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"Hearing" Bond Breakage. Measurement of Bond Rupture Forces Using a Quartz Crystal Microbalance

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We present a new method of measuring bond rupture forces. It is based on the use of microparticles attached by many bonds or interactions of one type to an oscillating surface. We have used a quartz crystal microbalance and linearly increased the driving voltage resulting in bond rupture which can be detected both optically and acoustically. The rupture force spectra give sharp peaks which depend on the mass of the microparticle and the strength of the interactions attaching it to the surface. This method is applicable to weak interactions such as nonspecific adsorption through to strong chemical bonds. It has widespread potential applications in sizing, sorting, analyzing, and separations based on differences in rupture force.

Introduction

Direct measurement of bond or interaction strengths or rupture force has been largely made by using an atomic force microscope (AFM) and optical tweezers as well as limited studies using surface force apparatus.[1] While AFM is capable of measuring bond rupture forces for individual bonds, the technique has the disadvantage that only one measurement can be made at once and there is quite wide variation in the rupture force measured, reflecting differences in molecular environment and number of bonds broken. AFM has been used to study the avidin−biotin interaction,[2,3] DNA hybridization,[4] antibody−antigen interactions[5] and the interaction between adhesion glycoproteins[6] as well as more recently chemical bonds.[7] Optical tweezers are more limited since the force that can be exerted is only of the order of tens of piconewtons. They have been used to study forces exerted by single molecular motors.[8]

We have discovered a new method to measure molecular interactions which can measure anything from relatively weak interactions such as nonspecific interactions through to hydrogen bonds and then full covalent bonds. A schematic showing the principle of the method is shown in Figure 1. This effect is based on oscillating a surface (with microparticles sitting on it) with monotonically increasing amplitude and hence acceleration. This is done by means of driving the surface with a piezoelectric acoustic wave device, in this case a quartz crystal microbalance (QCM), to measure the onset of bond rupture. The same piezoelectric device is used as a sensitive microphone, operating at its third harmonic mode, to detect acoustic noise produced by a rupture event. The method averages over a number of interactions in one experiment and hence provides precise data in a single measurement. In addition the apparatus required is very low cost.

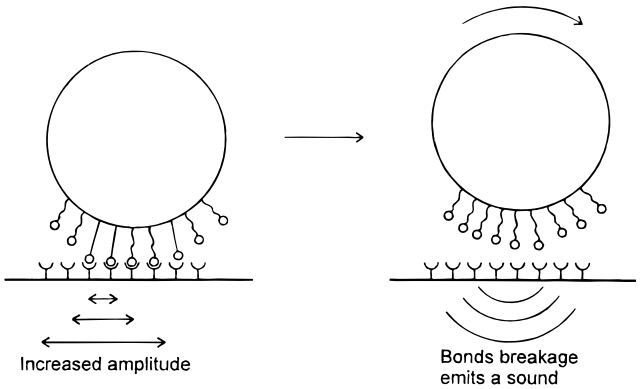

Figure 1. Schematic showing the principle of the experiment.

The rupture force recorded in any experiment is a function of loading rate. In these experiments we increase the loading by increasing the drive voltage with time. The rate of increase can affect the observed rupture force spectra since if increased slowly there is a longer time and hence greater chance that bond breakage can occur due to thermal fluctuations while there is still a barrier to bond breakage.[9] For this reason we have recorded all data presented in this paper at the same scan rate. The only other important point is, in comparison to other rupture force measurements, we apply a tangential force on our particles while in an AFM experiment the force is normal.

The quartz crystal microbalance used in these experiments is a disk of crystalline quartz with gold electrodes on the top and lower surface. It undergoes a shearing oscillation when an alternating voltage is applied to these electrodes due to the converse piezoelectric effect.[10] The QCM has a sharp resonant frequency and a high *Q* or quality factor. In most experiments to date with the QCM, changes in the resonant frequency or phase have been measured when the QCM is driven at constant voltage. These changes have been used to sensitively detect mass changes on the surface of the QCM. There has been

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previous work by Dybwad using a QCM to study the adhesive bonding between a single gold particle and a gold surface at fixed driving voltage.[11] In our experiments, in contrast to previous work, we increase the driving voltage and hence amplitude of oscillation of the QCM. In these experiments latex spheres are attached to the surface via multiple numbers of interactions or bonds of interest. We have studied a nonspecific interaction (latex−gold) and also the streptavidin−biotin interaction (multiple hydrogen bonds) and an amide linkage (covalent bond). In this paper we present the first results using this new technique and then go on to discuss possible mechanisms for our observations.

Experimental Method

The QCM's were made from polished quartz plates, AT cut at an angle of 35° (HyQ, Cambridge, U.K.). They were 8.25 mm in diameter. Layers of chromium 200−300 Å and then gold 1000−1200 Å were deposited on the surface in a 4 mm diameter circle in the center. The resonance frequency of the QCM was 14.3 MHz. The Q factor was determined by measuring the power consumed by the QCM as a function of frequency, at a fixed driving voltage. The full width, $\Delta f_{\text{resonance}}$, between half-maximum levels was determined and then the Q factor calculated by the formula $f/\Delta f_{\text{resonance}}$. In solution, experiments were performed by positioning the QCM's working gold surface face down and adding water to a reservoir until wetting at the edges of the QCM was observed due to capillary action.

In all experiments we used latex microspheres 5 μm in size. Importantly the spheres we use have only 1% variation in their diameter. The coverage of spheres used was uniform and about 1% of the surface area of the QCM. For the experiments in air the QCM was horizontal with the microspheres on the top surface. In our experiments latex spheres are attached to the surface via multiple numbers of interactions or bonds of interest. The solution of latex spheres was deposited on the surface and dried in nitrogen flow to make the physisorbed interaction. To make the streptavidin−biotin interaction, 20 μL of biotinylated bovine serum albumin dissolved in PBS buffer (10 mg/10 mL) was placed on the surface. The surface was washed and then dried in nitrogen flow, and then 20 μL of streptavidin-coated microspheres (the attachment of the streptavidin to the latex is by a covalent linkage, Bangs Laboratories, Fishers, IN) in PBS buffer was deposited on the surface. This was left for 3−4 h and then washed with water and dried in nitrogen flow. To make the amide linkage the surface was first exposed to 1 mm 12-mercaptodecanoic acid in ethanol for 24 h. The acid group was activated with EDC−NHS for 40 min and then the solution removed and the amine terminated spheres (Bangs Laboratories) added. These were left for up to 1 h and then washed in water and ethanol.

The total scan time used in all experiments was 500 s, and we observed that the bond breakage takes place over a period of 1−10 s, which is a relatively short period of time. Since there are 10^4−10^5 balls on the surface, this means that several thousand oscillations are required on average between detachment of individual balls. This is not entirely a random process since it occurs near the moments of maxima of acceleration (and displacement). Noise will be detected when the sound due to individual spheres detaching from the surface occurs at the same frequency as the acceleration maxima. This noise is quite broad band, but maxima in the noise frequency spectrum are situated near the frequency of oscillation and its harmonics. This noise is weak and needs to be detected sensitively. Our quartz crystal is a sensitive microphone but only at frequencies near its resonant modes of vibration. The fundamental mode is not very good for detection of noise due to bond breakage since we apply a large excitation voltage at this frequency which interferes with noise detection. The second harmonic mode in our setup has a completely zero piezoelectric effect producing no electricity out of vibration. The first suitable mode therefore is a mode located near to the third harmonic. Resonance of this mode occurred at a frequency $-3F + \Delta f$, where F is the fundamental frequency of the QCM and where Δf was determined to be around of 85 kHz. This frequency was selected for the lock-in amplifier to detect noise. In our experiments we recorded this noise as the driving voltage to the QCM was linearly increased starting from 0 V or alternatively we monitored the noise, at fixed driving voltage, as a function of time.

The experiments were performed in a chamber equipped with two optical windows. One provided laser illumination of the sample and the other allowed observation of the scattered laser light by an optical microscope. The QCM was placed in the chamber. A signal generator, model DS345 (Stanford Research Systems) was used to drive the QCM. Motion and detachment of particles was observed with an Olympus BH-2 optical microscope equipped with a CCD Panasonic WL-SL300 video camera. In most cases we observed the center portion of the QCM. The main measuring device was a lock-in amplifier, SR 844 (Stanford Research Systems), operating near the third harmonic (3rd resonant mode). The lock-in detects sound detected by the QCM at the third resonant mode due to bond breakage. The reference signal was fed to the lock-in using a second synthesizer. All devices were interfaced to a computer for control of the experiment and collection of the data. All scans consisted of 1000 points with a dwell time of 0.5 s/point.

We measured the temperature rise in both air and water in these experiments using 50 mm diameter thermocouple wire (T1 and T2, Goodfellow) glued to the outer edge of the 4 mm diameter electrode using silver epoxide. Using thicker thermocouple wire 120 mm in diameter we confirmed that there was only a small change in the measured temperature, indicating the thermocouple was not conducting significant heat from the QCM. In air with a voltage of 1 V the temperature rise was 1 K, and for a scan from 0 to 10 V the temperature rose by 30 K. In water the corresponding figure for a scan from 0 at 10 V was 7 K.

Results and Discussion

Figure 2 shows the data obtained in air for the nonspecific interaction, the streptavidin−biotin interaction, and one amide bond. This is presented as both noise signal versus driving voltage, which we will refer to as the rupture force spectrum, and also plots of shift in resonant frequency of the fundamental mode versus driving voltage. The maximum voltage we can apply with the current experimental setup is 10 V. The rupture force spectrum for the nonspecifically adsorbed latex spheres shows peaks near 0.1 V. We found that the noise signal is linearly proportional to the number of spheres on the surface. The spectra for the streptavidin−biotin spheres show no peaks in this region but two peaks close to 6 V at high loadings as shown here (1% of surface coverage) and a single peak at low loadings (0.1−0.2% surface coverage). The amide linkage shows no peaks at all in the spectrum. Note the peaks are quite sharp in the rupture force spectrum. The second scan, following immediately after the first, of the streptavidin−biotin spheres shows no peaks at 6 V but only peaks below 1 V indicating the streptavidin−biotin interactions have not re-formed. If the spheres are left for 6−12 h after bond breakage we observe the same rupture force spectrum as in Figure 2a indicating the streptavidin−biotin interaction has re-formed (data now shown). There is experimental evidence that the mass sensitivity and amplitude of the QCM varies with spatial position in both air and liquids.[12,13] At resonance the amplitude of the QCM shows a Gaussian variation with the maximum amplitude at the center. This would mean that we should observe broad peaks in the rupture force spectrum with a similar Gausssian shape and not the sharp peaks that we do observe. However the

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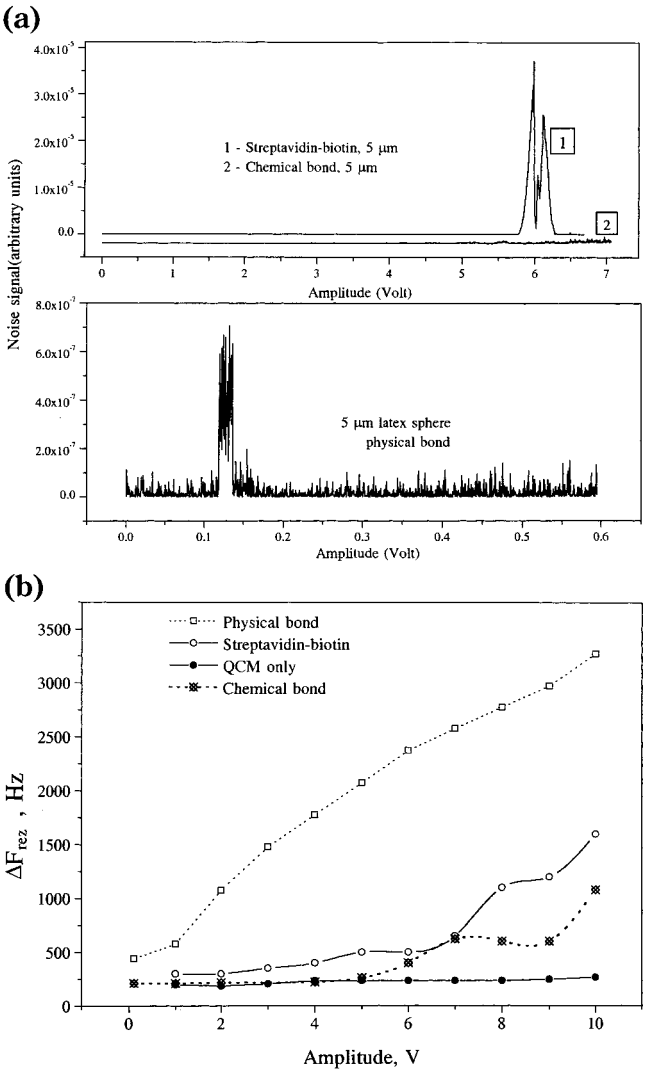

Figure 2. (a) Rupture force spectra of noise signal versus applied voltage for a nonspecific interaction (latex−gold), streptavidin−biotin interaction (streptavidin covalently attached to the latex sphere and biotinylated bovine serum albumin on the gold), and chemical bond (amide bond formed between an acid terminated thiol layer and an amine labeled latex sphere). All spheres are 5 μm in diameter. (b) Shift in the resonance frequency of the QCM versus applied voltage for the same nonspecific interaction, streptavidin−biotin interaction, and chemical bond as described above.

QCM operating as a microphone at the third harmonic will show a similar spatial dependence being most sensitive at the center and the sensitivity decreasing radially toward the edges. To test this we performed experiments depositing the nonspecifically adsorbed latex spheres at the center of the QCM and at the edge. These experiments confirmed that the dominant contribution to the detected acoustic noise was from the particles at the center of the QCM.

The plots of frequency shifts versus voltage amplitude are shown in Figure 2b. The QCM with no spheres shows no change in frequency with voltage. In contrast the nonspecifically adsorbed spheres show a shift in resonance frequency between 0 and 1 V and the size of the shift increases with applied voltage. The streptavidin−biotin spheres only start to show a shift near 6 V—the same voltage as measured in the rupture force spectra. This shift also increases with applied voltage. The QCM with chemically attached spheres shows a change near 6 V and a second change near 9 V although no noise was observed

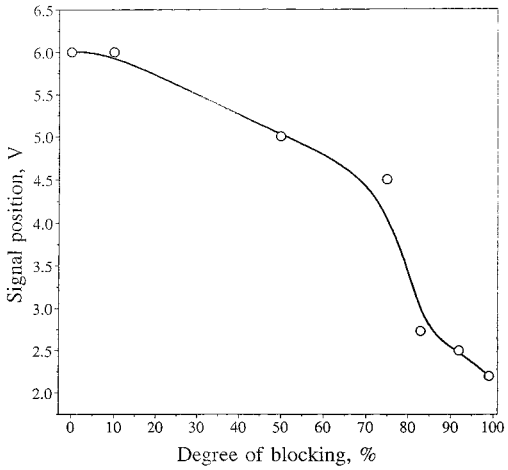

Figure 3. Shift in the position of the first and main peak in the rupture force spectrum with blockage of streptavidin sites on the latex microsphere. The line is an aid to the eye.

at these or any other voltage. We assign these changes to the reduction in mass due to the detachment of the spheres from the surface and then the spheres rolling around on the surface and eventually rolling off. At high amplitude we can observe this to take place within a few seconds, and that it takes longer at lower amplitude. The removal of the spheres from the surface explains why we observe a reduction in frequency while Dybwad[11] observed an increase in frequency with a single particle weakly mechanically coupled to the surface. Further work is required to understand why we observe frequency shifts for the chemical bond but no detectable noise or observation of detachment of spheres. Our results suggest that while both methods can detect detachment of the spheres from the surface the noise is a more sensitive method than observation of shifts in the resonant frequency. Both these experiments clearly demonstrate we can detect breakage of both the nonspecific and streptavidin−biotin interactions. The stronger interaction requires the higher voltage (×60 higher), and there is not sufficient force to break the chemical bond in this experiment.

We have performed a series of experiments where we titrate the streptavidin sites on the sphere with biotin and then attach it to the surface. We assume that the blocking is a random process so that the fraction of streptavidin sites blocked in the contact region between the microsphere and the surface is the same as the fraction of sites blocked over the entire microsphere. The variation in the position of the first peak is plotted against coverage in Figure 3. As the number of available streptavidin sites is reduced, the peak shifts to lower voltage. However this does not show a linear dependence. If the force was proportional to the number of streptavidin−biotin interactions, then as the number of these interactions is reduced due to blocking one could expect to observe a linear decrease in the rupture force with degree of blocking. We observe a sharp rollover near 70% blocking suggesting that it is the breakage of the remaining 30% of the streptavidin−biotin interactions which is important in determining the rupture force.

To gain further insight into the mechanism of detachment we have monitored the noise signal versus time when the QCM is driven at a voltage slightly above that required for detachment. The driving voltage is jumped from 0 to 1 V in the case of the nonspecific interaction and 0 to 8 V in the case of the streptavidin−biotin interaction. The results are shown in Figure 4. The nonspecific interaction shows immediate noise and then a series of peaks at later

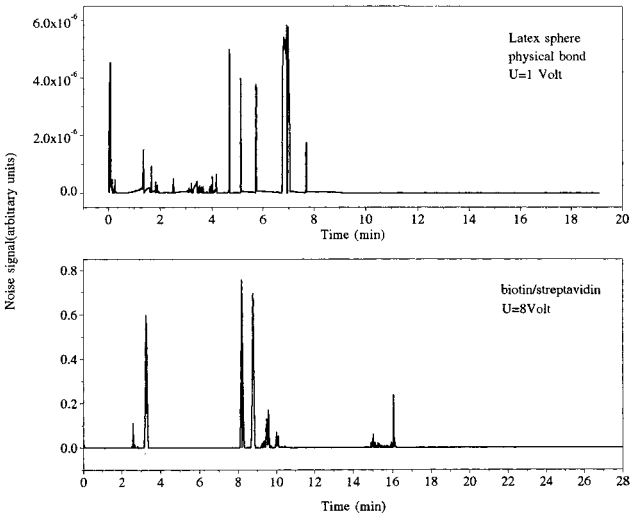

Figure 4. Noise signal versus time plots for the nonspecific interaction and streptavidin−biotin interaction when the applied voltage is jumped from 0 to 1 and 8 V, respectively.

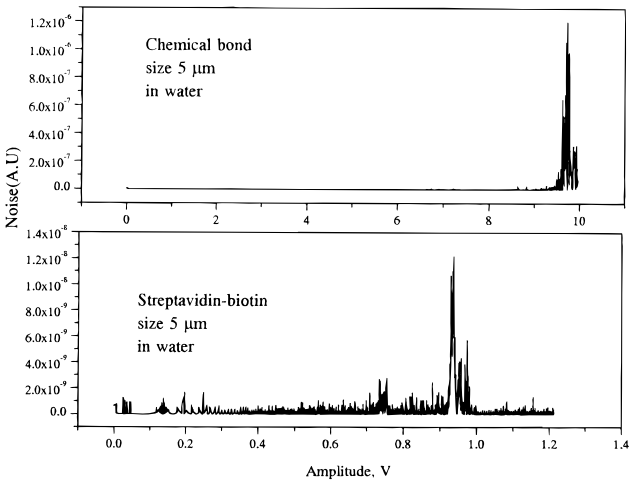

Figure 5. Rupture force spectra *in water* for thestreptavidin−biotin interaction and a chemical bond. These are the same as in Figure 2. All spheres are 5 μm in diameter.

times. In the streptavidin−biotin interaction experiment there is no immediate detachment, but after a period of time we observe noise as the spheres break off the surface. We observe two main periods of noise at different times. At the time that the potential is jumped, no interactions attaching the sphere to the surface have been broken, all interactions need to be broken before the microsphere can be observed to move under the microscope and we concurrently detect acoustic noise. For the nonspecific interaction this occurs immediately since the sphere already has enough thermal energy to break the interactions holding it in place. For the streptavidin−biotin interaction thermal energy is insufficient to break the interaction, and thus, we believe we observe a delay until sufficient interactions have been broken so that thermal fluctuations can break the remaining interactions or positive feedback can occur. Further experiments are needed applying smaller or larger voltage jumps to support this hypothesis. It is also noticeable that the peaks in Figure 4 are not random in time. This suggests that there may be some cooperative component to the observed detachment at higher coverages.

To make the measurement more quantitative we have estimated the amplitude of oscillation of the QCM on the basis of experimental data, and hence can estimate the force on the microsphere. This was performed by measuring the power consumption of the QCM and its Q factor (or merit factor—the reciprocal relative resonance bandwidth $= f/\Delta f_{\text{resonance}}$). The amplitude of the QCM, A, is given by

$$A = \sqrt{\frac{QP}{2\pi^3 f^3 M}}$$

This formula is based on energy balance assuming 100% efficiency in conversion of electrical energy to a mechanical work. Q is the Q or merit factor, P is the electrical power consumed by the QCM, f is the resonant frequency of the quartz crystal, and M is the effective mass of the QCM quartz plate (taking into account that the amplitude is greater at the center of the QCM). The Q factor in our experiment in air was determined to be 5000−15000 at 6 V, depending on the QCM balance used and loading. Assuming a Q value of 10 000 gives an estimate of the vibrational amplitude at the center of the QCM of 150 nm (for a 6 V excitation voltage).[14] Since the latex spheres have a mass, m, then the peak force is given by $m(^2/_7)A(2\pi f)^2$. This formula is derived by solving the equation of motion for a ball pivoting about its point of contact to the QCM and the factor of $^2/_7$ takes account of the fact that the center of mass of the sphere does not move the full distance of the pivot point. The mass of a sphere is 69 pg so the force on the sphere is therefore estimated to be 26 μN. This should be compared to the force needed to break the a single streptavidin−biotin interaction[2] of 160 pN and indicates that approximately 160 000 interactions are broken simultaneously. We also estimate that this corresponds to 50% or more of the initial streptavidin−biotin interactions between the sphere and the surface assuming a close-packed layer of streptavidin on the surface of the sphere. This is in reasonable agreement with the turnover we observed near 70% blockage of the streptavidin−biotin interactions in Figure 3. While this calculation is only approximate, it suggests that in these experiments the majority of the interactions attaching a sphere to the surface are broken simultaneously. This may be the reason that sharp peaks are observed in the rupture force spectra and that there is detectable noise on detachment.

All the data presented above were obtained in air. We have done preliminary experiments in water and have observed bond rupture for both the streptavidin−biotin interaction and also the chemical bond as shown in Figure 5. We observe noise and hence bond rupture at 1 V for the streptavidin−biotin interaction and near 10 V for the chemical bond. It is most likely that the bond broken is the gold−sulfur anchor since this is the weakest bond.[7] There are two competing effects when experiments are performed in liquids. First the Q factor of the QCM decreases due to liquid loading reducing the amplitude of oscillation at a particular voltage compared to air. In our case this was a reduction by approximately a factor of 7 but varied between experiments. However, in liquids there are viscous forces acting on the microsphere and in addition the effective mass is increased due to its associated layer of water increasing the force on the sphere. Our observation of the reduction in the critical voltage for

(14) The order of magnitude of this calculation is consistent with a more sophisticated calculation by Kanazawa (Kanazawa, K. K. *Faraday Discuss* **1997**, *107*, 77) where for a QCM with a resonance frequency of 5 MHZ and an unloaded Q factor of 100 000 and then for a 1 V peak rf voltage the displacement is calculated to be 132 nm. It is also in agreement with experimental measurements in ref 13.

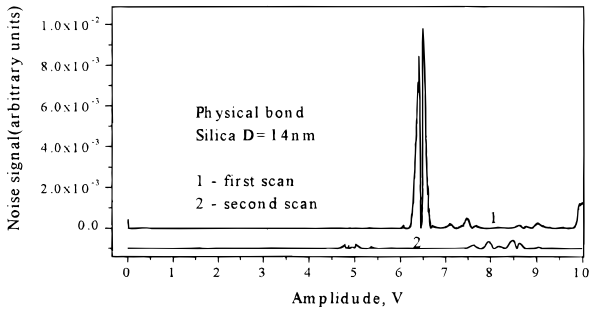

Figure 6. Rupture force spectra in air for nonspecifically adsorbed 14 nm silica particles. The first and second scans are shown.

the streptavidin−biotin interaction (by a factor of 6 from 6 to 1 V on going from air to water) and the breakage of a chemical bond in water indicates the latter effects are dominant. Thus performing these types of experiments in water or liquid should be straightforward.

Assuming the same density for the nonspecific interaction, the streptavidin−biotin interaction and the chemical bond and that the rupture forces are not changed on going from air to water, then we can obtain a relative scale of rupture force. This is 1:60:600 for the nonspecific interaction:streptavidin−biotin interaction:chemical bond, although the nonspecific interaction data were recorded at a slower scan rate than the other two bonds and so its strength may be slightly under estimated. This scaling appears reasonable and demonstrates the dynamic range of this method. In fact the published values are 160 pN for the streptavidin−biotin interaction[2] and 1.4 ± 0.3 nN for the gold−sulfur anchor bond.[7] Thus the ratio of rupture forces in our experiment is in good agreement despite the fact we apply a tangential force and the AFM applies a normal force so the two experiments are not strictly directly comparable. By suitable calibration experiments with known bond densities it should be possible to make these measurements quantitative. While the forces exerted on the latex spheres are large, compared to an AFM, due to their large mass, we have data which show that it is straightforward to work with nanometer size particles with far fewer bonds or interactions attaching the particle to the surface. To illustrate this the data for nonspecifically adsorbed silica particles 14 nm in diameter in air are shown in Figure 6. In the first scan a peak was observed at 6.5 V in contrast to the 5 μm diameter latex spheres in air, where the peak was observed at 0.1 V. In the second scan all the particles have been removed from the surface and no peak was present.

Further work is clearly required to elucidate the mechanism of bond rupture. At this stage we can however rule out some possible mechanisms. Heating could give rise to bond rupture, but the temperature rise in our experiments is 30 K in air and 7 K in water and this is far too small to explain the observed breakage of a chemical bond. This means that the force applied to the particle as a result of the large acceleration to which it is subjected is the most likely explanation of our results. Our experiments suggests that, at a certain critical amplitude, the acceleration and hence force on the spheres in the center region of the QCM are sufficient to rapidly break the bonds or interactions attaching the sphere to the surface. We would speculate that initially all the bonds attaching the sphere to the surface are in place and that during the scan some of these bonds or interactions are broken. At this critical acceleration, positive feedback occurs since once one bond is broken the same force is applied to less bonds so another bond breaks resulting in rapid breakage of the remaining bonds. This model is consistent with all our experimental data to date and explains the sharp intense peaks we observe in the rupture force spectra, but more experiments are required to confirm its validity.

Concluding Remarks

This work provides a new, sensitive, low-cost, and potentially quantitative tool to probe the forces involved in molecular recognition and to measure bond strengths. Further experimental and theoretical work is clearly required to gain more insight into the mechanism of sphere detachment, and to understand how to interpret the observed rupture force spectra. We have shown that the rupture force spectra we observe have sharp peaks and that the positions of the peaks are a function of the strength of the interactions attaching the sphere to the surface and the mass of the particle. This method appears significantly more sensitive than impedance measurements for the detection of bond breakage, and the relative rupture forces we measure are in good agreement with measurements using atomic force microscopy.

The method presented here also has widespread potential applications for separation, sorting, and sizing. The experiments show that in air we could separate streptavidin-labeled spheres from normal latex spheres using a QCM with a biotinylated surface and with a driving voltage above 0.1 V but below 6 V. The normal latex spheres would be removed from the surface leaving only the streptavidin-labeled spheres attached to the surface (by the stronger streptavidin−biotin interaction). This opens up a new form of separation science based on variable force applied for a certain length of time which could have widespread application, for instance, in particle sizing and sorting as well as the design of new biosensors. Such a separation method would be low cost and could easily be multiplexed and automated.

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