Specific Aims

There are few experimental techniques that allow the application of force on biological molecules. Among them, optical or magnetic tweezers and atomic force microscopes (AFMs) have provided much insight into the mechanics of DNA, RNA and chromatin [1] [2] [3] [4], friction and wear in proteins [5] [6], and stepwise motion of motor proteins [7], all of which are important for understanding disease.

However powerful, these methods are not able to be deployed as integrated devices to probe the mechanical properties of heterogeneous samples in a wide range of applications; tweezer and AFM experiments rely on highly trained experimentalists, are not widely applicable as analytical tools, and are often constrained to the analysis of well prepared homogeneous samples.

Among direct mechanical transduction methods, the sensitivity, low cost, ease of use and integrability make the quartz crystal microbalance (QCM) ideal for real time monitoring of biomechanical properties such as viscoelasticity, conformal changes [8], and contact rigidity [9]. Naturally, these benefits do not come without disadvantages. The underlying mechanical properties of the sample are often not revealed by the stepwise changes in the QCM sensorgram, an issue complicated by the choice of theoretical model. Operation in the liquid phase is also associated with a rather low-Q resonance precluding their use for single molecule detection; up to now it has not been possible to integrate force on biomolecules in QCM measurements.

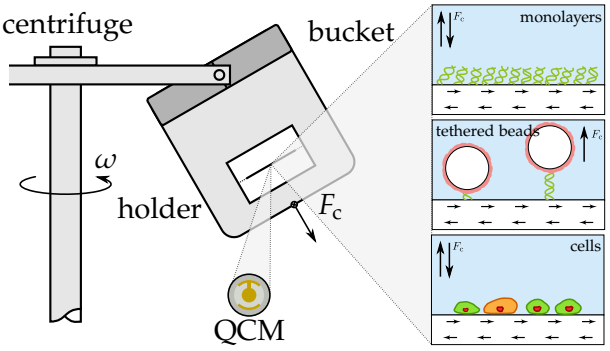

Figure 1: Specific aims of the proposal. A QCM is integrated into a centrifuge for realtime operation in the liquid phase, applying force on (a) protein/DNA monolayers (b) protein coated microparticles and DNA/chromatin complexes and (c) collections of cells for cancer assays.

In light of these issues and the analytical power of force based techniques, this proposal concerns the development of a novel type of instrument that uses a QCM as a direct mechanical transducer for the response of biomolecules placed in a variable force field provided by a standard commercial centrifuge. This centrifugal force quartz crystal microbalance (CF-QCM) concept enables direct introduction of pico- to nanoscale forces in the liquid phase for analyzing the mechanical properties of biomaterials ranging from molecules to cells. We will deliver a research program centered around the following three aims, see Figure 1

Aim 1: To build and characterize a functioning CF-QCM prototype, we will integrate a state of the art QCM into a commercial centrifuge for real time operation in the liquid phase.

Aim 2: To characterize the response of the CF-QCM in homogeneous and heterogeneous molecular samples, we will conduct measurements on protein monolayers, protein coated microparticles, and DNA chromatin complexes.

Aim 3: To understand and obtain quantitative mechanical properties of a complex sample, we will extend our analysis to encompass collections of cells to detect biomechanical changes associated with cancer.

The successful development of the proposed project will have a significant impact on the fundamental understanding of force relating to biomechanical properties in a novel public health diagnostics device.

Research Team: We have formed a team of two investigators led by PI Dr. Frank Vollmer (Brigham and Women's Hospital/Harvard Medical School), an expert in biosensing and biophysics, and co-investigator Dr. Yuki Sato (Rowland Institute at Harvard University), an expert in physics and device engineering.

Research Strategy

Significance

Mechanical properties such as viscoelasticity, contact stiffness and adhesion, and force-dependent conformational changes have been shown to be instrumental in understanding biofunctional behavior from single molecules to complex collections of cells. These properties are often descriptive for specific molecules and cell types. For example, force extension curves of DNA vary with assembly of histone proteins into chromatin. In cells, viscoelasticity alone can discriminate between healthy and cancerous tissue. Biosensors that can probe mechanical properties are therefore ideal for both fundamental studies in life sciences as well as diagnostic assays improving public health.

Perhaps the simplest way to probe a biomechanical property is by monitoring its response to direct application of force. Current force based approaches, for example those based on atomic force microscopy or optical or magnetic tweezers, are powerful but limited in their applicability as biosensors. In particular, their operation requires significant expertise on the part of the investigator, and is often constrained to well prepared samples not amenable to multiplexing.

Among tools suitable for direct mechanical transduction, the quartz crystal microbalance (QCM) has seen increasing real-world utility as simple, cost effective, and highly versatile mechanical biosensing platforms. A QCM typically presents itself as thin disk-shaped piece of strategically cut piezoelectric quartz with electrodes on either side. When part of an electronic oscillator circuit, the quartz can form a mechanical resonator which vibrates at its fundamental frequencies. Changes in these frequencies and their associated bandwidths upon sample adsorption or desorption are related to the properties of the sample and the strength of its coupling to the QCM. Since its introduction by Sauerbrey [10] in 1959 as sub-monolayer thin-film mass sensors in the gas phase, the understanding of these piezoelectric devices has been repeatedly enhanced to study phenomena such as viscoelastic films in the liquid phase [11], non-destructive contact mechanics [12], and complex topologies of biopolymers and biomacromolecules [13]. However, despite their popularity QCMs suffer from low-Q resonances which negatively impact their sensitivity. In addition, extracting quantitative mechanical information from biomolecules is often confounded by non-trivial interpretation of the discrete shifts in the system's frequency and bandwidth.

Our response to these issues concerns the development and application of a new type of instrument which places a quartz crystal microbalance in a standard commercial centrifuge. When spinning, controllable centrifugal force is applied to a biomaterial under assay, and the QCM signal as a function of applied force is monitored *in situ* and in real time.

We have tested this new sensing concept in a prototype instrument where we have observed marked difference in mechanical responses from protein monolayers, lambda DNAs, and protein coated microbeads as a function of applied centrifugal force. We have evidence that the force is acting to directly probe both the biomechanical properties of the sample and its coupling with the QCM. By testing different load scenarios, we have identified a simple mechanical model based on coupled oscillators to interpret the changes in frequency and bandwidth of the QCM device. This model favorably indicates that the QCM-sample coupling can be modified in situ to significantly enhance the sensitivity of the device when compared to traditional QCM experiments.

The preliminary results obtained in collaboration with Dr. Sato at the Rowland Institute at Harvard University suggest the immense capability of this platform with potential applications ranging from nanotribological study of protein layers to characterizing mechanical properties of DNA and chromatin. Furthermore, when applied to cell cultures, this method will allow for rapid identification of cell types from their mechanical response. In such a way it would enable the identification of cancer cells, possibly within a mixture of cells. With the intention to make the platform operational in standard laboratory centrifuges, the barrier to use will be lowered allowing researchers to address similar questions in a wide range of scientific fields.

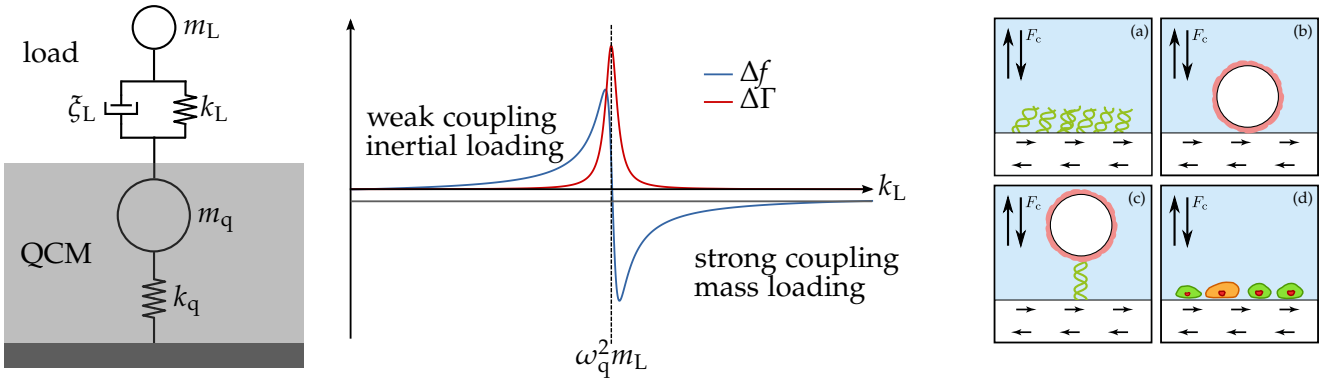

Figure 2: (left) Mechanical model QCM behavior under load. (center) The changes in frequency and bandwidth of the system as a function of the contact stiffnes k_L. (right) Examples of different types of sampes which can be probed with the CF-QCM.

We anticipate this novel biotechnology platform to not only address fundamental questions regarding proteomics and DNA/chromatin dynamics listed in the proposal, but also to formulate new sets of scientific questions thus far unexplored, contributing to advancing knowledge in diverse areas of medical sciences. Our findings on mechanical and thermodynamic properties of biological objects such as bacteria and cells should also feed back to studies related to disease pathways and computational biology. When applied to measurements with cells, the platform has the potential to enable a unique and rapid cellular assay.

Innovation

Our thrust borrows from recent advances in QCM biosensing and brings to the table a new mechanism for dynamically probing and manipulating a sample *in situ* and in real time. Our study is designed to demonstrate these new sensing capabilities by examining the mechanical responses of protein monolayers, functionalized microbeads, and DNA/chromatin complexes under applied load. In parallel, our program will develop a physical model to describe the quartz crystal sensor response, where mechanical parameters are directly obtained from the response of the CF-QCM.

Where typical mechanical biosensing produces a single, stepwise sensor response that can be linked to, in the case of a QCM, the static adsorbed mass, contact stiffness, or viscoelastic compliance, our proposal allows for the direct manipulation of elective dynamic versions of these parameters through centrifugal force gradients. Examples of loads which could be probed and the possible action of centrifugal force are are shown in Figure 2 (right). Note that the centrifugal force can be such that it either pushes or pulls the sample with respect to the sensing surface. They are:

	load	action
(a)	monolayer of DNA molecules	change the conformal shape of DNA
(b)	protein coated microparticles	affect the contact stiffness
(c)	microparticles tethered with lambda DNA	extend and stretch DNA
(d)	heterogenous collections of cells	modify cell adhesion and contact area

We propose a coupled resonator model to describe the changes in frequency and bandwidth when spinning. This model is shown in Figure 2 (left). Here, the resonance of the QCM $\omega_q = \sqrt{k_q/m_q}$ is represented by a simple mass-spring system. The QCM is coupled to an external load with mass m_L though a parallel spring k_L and dashpot ζ_L (drag coefficient) representing a Kelvin-Voigt viscoelastic material. As a function of the load's coupling to the QCM, k_L, the resonance of the system takes the form of a complex Lorentzian, shown in Figure 2 (center). These parameters are designed to be descriptive of an arbitrary load. About the zero

crossing frequency at $m_L\omega_q^2$ this model describes two important limits, called "strong" and "weak coupling", respectively.

The limit of strong coupling is the classic way in which QCM data is interpreted; a negative frequency shift is related to mass adsorption [10]. This is typically seen for most homogeneous films in the liquid or gas phase. In the limit of weak coupling, which occurs for discrete micron sized objects, a positive frequency shift is observed [14]. In either case, control over k_L grants several advantages:

1. By increasing the spin speed and centrifugal force, k_L can be increased or decreased such that its value moves toward the zero crossing, increasing Δf, $\Delta\Gamma$ and consequently the device's sensitivity.
2. By considering the way frequency and bandwidth change with applied force, the underlying mechanical model is elucidated.

Applying this model for increasing centrifugal forces, our study will theoretically and experimentally identify different sensing regimes at low and at high g-force values, both in a "loading" configuration, where the centrifugal force is in to the plane of the crystal ("pushing"), and in an "unloading" configuration, where the centrifugal force is out of the plane of the crystal ("pulling"). For sensing at low g-force values the predicted increase in signal (frequency and bandwidth changes), and therefore sensitivity for extracting the elasticity and viscosity parameters of the biomaterial, has been indicated by our preliminary experiments. We anticipate that we can extract these mechanical parameters (viscosity, elasticity, mass, etc.) at much higher precision to the both the baseline increase in the values as well as by fitting parameters to their force-dependent behavior. This method and principle is unique to CF-QCM. We plan to use this capability in an innovative approach that allows discriminating different cell types from their mechanical response, thereby detecting much softer cancer cells within a collection of cells that have not transformed. Our study will open up avenues for further developments of sensitivity enhancements of QCM devices, for example by engineering quartz crystals that can be placed in even higher centrifugal fields exceeding 100 g, bringing QCM based single virus measurements and possibly single molecule detection within reach.

We have designed our study to result in a CF-QCM platform applicable to a wide range of materials (molecules, cells) and in liquid samples operational in a standard laboratory centrifuge. Furthermore, our study was designed to use the CF-QCM to address fundamental questions in the life science. Amontons' law states that for any two materials the lateral friction force is directly proportional to the normal applied load, yet our preliminary results from monolayers and DNA already indicate deviations from this linear relationship. Our study is designed to gain novel insights into the thermodynamic nature of friction in nanoscale protein and DNA layers.

In summary, the broader impact of this project is in its potential to establish a new generation of biosensors with a completely novel transduction mechanism. This will be a boon for biosensing, diagnostics, and public health. The proposal is transformative as it describes novel bio-sensing paradigm that takes advantage of the mechanical response of coupled mechanical resonators under centrifugal load. It is widely applicable since the stand alone detector only requires a standard laboratory centrifuge.

Approach

Aim 1: Development and Characterization of Prototype Instrument

Our first aim concerns the development of an advanced CF-QCM instrument. The design of this instrument is based upon experience gathered from exploratory experiments with a prototype developed by Dr. Yuki Sato at the Rowland Institute at Harvard. This prototype is pictured in Figure 3. It consists of a QCM in a standard bucket centrifuge capable of generating a controllable accelerations in the range of 0 g to 100 g.

The QCM is a 5 MHz quartz crystal microbalance with gold electrodes. The QCM is connected in proximity to a remote driver which is tethered via a cable and slip-ring connector to a SRS QCM200 PLL based driver circuit outside of the centrifuge housing. The QCM200 outputs Butterworth van Dyke (BvD) equivalent relative frequency Δf and resistance R values which are recorded by a computer.

The crystal is mounted radially by its edges in the centrifuge such that the centrifugal force is always normal to the surface of the crystal. On the sensing side of the crystal is a 125 µL PDMS/glass cell containing the specimen under investigation. The non-sensing side of the crystal remains in air. When in operation, the crystal is mounted in either the "loading" configuration, where the centrifugal force is in to the sensing side, or in the "unloading" configuration, where the force is away from the sensing side.

We will improve and advance this design in many ways. Figure 4 shows a block diagram of the proposed design. Of prime importance for the success of new instrumentation is maximizing its accessibility, meaning the ability for other researchers from different fields to operate the instrument and interpret its data to make meaningful measurements. The former aim will be addressed primarily by miniaturizing the CF-QCM control electronics and engineering the sensor into a standard 50 mL centrifuge tube form factor, eliminating the need for a dedicated or otherwise modified centrifuge.

On the top, a battery provides power to on-board integrated electronics system below. The electronics system drives and monitors the QCM signal as well as other environmental parameters (vectorial acceleration, vibration, pressure, temperature, etc.) which are recorded to increase the fidelity of the measurement process. These signals, along with the output from the QCM, are interfaced to an external computer using a wireless link. This allows the centrifuge to reach high spin speeds; our prototype instrument is currently limited in this aspect because of its manual tethered connection. Due to recent advances in consumer electronics such as cell phones, all of these electronic sensors are presently available as easily integrable commodity components.

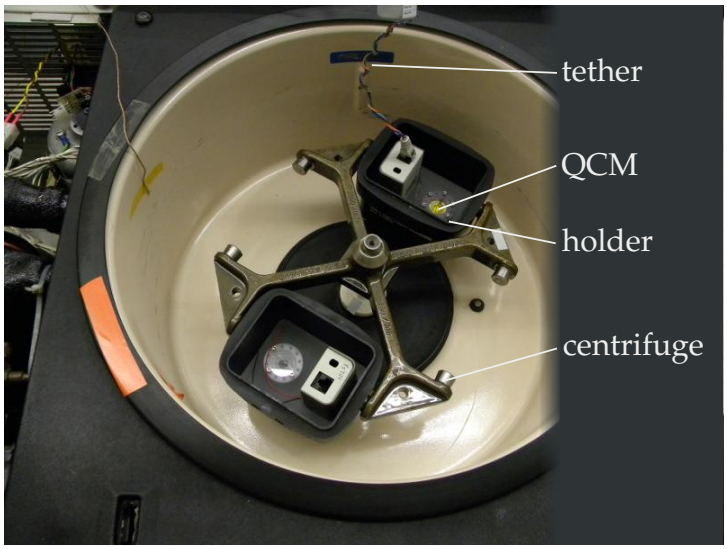

Figure 3: Prototype instrument.

Below the electronics is the QCM itself whose sensing area is encompassed by a microfluidic flow cell. This cell will be manufactured using standard PDMS soft lithography techniques applied directly to the surface of the crystal. PDMS is ideal for such applications, as it readily bonds to smooth dielectrics such as quartz and is biologically inert. Such a strategy will additionally reduce thermal noise by essentially nulling liquid phase convection. The cell, which can be removed from the main instrument body, will have the ability to be externally accessed for pre-centrifuge calibration. The calibration procedure will also be necessary to mitigate spurious signals caused by nucleated bubbles due to dissolved gases in the liquid sample.

Of particular interest is the system's bandwidth, Γ, which is related to the "dissipation factor" D (as recorded by some QCM devices with dissipation monitoring) by $D = 2\Gamma / f_0$. This parameter is much better at determining viscoelastic properties than R, which is only approximately related in some regimes. The electronics will leverage advanced QCM drive circuits giving access to Γ, such as QCM-D, which have already been integrated into a single chip [15] [16]. Clear interpretation of the data will be made possible by developing and extending the mechanical model and understanding its limits and applicability. High bandwidth, low latency wireless data links, environmental sensors of temperature and acceleration, and image sensors are now commodity items and may easily be incorporated into existing designs. Furthermore, centrifuges allow for very good temperature control down to 0.1 °C.

The shift in the resonance due to mounting stress is much larger than the intrinsic acceleration response, but is also much more difficult to assess because it is highly dependent on the configuration which specifies the forces involved on the crystal. In the prototype instrument, this was not seen as an issue. This is primarily because, for symmetric annular mounting microliter sample volumes, the absolute shifts were small compared to the phenomena under investigation. In addition, the frequency- and bandwidth-force curves traced out were reproducible and could be subtracted for calibration.

The sensitivity of a QCM increases with its resonant frequency, and a higher resonant frequency comes at the cost of a thinner and considerably more delicate crystal. The crystal must be housed in such a way that it is indifferent to high-g environments. Fortunately, the use of quartz crystals as accurate timepieces has lead to their extensive characterization in high-g environments for military applications such as missile guidance, radio communications, and aerospace. Different mounting strategies and crystal cuts will be investigated if these effects are found to need compensation, aiming for the highest frequency operation possible.

Below the crystal opposite to its sensing side is a small integrated microscope. This allows monitoring of the micromolecular state and motion of the system in parallel with the data from the CF-QCM. The microscope will provide, where applicable, a secondary validation of the behavior at the interface.

The device is designed to be modular, and as such several additional configurations may be explored besides the one indicated. Some examples include a large sample volume which could used to measure bulk sedimentation velocities, zero point velocity flow cells for depositing particles and studying diffusion limited reactions, and a module to mount the QCM at an angle to the centrifugal force for kinetic friction measurements.

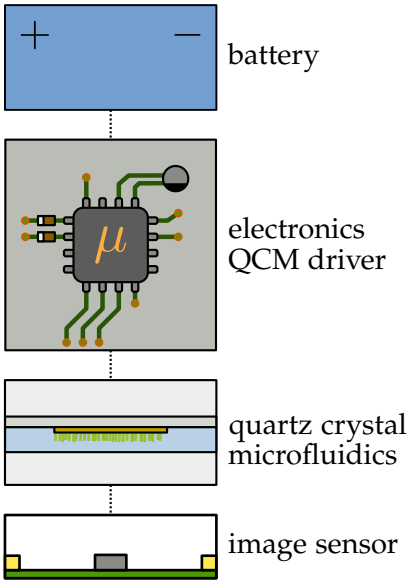

Figure 4: Block diagram of the proposed centrifugal force quartz crystal microbalance. The device will be integrated into a standard 50 mL centrifuge tube.

To further the goal of making the proposed instrument accessible, all information relevant to its development such as mechanical drawings, schematics, PCB artwork, documentation, and source code will be made available on the internet under free, non-encumbering licenses such as the GNU General Public License, version 3 [17] and the CERN Open Hardware Licence [18]. This will facilitate others interested in similar studies to be able to replicate, modify, and redistribute improved versions of the proposed work.

This aim will be successful if the instrument is able to reproducibly acquire data from the QCM in an unmodified centrifuge spinning at 10 000 RPM without introducing non-nullable environmental effects.

Aim 2: Nanotribology with Proteins and DNA

Aim 2.1: Protein Monolayers

Friction is relevant to many nanotechnological applications and it is critical to the performance of macroscopic materials and micromechanical devices. Amontons' law, which describes phenomena that were under investigation since the time of Leonardo da Vinci, states that for any two materials the lateral friction force is directly proportional to the normal applied load. No theory has yet satisfactorily explained this surprisingly general law which seems to apply across several orders of magnitude in length scale and in many different types of materials [19] [20] [5]. This aim will use nanotribology measurements with centrifugal force QCM to study Amonton's law in protein monolayers. A linear relationship between friction force (extracted from measurements of cou-

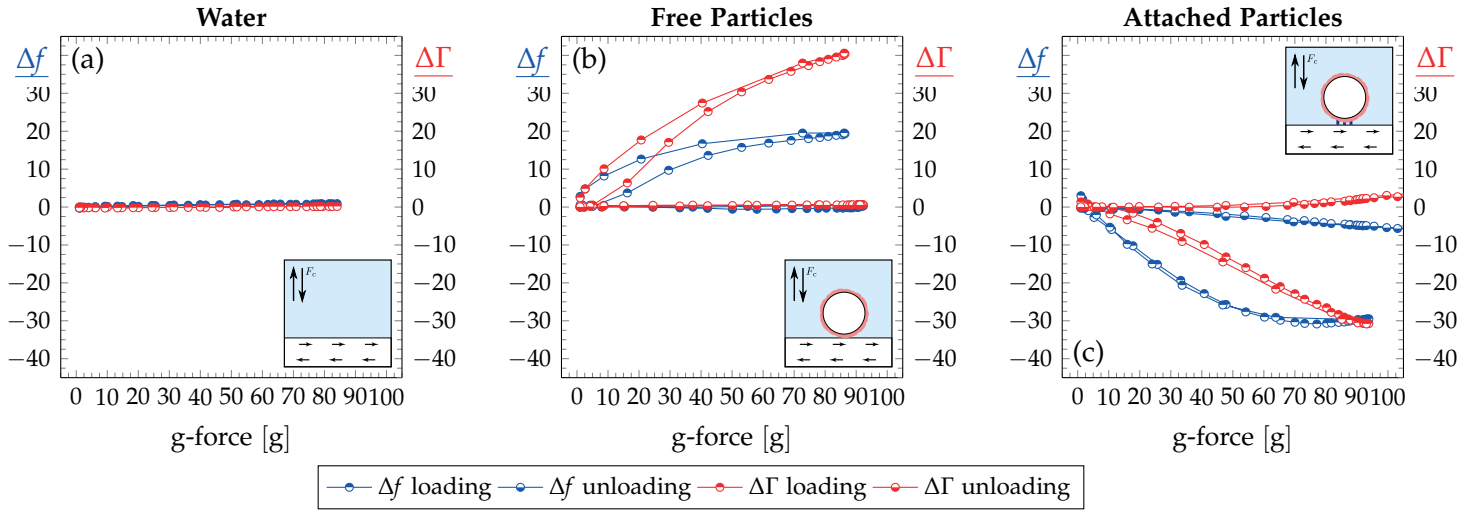

Figure 5: Change in the frequency Δf and bandwidth $\Delta\Gamma$ (inferred from motional resistance) of the CF-QCM under different load situations as the centripetal acceleration is directed in to (*loading*, represented by circles with the top half colored) and out of (*unloading*, represented by circles with the bottom half colored) the plane of the crystal. The situations are (a) deionized water, and (b) free 1.89 µm diameter streptavidin coated paramagnetic particles, and (c) 1.89 µm diameter streptavidin coated paramagnetic particles attached with 25 mer oligonucleotides. The response of deionized water is the QCM's response is almost identical to the intrinsic acceleration-dependent frequency shifts in AT cut quartz.

pling constant) and applied load will allow one to determine the effects of wear in the protein monolayer by measuring deviations from Amonton's law over time. Furthermore, any deviations from the linear regime in Amonton's law by increasing centrifugal load may also reveal further insights in the thermodynamic nature of friction on the nanoscale [19], an area of investigation largely unexplored.

We will first focus on extracting mechanical properties of protein monolayers using the CF-QCM approach. This will be done with normal planar monolayers as well as coated microbeads used as a transduction mechanism. There have been several studies already confirming that accelerations as small as 1g have a measurable effect on the output of a QCM sensorgram under load. This has been observed for Newtonian liquids, DNA, as well as for force-based techniques involving nanoindentors or AFM probe tips [21]. All of these responses have been found to be significant compared to the baseline acceleration sensitivity of the QCM itself. First proof of principle experiments will be implemented with simple "off the shelf" protein monolayer coatings such as BSA and actin (or myosin) to develop the theoretical basis for a description of frictional forces and apply this analysis to the interaction between two protein layers by respective protein coating of beads and QCM. These pioneering studies will provide the fundamental basis and understanding to study frictional forces in biomolecular coatings in nanotribology experiments.

Microbeads weakly coupled on a CF-QCM coated with protein will allow the variation of frictional forces between the protein and the weakly coupled microbead by applying centrifugal load. This will enable examining loading regimes where protein friction might vary linearly with load, and perhaps identify regimes where such response is nonlinear or may exhibit hysteresis due to conformational changes or elastic memory effects in the biological material, here a protein monolayer coating. Furthermore, by monitoring the coupling between the protein layer and a microbead over prolonged time [22], it is possible to determine the changes related to wear in protein coatings. Such study allows for a first quantitative analysis of biological monolayer coatings under frictional load. Some preliminary data to this aim is shown in Figure 5 (b). Here, free 1.89 µm diameter streptavidin coated polystyrene particles in water are on the sensor surface. As the centrifuge spins up, Δf and$\Delta\Gamma$ increase significantly with applied force. This behavior indicates that the system is in the *weak* coupling regime – the bead is "clamped" by inertia. It is important to note that, even under centrifugal load, the QCM does **not** sense the mass of the particle, rather it senses the stiffness of the contact. If the inertial mass of the particle was changed under centrifugal force, a negative frequency shift would be expected rather than the

positive one observed.

It will be also possible to use beads as a transduction mechanism in the strong coupling regime when using certain surface modifications. In Figure 5 (c), 1.89 µm diameter streptavidin coated polystyrene particles been attached to the gold electrode of the QCM by means of 25 mer thiolated oligos. The effect of rigidly attaching the beads is expected to sufficiently increase k_L such that the system is now in the strong coupling regime. Δf and $\Delta\Gamma$ are both negative and increase with centrifugal force, signifying that the centrifugal force is decreasing the rigidity of their coupling to the QCM. If the orientation of the crystal is reversed, opposite signals are seen in $\Delta\Gamma$. Through this configuration it would also be possible to (destructively) measure bond strength [23]. We will use this technique to study monolayers of proteins and DNA which couple strongly to the CF-QCM.

It is important to recognize that in each of the load situations (with the exception of the buffer), the sensor's response is *enhanced* by the introduction of centrifugal force. For unattached beads, the response in frequency and resistance has been observed in these experiments to be upwards of a factor of 10 larger at 100 g than for gravity alone, depending on the bead size and thus the initial value of k_L. This is clear from Figure 2 (center): in the weak coupling regime, moving towards strong coupling increases Δf and $\Delta\Gamma$ (loading with a centrifuge), while in the strong coupling regime moving towards weak coupling does the same (unloading).

In our experiments it will be fruitful to look at the response of the CF-QCM to situations under different functionalizations between the bead and surface. As is evident from loading with unattached streptavidin coated beads, it is possible to sense sub-monolayer films by loading with centrifugal force. Specifically by running the experiment using QCM-D and monitoring the loss tangent $\Delta D/\Delta f$, it would be possible to accurately measure the interfacial friction and "slip time" [24] – the ratio of m_L and ξ_L. By sweeping k_L over a wider range with greater values of centrifugal force, it is perhaps possible to separate the contributions in a heterogeneous environment [25]. In extension, similar measurements are applicable to the study of different cells, bacteria, and viruses.

Aim 2.2: DNA

We will use the CF-QCM sensing concept to study the properties of DNA both alone and tethered to microparticles, potentially extending and compressing them under centrifugal force. Previous studies have shown that, through the use of dissipation monitoring, QCMs are sensitive to not only the adsorbed mass and viscosity, but the physical conformal state ("shape") of DNAs hybridized to the sensor surface [26]. We believe that the CF-QCM can affect such conformality. In Figure 6(a), 48 kbp lambda phage DNAs were attached to the gold sensor electrode via a thiol linker. In the preliminary experiment, even though the force on the lambda DNAs is on the order of femtonewtons, a significant signal is observed. In the low-g range the direction of the signal is consistent with the behavior of similar DNA observed on QCMs under the influence of gravity alone [27]. However, under larger g-forces the sign of Δf reverses. The origin of this effect is unknown, but could indicate a large intrinsic value of ξ_L related to the elastic compliance of DNA under load. By using an advanced CF-QCM, we will investigate the origin of this force behavior and the range over which these parameters can be pertrubed and sensed.

With the sensitivity to the conformational change of the load and the coupling strength, the instrument raises a possibility for using beads tethered by lambda DNA as a transduction mechanism to investigate its kinetics. One such example is shown in Figure 6(b). 24.5 µm streptavidin coated polystyrene particles were tethered CF-QCM by means of a 48 kbp lambda phage DNA. Experiments were done in STE buffer whose density reduced the maximum force on the bead to about 40 pN. In contrast to typical force-extension measurements made by optical or magnetic tweezer experiments, the QCM will be sensitive to both the analyte within the transverse shear wave penetration depth (typically 95 nm in H_2O), and acoustic transduction through larger attached targets. The penetration depth is equal to about twice the persistence length of DNA – 40 nm, or about 150 base pairs in the fully stretched state, which is almost reached for a single tether at 40 pN as predicted by the worm-like chain model [4] ($z/L \approx 0.98$, where z is the extension and the total length $L \approx 16$ µm). Unstretched

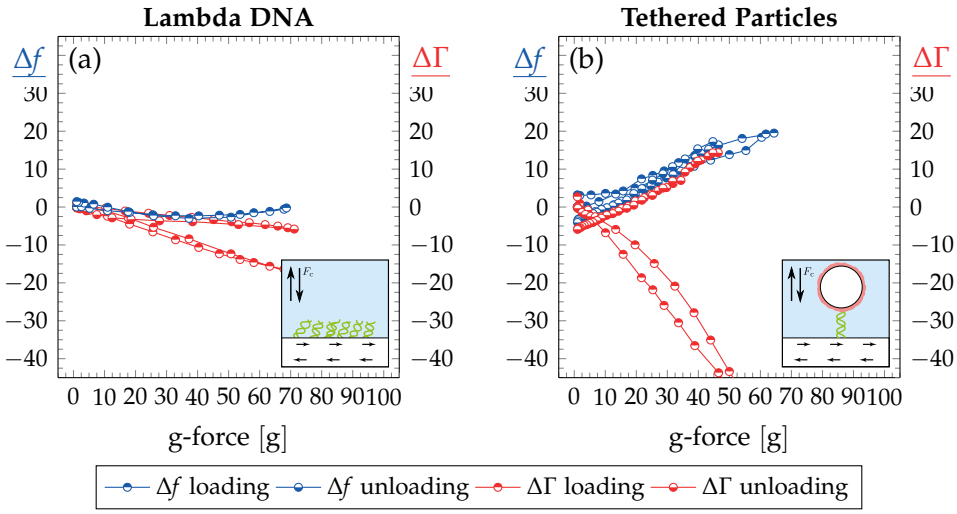

Figure 6: Change in the frequency Δf and bandwidth $\Delta\Gamma$ (inferred from motional resistance) of the CF-QCM under different load situations as the centripetal acceleration is directed in to (*loading*, represented by circles with the top half colored) and out of (*unloading*, represented by circles with the bottom half colored) the plane of the crystal. The situations are (a) lambda DNA only attached to the gold electrode, and (b) 24.5 µm diameter streptavidin coated polystyrene particles tethered to the sensor surface with 48 kbp lambda DNAs.

lambda DNA however, is randomly coiled into a ball 2 µm to 4 µm in diameter [28].

Though the instrument has not yet been developed enough to make accurate quantitative measurements in this load situation, the behavior of the data is a clear indication of its potential. As the tethered bead extends the DNA under centrifugal force, the effective inertial mass and intrinsic viscosity on the surface decreases, causing Δf to increase and $\Delta\Gamma$ to decrease. In the case where the DNAs are trapped and pushed between the bead and the surface, a sign reversal of $\Delta\Gamma$ occurs, but not Δf. The signs of these shifts are consistent with experiments we have carried out with tethered paramagnetic particles pulled away from or pushed towards the sensor with a magnetized source.

At the maximum spin speed, the frequency shift indicates an effective density decrease of 10 % or about 1 µg. For the number densities involved, the equivalent interfacial mass lost for a fully extended lambda DNA predicted by the WLC model are in the picogram range and cannot account for the more than 10^6 signal difference shown here. If indeed the response is due to lambda DNA extension, future experiments involving high frequency, large centrifugal force CF-QCMs could easily detect the kinetics of a single tether.

Aim 2.3: Chromatin

Chromatin has long been thought to be a static, non-participating structural element [1]. However, it is now clear that chromatin is a dynamic structure and histones are integral and dynamic components of the machinery responsible for regulating transcription in eukaryotes [29]. Yet, the different levels of chromatin structure and its mechanical properties due to histone modifications still remain poorly characterized.

The CF-QCM technique is particularly suitable for characterizing mechanical properties of biopolymers and chromatin in particular since one can directly measure the entropic energy changes related to removal of internal degrees of freedom by the centrifugal load. For double stranded DNA (dsDNA), stretching costs approximately $0.7 k_B T$ per base pair [4]. Though stretching dsDNA typically involves length scales far beyond the acoustic wave of the QCM, it is nonetheless sensitive to the exact conformal and viscoelastic properties of the tether on the QCM sensing surface. It is therefore an aim of this proposal to establish measurements with DNA tethered beads in the CF-QCM for studies of the mechanical and thermodynamic properties of chromatin.

First, force extension measurements will be established with the CF-QCM technique using bare lambda DNA as a toy model for extracting thermodynamic properties such as changes in entropy related to the removal of mechanical degrees of freedom under centrifugal load that extends tethered lambda DNA. After establishing the QCM centrifugal force platform, lambda DNA will then be assembled into chromatin using the well established techniques. Vollmer et. al. have used *Drosophila* chromatin assembly extracts to reconstitute chromatin with lambda DNA [30].

Figure 7 shows that regularly spaced chromatin can be assembled using *Drosophila* or *Xenopus* extract, even on a long 48 kbp lambda DNA molecule. As a control is shown the assembly of chromatin on a 3 kb plasmid (pBSKS(+)tRNA). Applying the centrifugal force QCM technique on microbead tethered chromatin will allow us to study the mechanical properties of chromatin under load. By pushing on the beads the nanotribology experiments established in previous sections on proteins will be applied to the DNA-protein chromatin complex. Next, the experimental geometry will be inverted so that pulling on the microbead with centrifugal force will allow the establishment of force extension measurements where one can directly measure changes in entropy as mechanical degrees of freedoms are removed by unbinding and unfolding of chromatin superstructures (solenoid model [31]). It is advantageous that experiments can be performed for many chromatin molecules in parallel where obtaining an ensemble average is extremely valuable since the individual conformation of chromatin complexes may vary to some extent under load.

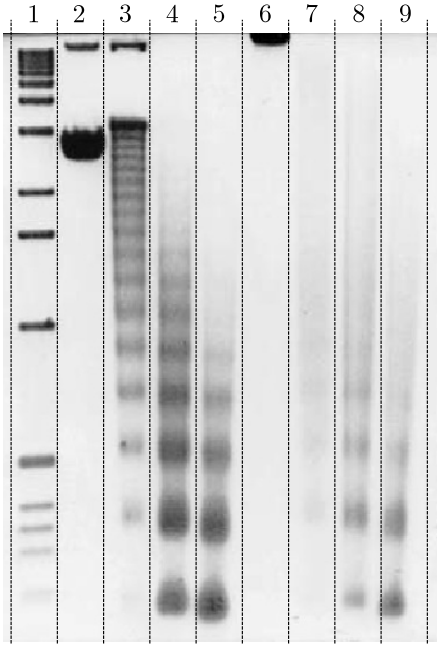

Figure 7: Micrococcal analysis of chromatin assembled with *Drosophila* extract on a plasmid versus assembly on lambda DNA. Lanes 1: Boehringer Mannheim Marker VI (220, 298, 344, 396, 517...) 2: Input Plasmid pBSKS(+)tRNA, 3-5: Timecourse Micrococcal digestion 45", 1'30", 5' 6: Input lambda DNA 7-9: Timecourse Micrococcal digestion 45", 1'30", 5'

It is possible to test chromatin models that describe the DNA-protein complex as a spring that is able to bend, twist and stretch (as opposed to WLC where segments of lambda DNA only can bend and twist but not stretch). The chromatin model is fully determined by the local density of elastic energy of the chromatin fiber (an energy per unit length). We will be able to determine the energy per unit length for the acoustic wave penetration depth of chromatin attached to the QCM, under varying loads (stretching). Such analysis can yield parameters to extend WLC model by including stretching of the protein/DNA Chromatin complex. This is the first time nanotribology as well as force extension measurements can be applied in the same experimental system.

Aim 3: Nanomechanical Assays with Cells

Thus far we have assumed that the mechanical properties each constituent of a biomaterial under investigation assume the same value. For example, in an experiment where many discrete proteins, microspheres, DNAs etc. interact simultaneously with the QCM, they all contribute equally (and linearly) to the sensor response. This simple assumption is of course incorrect; in reality such parameters will be distributed about some mean. Though our prototype instrument is currently limited to relatively low g-forces, a more advanced version with a higher spin speed would be able to reveal this distribution by sweeping k_L from weak to strong coupling (or vice-versa).

Building upon this concept, we hypothesize that such a distribution could also be assessed for mixed loads whose members assume sufficiently different mechanical properties. An example heterogeneous resonance with cells (a) and (b) is shown in Figure 8. It is known that, for example cancer cells have markedly different

viscoelastic properties than their healthy counterparts [32], and such properties are already accessable for homogeneous samples with QCMs [33]. By exploring the full parameter space with the CF-QCM technique, it should be possible to determine the presence and quantity of such members. Such analysis would be inherently rapid and parallel, being applied to many discrete members of the load simultaneously.

We will first test this principle by using binary mixtures of beads coated with different protein monolayers. The interaction with such discrete loads is better understood and will help to assess the range of forces required to accurately estimate the different distributions.

We will then focus on collections of cells. We predict that cells will form different contacts with the sensor surface. First, by focal adhesion rigid contacts will be naturally present in the strong coupling limit. However, because cells are deformable under centrifugal load, the membrane of the cell not in stiff contact with the surface but located within the acoustic evanescent wave will contribute to the CF-QCM signal in the weak coupling regime. This signal is a signature of cell deformation.

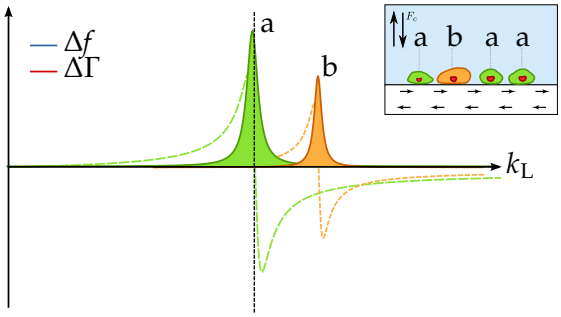

Figure 8: Bifurcation of the CF-QCM signal in response to a heterogenous load represented cells (a) and (b).

It is known that the mechanical properties of cells change when they transform into cancer cells [32]. In particular, it has been shown by AFM experiments that the elasticity modulus and viscosity will differ when comparing cell in normal state to cell in cancer state. Both of these parameters are accessible with QCM and can be modified in the CF-QCM.

Specifically, we will use common cell culture cells such as HeLa and HEK cells for initial studies; both cell lines are available in the Vollmer lab. With support from the Bonventre lab at Brigham and Women's Hospital we will have access to white blood and splenocyte cells from mice (see support letter from Dr. Bonventre). We will devise schemes to reconstitute a collection of cells in order to test if CF-QCM can discriminate the presence of cancer cells within a mixture of tissue cells. Enhanced specificity may be achieved by coating the CF-QCM gold surface with a biorecognition element specific to a subset of cells present in the sample. By varying the direction and magnitude of centrifugal force in the experiments, and independently quantifying the number of cells on the sensor by imaging, we will be able to identify circulating cancer cells form their significantly different mechanical properties.

We also believe that the technique can find applications for analyzing blood samples rapidly and at high throughput for circulating cancer cells. For such applications we will design a modified device that allows direct application of blood on the CF-QCM, separate red blood cells from larger circulating cells (lymphocytes etc.), and concentrate these cells on the sensor surface.

Timeline and Milestones

The proposed work is divided into four sections (Table 1). The first section will comprise instrument development, prototyping and CF-QCM assembly. The second section will commence together with the first section and comprise of experiments to identify surface chemistry for modification of gold coated quartz crystals with monolayers of proteins, microfluidic incorporation, and exposure to microbeads under load. Chamber design will be optimized to contain liquid samples which will require testing of different microfabricated geometries and materials. The second section will then continue with tribological studies of protein monolayers and testing of their friction and dynamics. The third section will commence after one year and will establish protocols for tethering DNA to the sensor surface, with or without microbeads. In vitro systems for chromatin assembly will be established. Different DNA linkers and functionalized microspheres and surfaces will be tested. In the

Proposed Research		Estimated Time
1st section (Aim 1)	CF-QCM instrument assembly (overall mechanical and electronic design, series of prototyping and characterization, developing software for sensor readout, integration of custom sensor electronics and optical elements)	July 2014 – July 2015
2nd section (Aim 2.1)	Nano-tribology of protein with centrifugal gravimetric sensor (establish protocols for surface functionalization, design/testing of optimized experimental chamber geometry, tribological study with proteins, testing protein friction and dynamics)	July 2014 – July 2016
3rd section (Aim 2.2-2.3)	Application to DNA dynamics (establish protocols for surface functionalization and DNA linkers, DNA stretching with micro-spheres and characterization, demonstrate detection of DNA dynamics in chromatin	July 2015 – July 2018
4th section (Aim 3)	CF-QCM study of mechanical properties of cells (cell culture on CF-QCM, functionalization of sensor surface, cell culture, compare cells, identify and detect cancer cells, detect cancer cells within mixture of cells)	July 2016 – July 2018

Table 1: Estimated Timeline of grant proposal for the completion of each sections. First section will be completed within 12 months of the project. second section will be completed within the first two years and third section commences on July 2015 and will be completed in July 2018. Fourth section will commence on July 2016 and will be completed in July 2018. Optimization of instrument design will continue throughout project period.

second year of the third section we will demonstrate detection of DNA dynamics (conformational changes) and dynamics in chromatin assembly/disassembly under centrifugal loads. The fourth section will commence one year after the third section and will continue for a total duration of two years. The 4th section will require initial results obtained form protein monolayers which will now be applied to the more complex analysis of cells attached to the CF-QCM under load. Analysis of simultaneous weak and strong couplings will allow extraction of mechanical parameters for different cell types. In the second year the approach will be applied towards detecting and identifying cancer cells. Furthermore, the approach will be applied towards detecting cancer cells within a mixture of cells.

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