Supplementary Materials

Molecularly Resolved Label-free Sensing of Single Nucleobase Mismatches by Interfacial LNA Probes

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AFM characterization of silicon substrate: All the AFM images were recorded at ambient condition (except for scratching experiment, which was performed in fluid). AFM experiments were performed using the picoLE AFM equipment of Agilent Corp. with a 10 μm × 10 μm scanner. Imaging was performed in the intermittent contact mode (acoustic alternating current or AAC). The cantilevers (μmasch, Estonia) having the backside coated with Al, frequencies within 150-232 kHz, and force constant values 3.5-12.5 N/m were used for all the imaging experiments. The AFM probes were cleaned in a UV-ozone cleaner (Bioforce Nanosciences) immediately before imaging. The scan range was made zero during the tip engage step to avoid tip contamination. The amplitude set point was 85-90% of the free oscillation amplitude (7.5-8.0 V). Scan speed was typically 0.5-4.0 lines/s.

AFM contact mode scratching procedure: The scratching experiment was performed in 20 mM sodium phosphate buffer with 100 mM NaCl, pH 7.0. The operation mode of the AFM was switched to contact mode and a 400 × 400 nm^2 area of the LNA film was scanned under large tip force (~120 nN) using a triangular silicon nitride cantilever having tip radius < 10 nm, spring constant 0.01-0.6 N/m (Bruker). The applied force is sufficiently large to scratch away the LNA molecules, but not sufficient to scratch the underlying substrate surface. Following the contact mode scratching, scanning was resumed over a large area under low tip force, centering around the resulting scratched region, and obtained the AFM topographic image of the scratched area under the same tip.

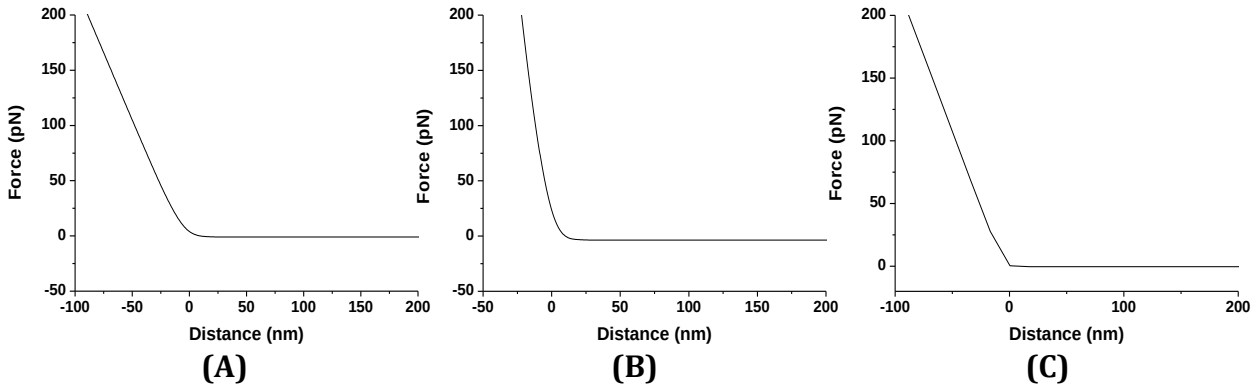

Figure S1. Control experiment between (A) bare tip – bare gold substrate, (B) bare tip – LNA modified gold substrate and (C) target DNA modified tip - fully non-complementary LNA modified gold substrate. The force distance curve displayed no adhesion in >95% of measurements in each case.

Table S1. Unbinding forces of fully complementary LNA-DNA duplexes for different NaCl concentration. The force curves were recorded in 20 mM sodium phosphate buffer with desired concentration of NaCl, pH 7.0 at 0.5 μm/s.

NaCl concentration (mM)	Unbinding Force (pN)
100	165 ± 5
150	181 ± 4
250	221 ± 5
500	313 ± 3
1000	338 ± 4
2000	271 ± 4

Table S2. Unbinding forces of fully complementary LNA-DNA duplexes for different $MgCl_2$ concentration. The force curves were recorded in 20 mM sodium phosphate buffer with desired concentration of $MgCl_2$, pH 7.0 at 0.5 μm/s.

$MgCl_2$ concentration (mM)	Unbinding Force (pN)
10	317 ± 5
15	355 ± 4
20	360 ± 6

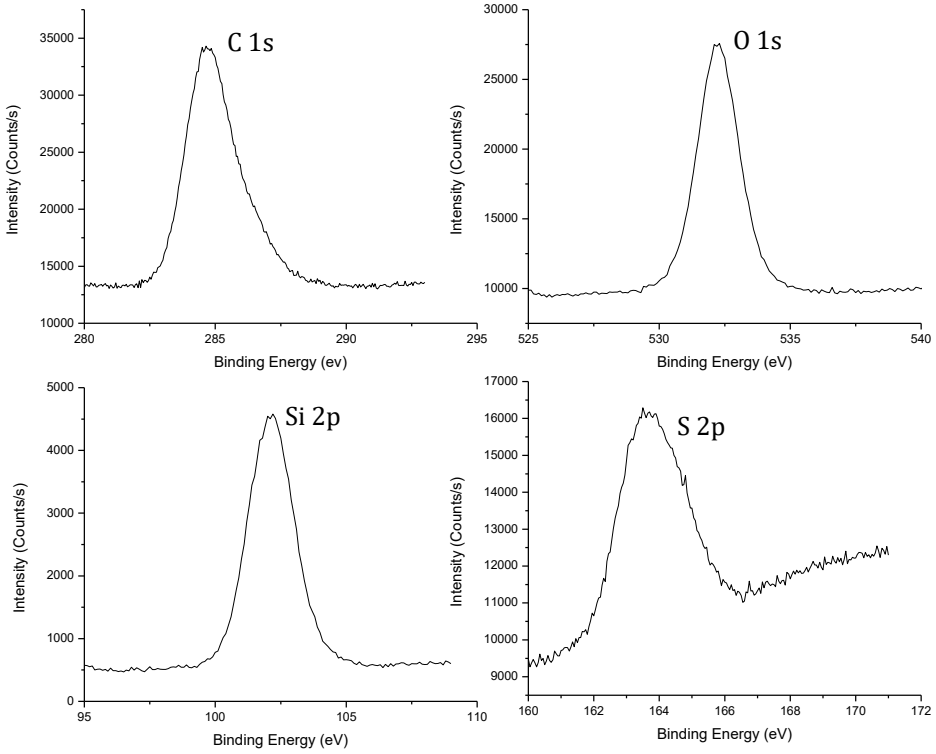

Figure S2. High-resolution XPS spectra of 3-MPTMS layer on silicon prepared by self-assembly of the MPTMS molecules.

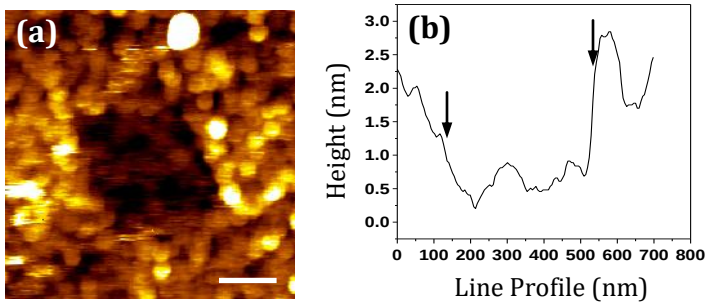

Figure S3. AFM topograph (a) and line profile (b) of ssLNA monolayer on silicon surface from which a square part (400 × 400 nm^2) was removed by the AFM tip enabling an estimate of the monolayer thickness (see the cross-section). Scale bar: 200 nm and Z-range for (a) 0-1.8 nm. The two downward arrows identify the boundary region of the scratched area.