Surface-to-Surface Bridges Formed by Reversibly Assembled Polymers

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Polymer bridging between surfaces and particles mediates a range of fundamental processes in the material and life sciences, including adhesion, tribology, and polymer flow; microtubule formation and function; and cell surface interactions.[1] Bridges occur when a polymer chain is either physisorbed or covalently bound to two separate surfaces, and covalent polymer bridging has received extensive theoretical[2] and experimental[3] attention. When the main chain of a polymer is defined by reversible, noncovalent interactions, bridging structure and the resulting material properties reflect the chemistry of small-molecule self-assembly.[4] These dissipative structures (hereafter reversible polymers, or RPs) potentially can adjust their size and shape in response to the steric constraints imposed by the surfaces.[5] They also can equilibrate on much shorter time scales than their covalent counterparts. Here we report that bridges formed by the self-assembly of multiple molecular components create short- to long-range interactions (several to >50 nm) reminiscent of covalent polymers. The bridges result from specific molecular recognition events at the surfaces and in the intersurface milieu, and the statistics of stochastic bridging events are revealed by force microscopy.[6]

We recently reported that reversible polymers formed from oligonucleotide-based monomers (OMs) are a useful model system for delineating molecule-to-material relationships in RPs.[7,8] Here, we employ the OMs to reveal polymer bridging as described in Figure 1. A gold-coated silicon substrate and AFM tip (Si_3N_4, Thermomicroscopes) were chemically modified with oligonucleotide surface linker **1** (Table 1) in a self-assembled thiol monolayer, using the method of Franzen et al.[9] The expected surface density of ~10^{12} mol/cm² was confirmed by fluorescence wash-off experiments, and the background was passivated to nonspecific adsorption with 6-mercaptohexanol. The tip and substrate were mounted in a homemade AFM, and the system was immersed in a buffer solution (1 M NaCl, phosphate, pH 7.0) of 5 mg/mL OM **2**. The tip was positioned ca. 5−10 nm from the surface, and the system was allowed to equilibrate for 10 s. The tip was then retracted at a rate of 150 nm/s (3.0 nN/s loading rate) and immediately returned to its original position.

The resulting force vs distance curves (Figure 2) reveal RP bridging. Individual bridging events are observed as characteristic sawtooth features attributed to RP chain extension and rupture. Bridges were observed in >90% of all retract cycles (Table 2), with multiple bridges present in roughly half of those cycles. Several control experiments attribute these binding events to specific RP bridges of the type depicted in Figure 1. First, experiments without either thiol **1** attached to the surface or OM **2** in solution show no bridging peaks. Second, the addition of 1 mM **3** to the OM **2** solution caps the RPs and inhibits binding to the surfaces. This dynamic chain termination results in a greatly reduced probability
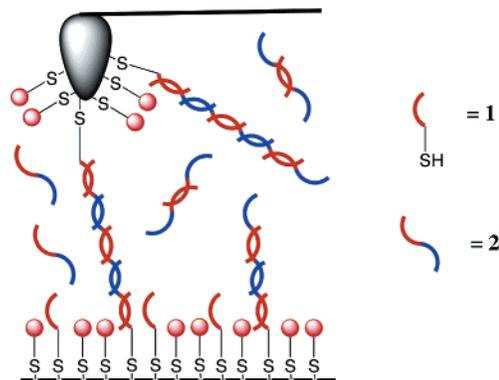

Figure 1. Pictorial representation of solution-phase, reversible polymer bridging between a gold substrate and a gold-coated AFM tip via OM **2**. Colors denote self-complementary base sequences, and the drawing is not to scale. The red spheres represent a passivating 6-mercaptohexanol monolayer that prevents nonspecific adsorption.

Table 1. Composition and Complementarity of OMs[a]

OM	Sequence
1	(5'→3')GGTATACC–C_3H_6SH
2	(5'→3')GGTATACCGCTTAAGC
3	(5'→3')GGTATACCGC
4	(5'→3')GCCCGGG – C_3H_6SH
5a	(5'→3')GCCCGGGCTCTCAAAAACTCTCGGGCCCTAGAGGGGCCCTAGA
5b	(5'→3')CCCGGGCTCTAGGGCCCCTCTAGGGCCCGAGAGTTTTTGAGAG

[a] Red and blue colors denote complementarity within OM systems 1−3 and 4−5.

of bridge formation (Table 2); over 60% of all retract cycles exhibit no bridging peaks. In a third control experiment, the substrate is functionalized with thiol **4**, while the AFM tip still displays thiol **1**. Thiol **4** is not complementary to OM **2**, but does associate with one end of OM **5a/5b**, an RP whose structure has been studied previously in our group.[7] In a 1:1:1 solution of **2/5a/5b**, therefore, the substrate and tip feature OM brushes of similar length that are chemically incapable of forming direct bridges of the type shown in Figure 1. In this case, no adhesive bridging peaks are observed by AFM, ruling out contributions from nonspecific adsorption, brush electrostatics, and physical entanglements between brush layers.

Analysis of force curves was performed using Igor Pro (Wavemetrics). All curves were analyzed using the retract cycle data only. Forces less than 15 pN were treated as nonbridging events. No differences were observed between the statistics of the first 50 and last 50 measurements, confirming that the system is reversible and that ruptures are repaired from measurement to measurement. A histogram of the observed forces is shown in Figure 2. The most probable unbinding force in these conditions is ~30 pN, a value we attribute to the force necessary to break the 8-mer overlaps
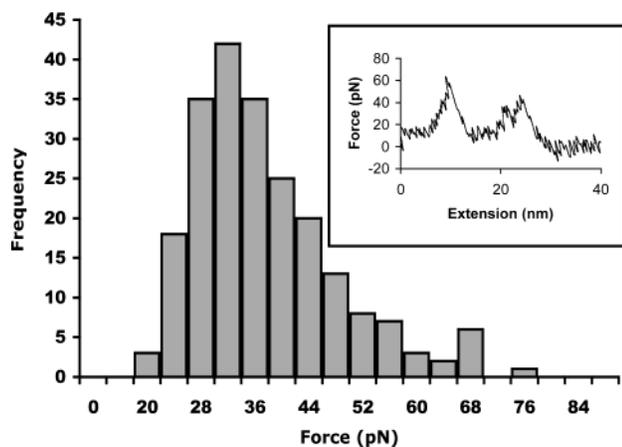
Figure 2. Histogram of observed rupture forces for reversible polymer bridges (OM **2**, 5 mg/mL in phosphate buffer pH 7.0) upon AFM tip retraction. A representative force vs distance data plot is shown in the inset; positive value indicates an attractive force between tip and surface, and the extension is corrected for tip deflection.

Table 2. Distribution of Single and Multiple Unbinding Events (Bridge Formation) between AFM and Substrate with 5 mg/mL OM **2**, with and without Competitive Chain Terminator OM **3**, 5′-GGTATACCGC-3′

no. of unbinding events per retract cycle	frequency	total retract cycles (%)
5 mg/mL OM **2**		
0	8	7.0
1	51	44.3
2	29	25.2
3	19	16.5
4	8	7.0
5 mg/mL OM **2** + 5 mg/mL OM **3**		
0	30	68.2
1	12	27.3
2	2	4.5

defining the RP chains. This value agrees well with the work of Strünz et al.[10] who previously examined isolated 10-mer DNA overlaps whose free energies of association are ~30% higher than the 8-mers employed here. Extrapolation of their results to 3 nN/s loading rates gives a very similar, most probable rupture force of ~30 pN, although a precise comparison of the two results is complicated by differences in the buffer ionic strength and compliance of the chemical attachments in the two studies.[11]

Nearly identical rupture forces are observed for different RP bridging lengths (Supporting Information), suggesting that the sequential associations along the main chain are effectively independent binding events. The most probable extension at break is an additional 10 nm beyond the 5−10 nm equilibrium separation, but bridges of up to 50 nm are also observed. Each unit of OM **2** adds only ~2.7 nm to the length of the RP bridge, and an even number of monomers must be present to satisfy the complementarity of the surface attachment chemistry. The length scale of the observed interactions is therefore indicative of truly polymeric assemblies between surfaces. Light scattering studies have shown that the most probable equilibrium length of the RP formed by OM **2** in a 5 mg/mL solution is ~66 nm,[8] and therefore the length

distribution of the bridging RPs is greatly perturbed from that in solution. Because the AFM tip is curved, the length of the actual bridges may be somewhat longer than reported by the tip−surface separation. It is not possible, however, to offset the observed bridge length distribution and fit the solution distribution of RP lengths; the bridging probability is clearly greater for RP lengths that are closer to the tip−surface separation, perhaps as small as only a few monomer units.

In conclusion, we have shown that DNA-based reversible assemblies form bridges between surfaces. The forces associated with the rupture of these assemblies are independent of polymer bridge length, and they resemble those expected for the isolated associations defining the polymer bridges. The assembly is reversible and is inhibited by a competitive, nonpolymerizing oligonucleotide. Noncomplementary polymer brush layers do not bridge, and thus the forces are mediated by specific molecular recognition events. Further, the length distribution of the bridges differs greatly from that of the polymers in solution, and therefore the bridging is responsive to the spatial constraints of the environment. Reversible polymer bridging therefore reflects a concatenation of polymer physics and molecular self-assembly. The modularity of the OMs, surface chemistry, buffer ionic strength, and instrumental control system all provide mechanisms through which to study structure−activity relationships in this complex and important environment.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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