fs), which is much smaller than the decrease for promoter binding studies, and may be regarded as non-specific. Abbreviations: ssDNA - single stranded DNA, cDNA - complementary DNA strand



**Figure 4b.** Binding is observed for the interaction between T7RNAP and the strong promoter. There is a net rise of 3 ohms in Rm, and a 140 Hz decrease in Fs.



**Figure 4c.** When the same strong promoter sequence is made into an open promoter by deleting part of the non-template strand, the binding interaction becomes much stronger. A net increase of 12 ohms in Rm and a decrease of over 200 Hz in Fs can be observed.



**Figure 5.**  $\Delta F_s$  and  $R_m$  vs. Time graphs for the successive introduction of 0.07 M and 0.5 M NaCl solutions and Tris buffer (containing 0.07 M NaCl). Each sample introduction was completed by flowing through double distilled water to wash off ions completely from the crystal surface.

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