Single-molecule force spectroscopy of DNA-based reversible polymer bridges: Surface robustness and homogeneity

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1. Introduction

The sign and magnitude of the interaction energy between two solid surfaces directly affects adhesion, friction and colloidal stability [1]. For non-coated surfaces, the interaction energy is defined by the identity of the bulk material. Coatings can be added to the surfaces of bulk materials to alter their interaction potential. Covalent polymers have been used extensively for this purpose, and the surfaces of bulk materials to alter their interaction potential. Cova-

interaction energies are a result of many interactions over an area between two surfaces. In certain situations, however, it is desirable to deconvolute the ensemble energies into those of various single polymer-surface contributions to adhesion and other surface interactions. Single-molecule force spectroscopy [17–30] (SMFS) using the AFM has been used for this purpose. For example, Gaub et al. have recently used this technique to characterize bridging of single polyelectrolyte chains between surfaces and how the extent of bridging depends on the solution in which the poly-

Like covalent polymers, main chain reversible polymers (also known as “supramolecular”, “equilibrium”, or “living” polymers) can be used to modulate the interaction energy between surfaces. Main chain reversible polymers (hereafter, reversible polymers) are similar to their covalent counterparts in that they are high molec-

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polymers, because of their weakly interacting monomer units, are able to respond to their environment making their structure, and molecular weight amendable. Typically, this is exploited in the context of stimulus-responsiveness [41,42,53] but it can be used to alter surface interactions in ways not possible using covalent polymers. Beyond modulating surface interactions, reversible polymer self-assembly between surfaces holds promise for nanoscale device fabrication. For example, one challenge with hybrid top-down and bottom-up assembly methods is to adequately match the structure and function of molecular interconnects to the gap between device features. The weak, reversible interactions holding reversible polymers together can be exploited for this purpose: the reversible polymers can be designed to self-assemble across a gap, connecting two surfaces. Additionally, because the molecular weight (length) of reversible polymers is responsive to their environment, autonomous bridge repair mechanisms can be envisioned.

While the reversible polymer strategy is conceptually enticing, experimental probes of reversible polymer bridging between surfaces are limited. Previously, our group showed that SMFS using the AFM could be used to characterize the formation of DNA-based reversible polymers between an AFM tip and a surface, with a gap width \( d \) of 5–10 nm between the two surfaces, as shown in Fig. 1(a) [28]. The same DNA-based system was recently used to study the dependence of bridge formation on \( d \) and the time the bridges were given to form (\( t_{\text{wait}} \)) [29]. In a typical experiment, the substrate-surface and AFM tip were brought into gentle contact and the increase in \( d \) as a result of natural instrument drift, was used to probe the distance dependence of intersurface self-assembly. Force curves were collected by retracting the substrate from the AFM tip, as shown in Fig. 1(b). For each curve, \( d \) was calculated from the drift rate and the time at which each consecutive pull was executed. A typical pulling experiment lasted several minutes, until \( d \) reached \( \sim 50 \) nm, at which point the substrate was repositioned in the vertical axis and the procedure was repeated. The results showed that the bridging probability increased as a function of increasing \( t_{\text{wait}} \) and decreasing \( d \), which is consistent with theoretical predictions [41].

For the above and related systems, bridging probabilities need to be determined over multiple areas of a substrate surface, as well as on multiple areas on different surfaces on different days. The central concern is the homogeneity of the DNA monolayer. Along these lines, Franzen and coworkers [54] previously studied the composition of Au surfaces on which thiolated DNA was immobilized and subsequently backfilled with 6-mercapto-1-hexanol (MH). They added DNA-modified Au nanoparticles to the DNA-modified surfaces, with the DNA sequences on both surfaces fully complementary, and performed STM imaging. STM imaging of the surfaces revealed the nanoparticles to be distributed in an apparently random manner over the surface, such that different areas (\( \sim 50 \) nm\(^2\)) of a surface have significantly different numbers of hybridized nanoparticles (from \( \sim 0 \) to 10). In contrast, Li and coworkers used cyclic voltammetry to study self-assembled monolayers of DNA, with MH as a backfill [55]. Based on the resultant electrochemical properties of the film, the authors concluded that the monolayers contained no phase separated regions larger than \( 15 \) nm\(^2\) (the limits of the spatial sensitivity of the technique).

More recently, Bizzotto and coworkers investigated monolayers of DNA using electrochemistry, and in situ fluorescence microscopy. The results showed micron-scale heterogeneity, which was mainly attributed to large DNA aggregates physisorbed to the attached monolayer of DNA that remains after exposure to MH [56].

The coverage of DNA on the surfaces we are using to evaluate reversible bridging is therefore most likely heterogeneous on the length scale of the AFM experiments. The questions of surface heterogeneity and adequate sampling are, by necessity, persistent in single-molecule force spectroscopy or any other technique that has the potential to deconvolve ensemble behavior, and they present critical challenges for experimental design. Rather than rely on inefficient, \( ad \) hoc sampling strategies to confirm reproducibility in the data, we therefore sought to validate a robust surface sampling methodology in the context of reversible polymer bridging. Here we report an improved experimental procedure, which allows significant control over both the area of a surface being investigated and the distance \( d \) between the tip and surface. Numerous experimental factors are evaluated, including the effect of the frequency of repeated tip/surface contacts, the area of the substrate surface sampled by the AFM (Fig. 1(c)), and the use of multiple AFM tips and substrates on the observed bridging probability. The results support the notion that the surface coverage of DNA is heterogeneous, as reported by the Bizzotto group, and sampling methods that efficiently address the relevant heterogeneity are presented [56]. Finally, we demonstrate that relative bridging probabilities, for example those due to distance dependencies, are consistent even under circumstances in which significant day-to-day variations in the absolute number of events are observed—a result that has important consequences for series of experiments that would otherwise take longer than is practically feasible.

2. Experimental

2.1. Materials

MLCT-AUHW AFM tips were purchased from Veeco Probes (Camarillo, CA). Si wafers were purchased from Wafer World (West...
Palm Beach, FL). DNA was purchased from Operon (Huntsville, AL) and was purified by HPLC prior to use. Cleland’s Reductacryl reagent was used for reduction of any disulfides present in our DNA–thiol compounds and was purchased from Calbiochem (San Diego, CA). 6-Mercapto-1-hexanol and absolute ethanol was purchased from Aldrich (St. Louis, MO). Chemicals used to make buffer solutions were obtained from Fisher Scientific (Fairlawn, NJ). All water used in these experiments was deionized (DI) to at least 18.0 MS cm using a Nanopure system from Barnstead (Dubuque, IA).

2.2. Instrumentation

Automated pulling experiments were carried out on our custom 3-axis AFM, built in house. The AFM is equipped with a MultiMode AFM head from Digital Instruments (Santa Barbara, CA) and 2 piezoelectric positioning stages from Physik Instrumente L.P. (Auburn, MA). The x, y-stage (P-733.2CL) has a scanning range of 100 μm × 100 μm and a closed-loop resolution of less than 0.3 nm and the z-stage (P-753.11C) has a traveling range of 12 μm and a resolution of 0.05 nm. The AFM head is mounted on the x, y-stage which is suspended above the z-stage via 3 high-precision screws from Newport Corp. (Irvine, CA). Substrate samples are magnetically and adhesively secured onto the z-stage such that the AFM head remains stationary during approach/retract cycles. The control scheme for the AFM was designed in MATLAB’s (The Mathworks, Natick, MA) Simulink environment and was digitally implemented through the use of a dSPACE (Wixom, MI) DAQ card (DS1104).

2.3. Methods

Si substrates were cleaned by immersion in 3:1 H2SO4:H2O2 for 1 h (Caution: Piranha solutions react violently in the presence of many organic compounds and should be handled with extreme care). Si substrates were then rinsed with copious amounts of DI water and dried with a stream of nitrogen gas. 10 nm of Ti (used to promote adhesion of Au to the Si) was deposited on the Si followed by 300 nm of Au using a Kurt Lesker PVD 75 DC sputter coater (Clairton, PA). Au-coated Si substrates were cut into pieces and cleaned by exposure to UV irradiation for ∼10 min, according to a previously published protocol [57]. The Au-coated substrate was then dried under a stream of nitrogen gas, and a ∼30 μL drop of 1 μM DNA solution (1(5′-3′)GGTATACCC3H2SH) was added to the substrate surface for 4 h under a humid atmosphere. Prior to use, the 1 μM DNA solution was exposed to ∼4 mg Cleland’s Reductacryl reagent for 30 min, while agitation, to reduce any DNA disulfide bonds to thiols (thiols are known to oxidize to disulfides under ambient conditions). The reduction step was followed by filtering the solutions to remove the Reductacryl reagent. The substrates were then rinsed with DI water, dried under a stream of nitrogen gas and exposed to ∼10 mL of 5 μM MH solution for 1 h. The substrate was again rinsed copiously with DI water and dried with nitrogen gas. DNA-coated substrates were stored at −4 °C and used within 1 week. Previous studies, using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), showed DNA immobilization in this manner to be successful [29]. Also, immediately before use, Si3N4 cantilevers were exposed to UV irradiation for 10 min, soaked in absolute ethanol for 20 min, rinsed with DI water, and dried with nitrogen gas.

All experiments were carried out using the same general procedure, using all similar experimental conditions. The functionalized substrate surface and AFM tip were exposed to a solution (1 M NaCl, 0.01 M phosphate, 200 ppm NaN3, pH 7.2) of oligonucleotide 2 (1(5′-3′)GGTATACCGCTAAGG) with concentrations ranging from 10 to 20 μM, depending on the experiment. The AFM cantilever had a nominal spring constant of 20 pN nm⁻¹, as reported by the manufacturer.

Measurements were initiated once the drift rate of the surface away from the tip reached a value of less than ∼0.17 nm s⁻¹. The pulling process and distance-referencing procedure is shown schematically in Fig. 2, and was implemented using MATLAB’s Simulink environment. First, the vertical deflection signal, and hence the force value, coming from the cantilever was zeroed. This was done by collecting the vertical deflection signal measured from the quadrant photodiode with the cantilever fully disengaged from the surface for 2 s and averaging that signal. That value was then set to zero vertical deflection (zero force). The surface was then raised toward the tip at a rate of 50 nm s⁻¹ until contact was made and continued until the force readout from the cantilever reached ∼500 pN (force trigger). Once the force trigger was reached, the z-stage movement was stopped, and the position of the z-stage at contact was calculated by the software. This value is simply ∼25 nm below the z-stage position when the force trigger was reached (500 pN × (20 pN/ nm⁻¹)). The z-stage was then lowered ∼125 nm (100 nm past contact) at 150 nm s⁻¹ to remove nonspecific adhesion. The surface was then brought back toward the tip and stopped to leave the desired gap width (d).

The majority of the experiments was conducted over a grid on a surface, as depicted in Fig. 1(c). The grid area was defined by the number of spots on the grid in the x-dimension (x_g), the number of spots on the grid in the y-dimension (y_g), and the spacing between each spot (s). At each spot on the grid, the surface was found and d set, as indicated above. Then, n consecutive pulls were conducted. Here, n was typically set to 4, unless otherwise indicated. Four consecutive pulls at a given t_wait kept d within a range of ∼6 nm from its initial set point. Following n pulls, the surface location was found again and d was reset to counteract drift. The number of times the surface location was found at each grid spot (n_tot) multiplied by n gave the total number of pulls conducted at a given grid spot (n_tot).

The pulling velocity was 150 nm s⁻¹, the pulling distance was 100 nm, with the data collected at 5000 Hz. The raw vertical photodiode signal was filtered at 500 Hz by an 8-pole low-pass Bessel filter (Model 500CT, Frequency Devices, Ottawa, IL). Data from at least 2 grids (separated by 10 μM) under identical experimental conditions were used to calculate the average number of events per
pull (EPP). EPP was calculated by totaling the rupture events over the grids and dividing by the total number of pulls, typically ∼800 pulls (2 grids) or ∼1200 pulls (3 grids). The EPP value is used as the metric by which to assess bridging probability, with a relatively high value corresponding to relatively high bridging probability, and vice versa. To pick out a rupture event, we used the following procedure/criteria. For each pull, the vertical signal and z-piezo position was filtered using a 6-point window boxcar average and converted to force and tip/substrate separation, as seen in Fig. 3, inset. The absolute forces required for bond rupture were taken as either the differences of the peak for the rupture event and the baseline or the difference of the peak for the rupture and the onset of the next rupture event (for multiple bond rupture) and were only counted as a rupture event if that difference was ≥9.5 pN.

3. Results

3.1. Rupture forces

Unlike the majority of SMFS experiments, here the actual forces at rupture are not the data of interest; characterizing the number of events, and not the rupture force associated with those events, is the goal. Bridging was evaluated between a bare Si$_3$N$_4$ AFM tip and a DNA modified surface in the presence of 20 μM of 2. For these experiments, d was actively set to 20 nm using the surface finding algorithm detailed above, and t$_{wait}$ was set to 9 s. Fig. 3 shows a histogram for the observed rupture forces obtained for a typical experiment. The most frequently observed rupture force of ∼17 pN is comparable to that obtained previously with similar systems [28,29]. The consistency between the most frequently observed rupture forces for all experiments leads us to believe that the use of the nominal spring constant for calculating rupture forces has no effect on our interpretation of the results. The distribution of forces is monomodal, indicating that we are primarily probing single polymer bridges. Some contribution from the effectively simultaneous rupture of multiple bonds must be present, but we observe no evidence that it makes a meaningful contribution to the desired statistics.

At this point, it is unknown where along the DNA chain the force-induced rupture occurs, e.g. at the tip–DNA interface or along the DNA backbone. While this question is both interesting and potentially important in other contexts, the results presented here do not depend on the process that leads to the observed rupture event; only the presence of the bridges is significant. Investigations of the rupture mechanism are ongoing and will therefore be addressed in future work.

3.2. Surface robustness

Forces observed in the pulling experiments were much lower than those associated with gold–thiol dissociation and/or the extraction of gold from the substrate [58,59], and that comparison suggests that the thiolated DNA monolayer is not disturbed during the pulling experiment. In order to verify that neither the pulling nor the repeated tip–surface contact (during the distance-referencing procedure) damage or otherwise change the surface structure in a way that affects subsequent measurements, we conducted a pulling experiment on a single spot on the substrate and compared the bridging probability for the initial pulls to the bridging probability for the final pulls. Specifically, we pulled 400 times on a single spot following a typical protocol: 4 consecutive pulls were conducted on a spot, the surface contact point was relocated, and 4 more pulls were conducted, etc. This process was repeated at the same location such that 400 pulls were obtained (n = 4, z$_{tip}$ = 100, and n$_{tot}$ = 400; see Section 2). The EPP for the first 25 pulls and the last 25 pulls were nearly identical (0.88 ± 0.15 vs. 0.95 ± 0.20, respectively), an observation supported by analysis of variance (ANOVA) testing that confirms that the bridging probability for the pull conducted at the beginning of the experiment to be statistically equivalent at the 95% confidence level. We conclude that the surface was not affected by the repeated contact with the AFM tip under this experimental protocol. Similar observations have been made previously on related systems, although the details of the experimental procedure are different in all cases [28,29,52].

It is also possible that the initial contact and/or rupture events change the surface of the Au substrate and/or AFM tip, but only for a very short time: e.g. the very first pull, the effect of which might be “canceled out” in the multiple-pull averaging described above. To probe this possibility, we compared the EPP for a series of “first pulls” after contact to the EPP obtained by considering increasing numbers of pulls after contact. The experiment was conducted over a grid (grid parameters were x$_{grid}$ = 5, y$_{grid}$ = 5, s = 10 nm, n = 4, z$_{tip}$ = 4, and n$_{tot}$ = 400; t$_{wait}$ = 9 s and d = 20 nm), and the data were broken down into smaller data sets: one including only the first pull after each reference contact, a second containing the first two pulls after contact, and so on. This same procedure was repeated to give average EPP values for various numbers of pulls following contact. The results are shown in Table 1. An ANOVA was performed, and all values were found to be statistically equivalent at the 95% confidence level, indicating that whether 1–16 pulls following each tip–surface contact were considered (25–400 total pulls over a grid), the average observed EPPs still fall within the range of statistical equivalence. Again, it is apparent that tip–surface contact history does not influence the observed measurements.

<table>
<thead>
<tr>
<th>Pulls per Grid</th>
<th>Observed EPP</th>
<th>Std. error</th>
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<tbody>
<tr>
<td>25</td>
<td>1.12</td>
<td>0.19</td>
</tr>
<tr>
<td>50</td>
<td>1.26</td>
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<td>100</td>
<td>1.17</td>
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<td>1.38</td>
<td>0.08</td>
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<tr>
<td>400</td>
<td>1.34</td>
<td>0.06</td>
</tr>
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</table>

Fig. 3. Histogram of observed rupture forces for 20 μM DNA 2 in pH 7 phosphate buffer. The inset shows a sample force vs. extension plot depicting a single rupture event, where positive forces indicate surface–tip attractive forces.
The mean value does not change over time.

as \( n_{\text{err}}^{0.4} \), in good agreement with the inverse square root dependence expected for random variations in the absence of systematic changes as a function of number of pulls.

### 3.3. Surface heterogeneity

As discussed in the Section 1, the presence and possible effects of surface inhomogeneities are not obvious \textit{a priori}. The programmable grid size in the experimental design provides an easy mechanism by which to characterize surface heterogeneities and their effect on the bridging experiments, and so the bridging probabilities were measured and compared for multiple grids of various sizes. First, bridging on a 40 nm × 40 nm square grid was compared to that observed at a single spot from the grid corner (400 pulls each), as depicted in Fig. 1(c). The same surface finding/pulling algorithms described above were used for both the single spot and grid. The combined single spot and grid pulling routines were carried out back-to-back for each of 3 different locations of the same surface, with consecutive locations separated by \(~10\,\mu m\).

Fig. 5 shows that the observed EPP obtained for the single spot and grid at each of the three locations were statistically equivalent, as determined by ANOVA comparison tests at a 95% level. Thus, the surface appears to be effectively homogeneous over \(~1.6 \times 10^3\,\text{nm}^2\), at least for the experiments under consideration here. This conclusion is supported by spot-by-spot comparisons taken from a single grid, as seen in Fig. 6 for data taken from the third grid in Fig. 5.

Interestingly, while the EPP values for the spot and grid experiments are statistically equivalent at each location, the values at the three individual grid locations themselves are found to be statistically inequivalent (see Fig. 5). This is indicative of a surface that is \textit{locally} homogeneous but \textit{globally} heterogeneous (here, for locations separated by \(~10\,\mu m\)). Why should spots that are close to one another on a surface be more likely to have similar chemistry than those that are far away? One contribution likely comes from the fact that a “spot” is really much larger than a single point, because the surface area probed by the AFM includes a significant contribution from the AFM tip itself, which has a radius of curvature of 20–60 nm. When the AFM is used to sample multiple spots on a \(~40 \times 40\,\text{nm}^2\) grid, therefore, it is likely that the actual surface area being probed at any spot along the grid has considerable overlap with that probed at another, ostensibly different, spot. We note that recent results from the Bizzotto group \[56\] demonstrate that DNA-functionalized surfaces can be heterogeneous on these length scales.

We therefore went on to characterize the effect of grid size on the observed EPP. Here, we varied \( s \) to give 400 nm × 400 nm (\(~1.6 \times 10^5\,\text{nm}^2\)) and 800 nm × 800 nm (\(~6.4 \times 10^5\,\text{nm}^2\)) grids and compared the observed bridging probability to that obtained over 40 nm × 40 nm (\(1.6 \times 10^3\,\text{nm}^2\)) grids. For each grid size, measurements were taken on two different locations on the surface. In all cases, data were collected using the same surface and tip on the same day, all using the same solution of 2. The results are shown in Fig. 7. ANOVA tests were performed to compare the data obtained for a given grid size, on different surface locations. We found the statistical similarity between grid locations to increase as a function of grid size: the two EPP values obtained for different locations of the largest grid size (\(~6.4 \times 10^5\,\text{nm}^2\)) are statistically equivalent at the 95% level, while the values obtained for two different locations of each of the two smaller grids are statistically inequivalent. When the two grids of each size are combined, an ANOVA comparison shows that the two larger grid sizes are statistically equivalent at the 95% confidence level, but the smaller grids remain outliers.

The picture that emerges from these results is as follows. As expected, the charge and size of the DNA does not permit well-packed and highly ordered monolayers that are homogeneous over large length scales. Instead, the distribution of DNA is inhomogeneous, not just because of large-scale phase segregation and aggregation, but also due to the stochastic nature of thiolated DNA bond formation with the gold surface. Regions of different surface densities lead to different bridging probabilities \[52\].

An AFM-based bridging experiment on a single spot or small \(\sim40\,\text{nm} \times 40\,\text{nm}\) grid samples a surface area that, apparently, is somewhat smaller than that necessary to represent the surface as a whole. Picking points that span a larger surface area, on the order of
μm², appears to be sufficient to overcome the surface inhomogeneity. Alternatively, multiple grids can be sampled, but the smaller the grid, the greater the number of grids that should be averaged.

This level of surface heterogeneity appears to be similar to that observed by Franzen and coworkers [54] using a nanoparticle hybridization assay, although statistical analyses of the type presented here are not given in their paper. These results differ somewhat from the electrochemical study by Li and coworkers [55] in which no evidence for surface segregation over areas larger than 15 nm² was observed. The apparent discrepancy between the studies could be the result of slight differences in surface preparation or oligonucleotide structure, and it could also arise from the nature of the measurements. Unlike Li and coworkers, neither the hybridization assay of Franzen et al. nor our study examines the actual distribution of thiolated DNA on the gold surfaces. Instead, both measure the effective hybridization efficiency between regions of a gold surface to a second probe surface (DNA-coated nanoparticles in the work of Franzen et al. and an AFM tip here). Oligonucleotide packing density can have an influence on hybridization [60], and so it is possible that two surfaces (or two regions on the same surface) could have the same number of oligonucleotides but significantly different hybridization behavior.

3.4. Day-to-day reproducibility

The day-to-day reproducibility of the bridging statistics was studied by collecting data for at least 2 grids on each of three different surfaces, using three different tips on three separate days (grid parameters were $n_{\text{tot}} = 400$, $x_t$ and $y_t = 5$, $s = 10$ nm, $d = 20$ nm, and $t_{\text{wait}} = 9$ s). The results are presented in Fig. 8. It should be noted that the DNA-modified surfaces used for these experiments were coated with gold, cleaned, and modified with 1 on the same day; i.e. the surfaces are identical other than the fact that the surface used on the third day was two days older than the one used on the first.

For each of the samples, ANOVA testing revealed that the grids obtained on a given day were mostly statistically equivalent. Occasionally, a grid within a day was statistically inequivalent from the other grids, presumably as a result of the factors discussed above. As above, all of the grids from a given day were combined, and the average EPP value was used to represent the surface as a whole. The average EPP values for the three different days were compared using ANOVA, which showed them all to be statistically inequivalent, a result that we attribute primarily to variations in AFM tip geometry and accompanying tip/surface interactions, and/or surface inequivalencies that might result from prolonged storage time. These potential mechanisms are essentially impossible to resolve, due to the fact that samples prepared in parallel cannot be examined in parallel (instrument equilibration and data collection takes roughly a half a day or more per substrate), and the effect of time and/or additional handling of the AFM tip is an additional complication. Our goals here are mainly practical, rather than fundamental: whatever the root cause, day-to-day variations hinder our ability to compare multiple data sets taken on different days—a necessity due to the time required to get adequate statistics for each set of conditions.

3.5. Data normalization

To get around the day-to-day variation in bridging events, we investigated the possibility of normalizing the data against experimental conditions that typically give good statistics. For example, an experiment with $n_{\text{tot}} = 400$, $x_t$ and $y_t = 5$, $s = 10$ nm, $d = 20$ nm, $t_{\text{wait}} = 9$ s, and $[2] = 20$ μM typically gives EPP $\approx 1$. This standard grid is used as a reference grid, against which relative EPP values are determined for a desired set of conditions. We tested this protocol for test grids with $n_{\text{tot}} = 400$, $x_t$ and $y_t = 5$, $s = 10$ nm, $d = 15$ nm, $t_{\text{wait}} = 9$ s, and $[2] = 10$ μM. The results from two different days are shown in Table 2. While the absolute EPP values vary significantly (factor of $\sim 3$) from day-to-day, the ratio of the EPP values for the test and reference grids for each day were found to be constant (0.65 for the first day and 0.63 for the second). The effectively constant proportionality between the reference and test grids from day-to-day facilitates studies that necessarily span long periods of time and further validates the use of the AFM-based protocol to establish trends, rather than absolute values, in reversible-polymer mediated bridging between surfaces.

<table>
<thead>
<tr>
<th>Grid</th>
<th>Day 1</th>
<th>Day 2</th>
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<tbody>
<tr>
<td>Anchor</td>
<td>0.81</td>
<td>0.24</td>
</tr>
<tr>
<td>Test</td>
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<td>0.15</td>
</tr>
<tr>
<td>Test:anchor ratio</td>
<td>0.65</td>
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4. Conclusions

Here, a new MATLAB program was developed that allows the AFM tip–surface contact point to be automatically determined, thus allowing the distance d between opposing surfaces to be actively controlled. Systematic experiments revealed the surfaces to be robust throughout pulling experiments, so that multiple touches could be carried out on a single spot with no measurable affect on the results. Differences were observed, however, both in different spots on the same surface and, more dramatically, from one day to another. Normalizing the data allows day-to-day differences in multiple sets of data to be overcome. While the results are relevant for future structure–activity studies of the system presented here, the effects of surface homogeneity may also be relevant to other AFM-based methodologies, including the development of very sensitive detection schemes using SMFS [61] for which surface inhomogeneity might have a significant effect on the probability of observing a given rupture events.

Acknowledgments

This work was supported by the NIH (EB-001307) and NSF (CBET 0835794). JRW acknowledges the support of a Duke GPNANO fellowship. We thank the NSF (IGERT Grant DGE-0221632) for funding. We thank the NSF (CBET 0835794). JRW acknowledges the support of a Duke GPNANO fellowship and NIH predoctoral traineeship (GM85555). MR and RLC thank the NSF (IGERT Grant DGE-0221632) for funding. We thank Matthew Heaton of the Duke University Statistical Consulting Center for assistance and advice in the processing of the error analysis of the large volumes of AFM data acquired.

References


