Sequencing Genomes by Pulling DNA Molecules

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Motivation

Sequencing a genome of every individual is of great importance to biomedicine and holds promise for making personalized medicine a reality. Due to the enormous task of sequencing a *three billion base human genome* in a short time scale and for a reasonable cost, the traditional method of Sanger sequencing has been abandoned in favor of next-generation technologies. These sequencing technologies are being pursued in hopes of attaining a human genome for under $1000.

DNA Sequencing Method

Single-stranded DNA (ssDNA) has different elastic properties and contour length than double-stranded DNA (dsDNA). One can quantify the double-stranded character of a single DNA molecule and detect 'yes' or 'no' events for binding short complementary oligos by repeatedly recording force-distance curves.

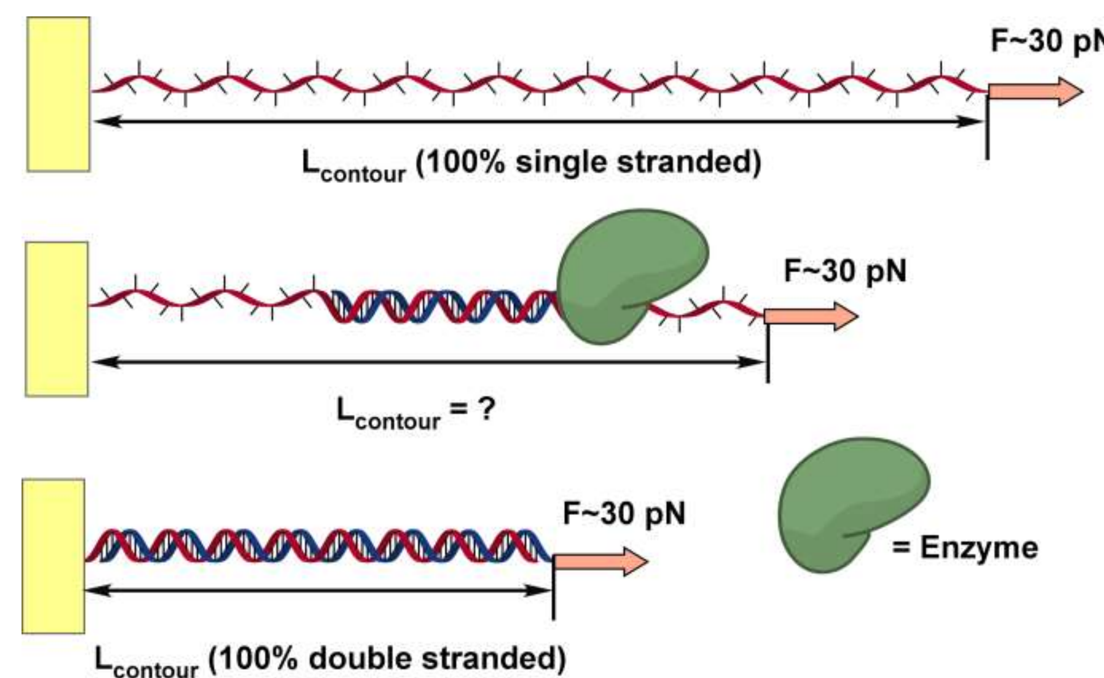

Figure 1. Basic idea behind the use of the conformational changes during polymerization or ligation to detect individual sequencing steps.

In order to stretch single DNA oligos in a highly-parallel manner, we use a magnetic tweezers instrument with evanescent field illumination (total internal reflection fluorescence, TIRF).

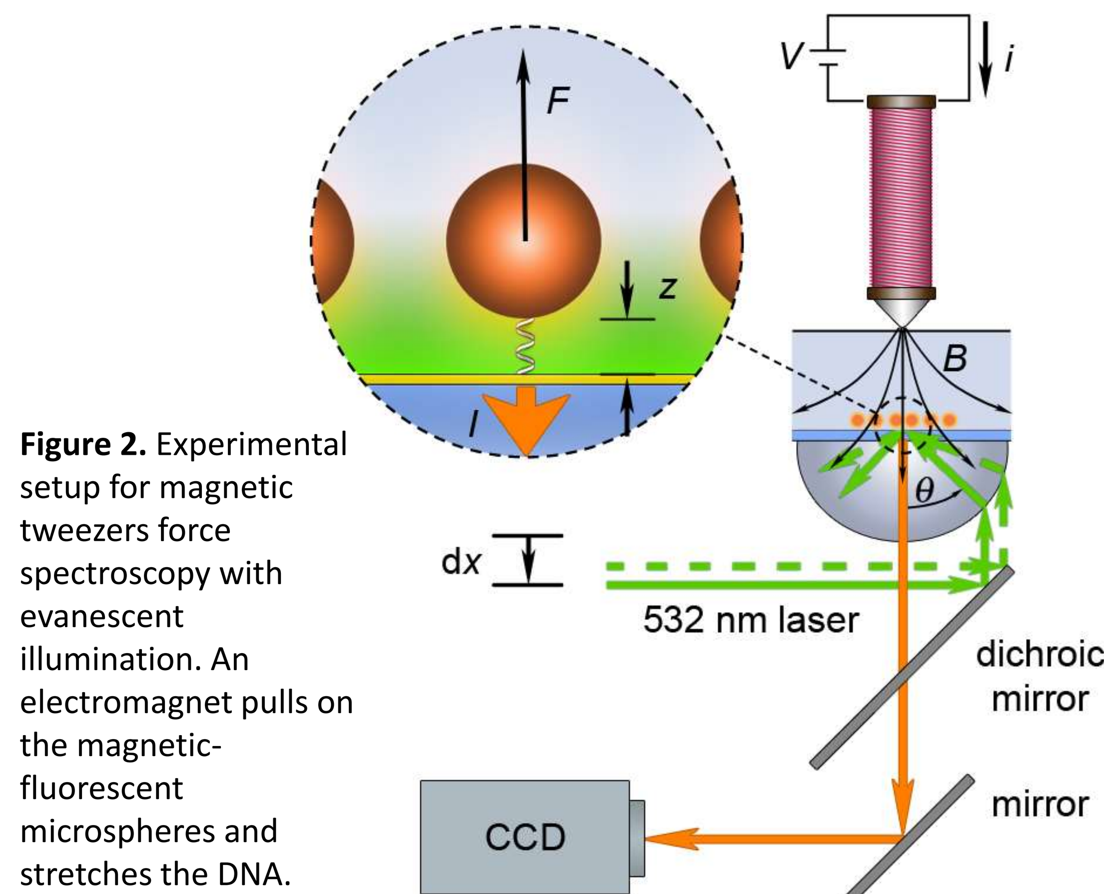

Figure 2. Experimental setup for magnetic tweezers force spectroscopy with evanescent illumination. An electromagnet pulls on the magnetic-fluorescent microspheres and stretches the DNA.

Making a DNA Chip

We require an array of well-spaced DNA molecules with appropriate blocking chemistry to avoid nonspecific binding of probes. Formation of self-assembled monolayers of organic thiols on gold is a facile method for controlling the surface functionality and density of DNA.

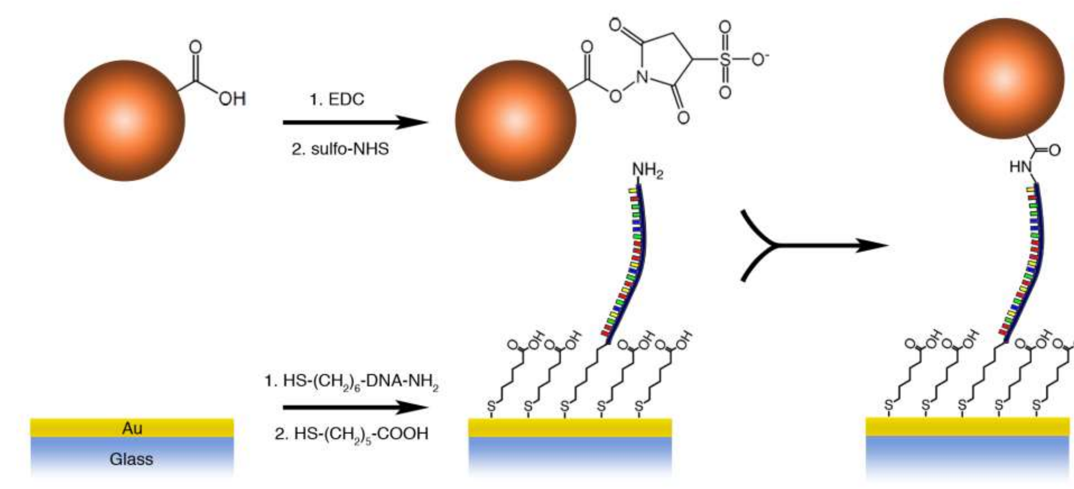

Figure 3. Reaction scheme for attachment of probe and DNA to gold-coated glass coverslip.

Since the thin layer of gold is optically transparent, we can measure the density of DNA molecules directly using fluorescent labels.

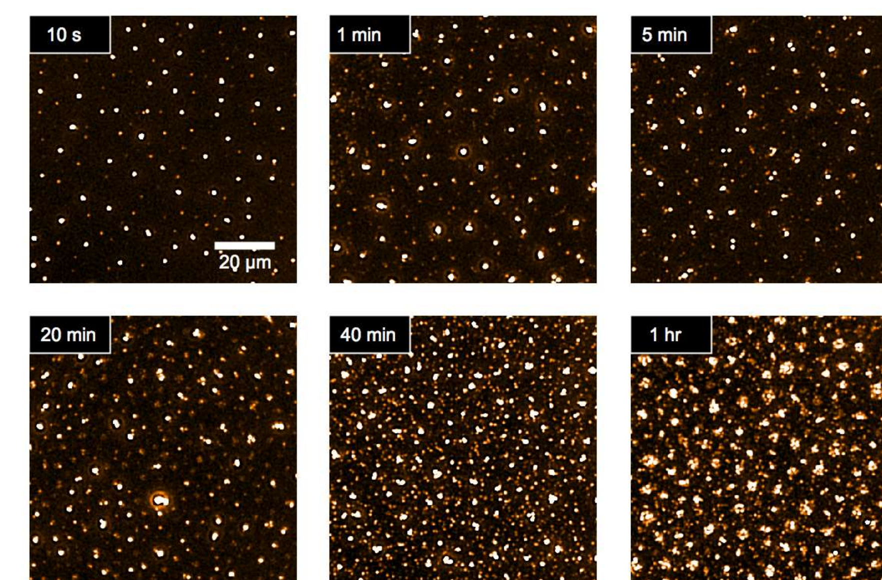

Figure 4. Fluorescence images from a time series of the reaction of 1 μM TAMRA-DNA-SH with a gold surface after addition of a blocking thiol.

Single Base Sensitivity

Using knowledge of the length of the oligomer and the system's equations of state (the freely-jointed chain model for ssDNA stretching), we converted the raw data to the standard format of molecular extension versus applied force.

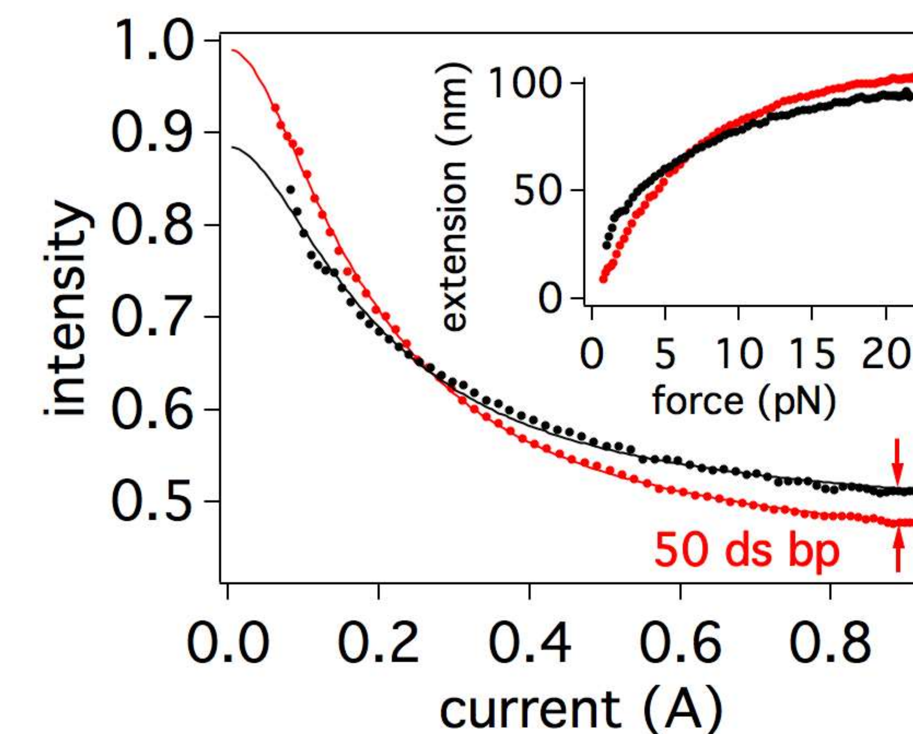

Figure 7. Experimental difference in intensity-current curves after hybridization of a 50-mer to the 200-mer (red - fully ssDNA, black - partially hybridized). Lines indicate fits to data. Inset: Plot of extension versus force for the same molecule using the parameters from fitting.

To test the sensitivity of our setup, we measured the hybridization of a 50-mer complement. We observed a change in the elasticity of the DNA strand for the double-stranded section. The resulting fit yielded excellent agreement with the expected fraction of double stranded character, with a difference of only 0.4 ± 1.2 bases. Therefore, force spectroscopy can detect additions of short oligomers and is a feasible method of sequencing.

DNA Stretching Results

We used a synthetic ssDNA oligomer (200 bases) as a model system for single-molecule DNA stretching. In our proof-of-concept experiments, we took movies of the probes (fluorescence) during stretching.

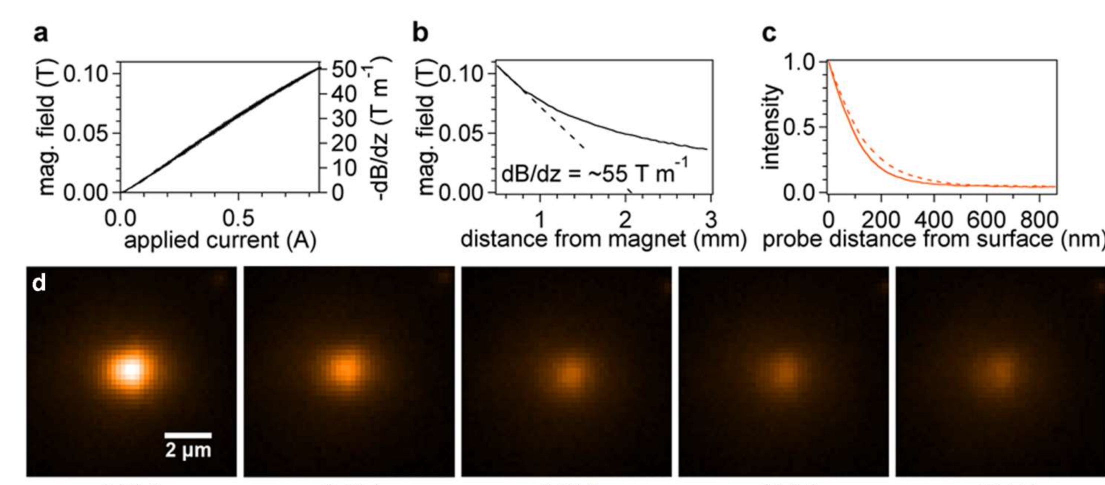

Figure 5. (a) Linear response of electromagnet field and its gradient with current. (b) Magnetic field vs. distance (fixed current) at 0.5 mm from the bead. (c) Intensity of probe fluorescence vs. distance at two incident angles. (d) Probe image vs. electromagnet current for 200-mer stretching.

We measured the integrated intensity of the probe versus current applied to electromagnet. The curves proved to be independent of stretching speed and were highly reproducible.

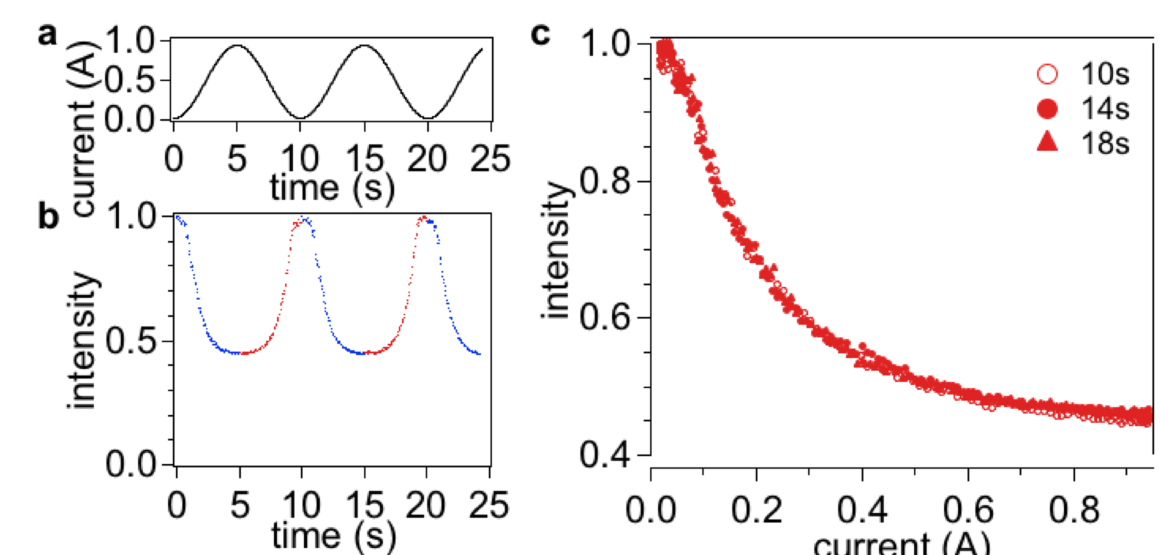

Figure 6. Time traces of (a) applied electromagnet current and (b) raw intensity of the probe (red – contraction, blue – extension). (c) Intensity as a function of applied current at different cycle times.

Sequencing by Ligation Scheme

By using short complementary strands with the enzyme ligase, we can interrogate the target DNA strand and obtain the sequence. After each probe strand is added, we look for a change in the force curve.

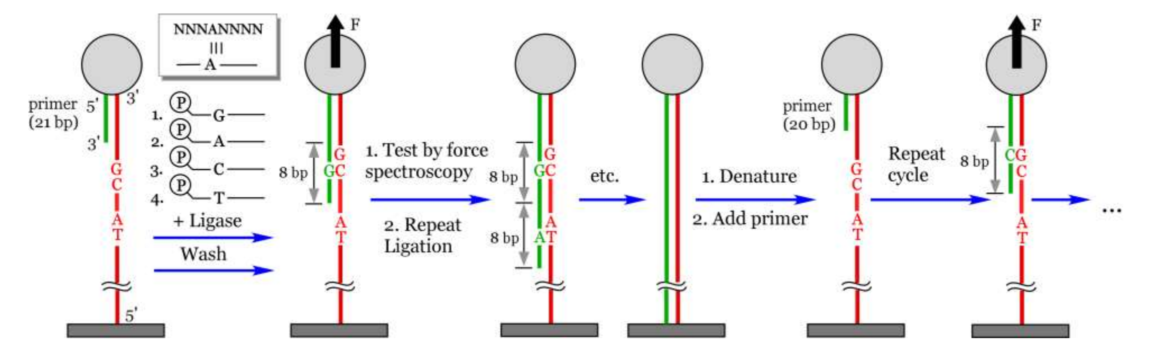

Figure 8. Scheme of the process flow for sequencing by ligation using single-molecule force spectroscopy.

Ligation in a Force Spectroscopy Array

Microfabricated wells allow us to space the probes in a regular pattern and enable us to use high flow rates for fast reagent exchange. The probes can populate nearly 100% of the micro-wells.

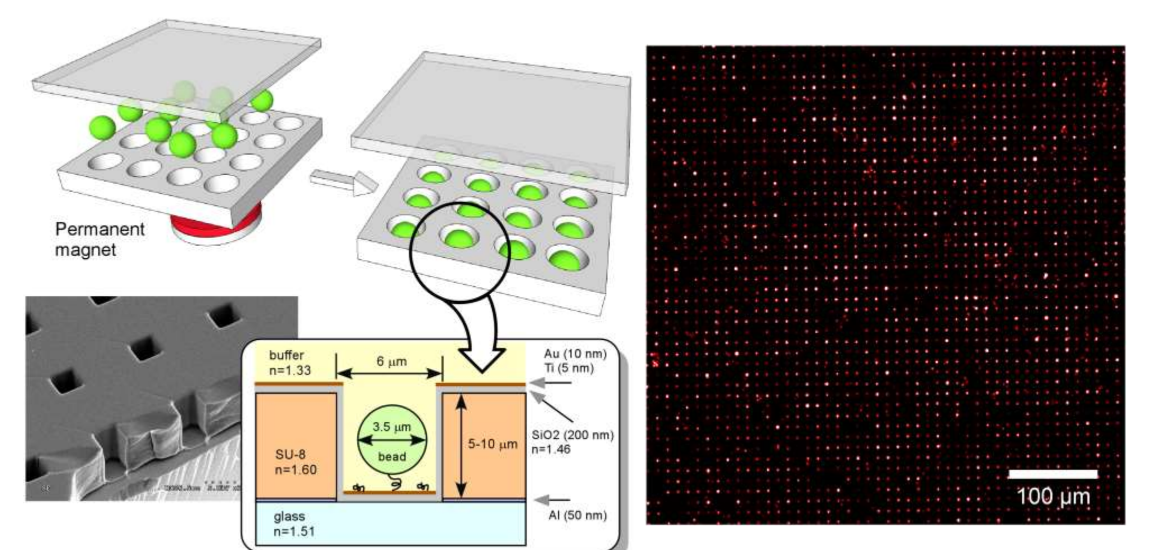

Figure 9. A force spectroscopy array populated by magnetic force probes.

The stretching behavior of the DNA in the micro-wells is similar to those experiments on a flat substrate. After addition of ligase and a DNA strand (8 bases long) to the target molecule, we observed a change in the intensity versus current plot of a probe in a well.

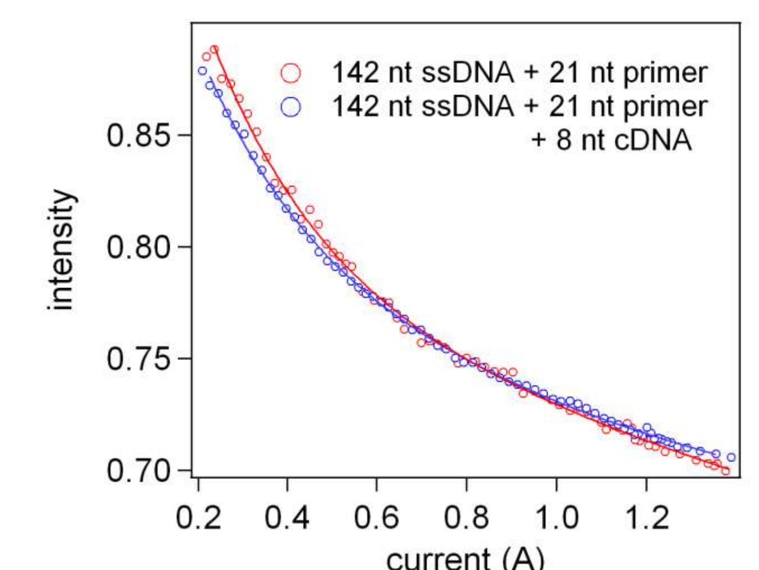

Figure 10. Experimental intensity-current curve obtained for a typical active bead in the well array, before and after ligation of an octamer.

Funding

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