Analysis of Exonuclease-DNA binding measurement distributions using a nanopore

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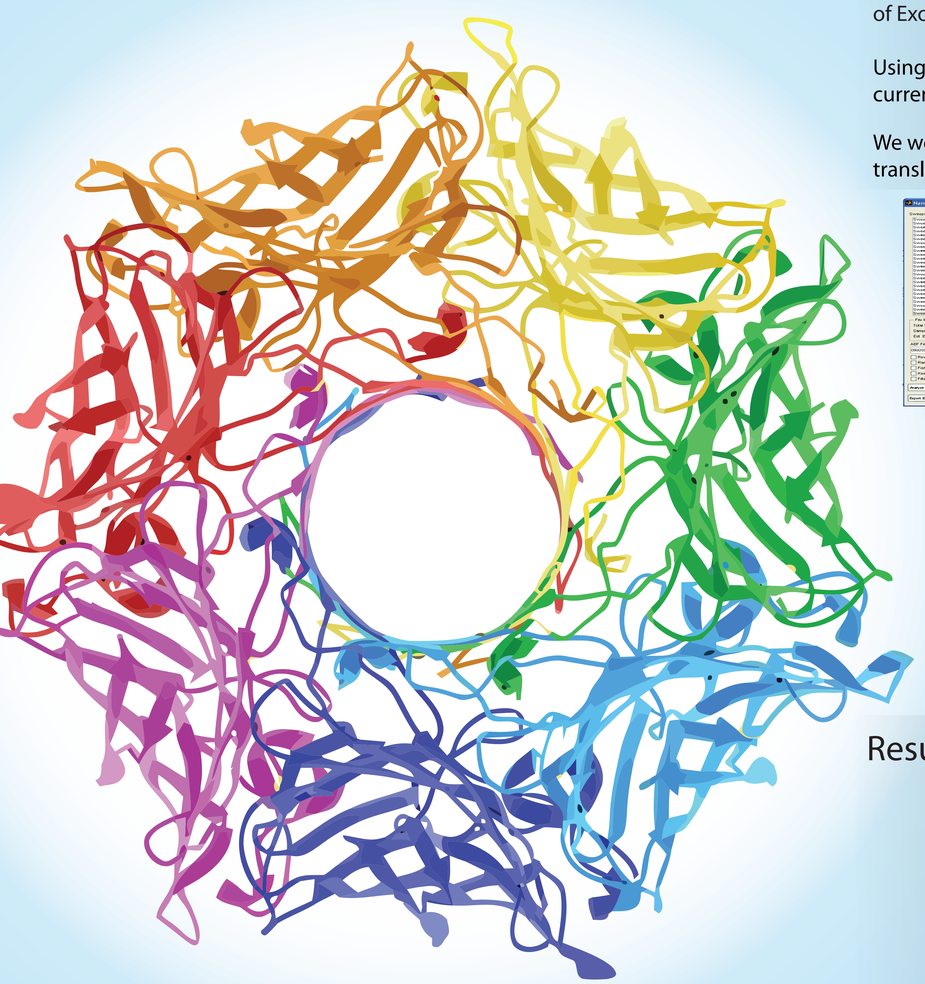
Background: Exo-bound DNA can be detected using a Nanopore

DNA-Protein interactions can be observed by applying a voltage across a membrane with a nanopore embedded in it. The electric potential allows for DNA to translocate through the pore by electrophoresis due to the inherent negative charge present in the DNA backbone.

A nanopore is a pore measuring nanometers in diameter and usually embedded in a membrane. Alpha-hemolysin is a biological nanopore and a toxin from bacterial sources that self assembles and embeds itself in a lipid bilayer.

Exonuclease is an enzyme that works by cleaving nucleotides one at a time from the end of a polynucleotide chain in the 3′ to 5′ direction.

In Exonuclease-DNA nanopore experiments, DNA-protein interactions can be discerned by a change in current which, results when a DNA molecule occludes the nanopore restricting the flow of electrolyte solution.

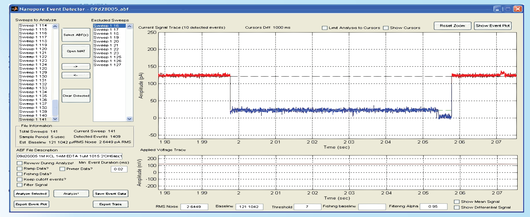


Methods:

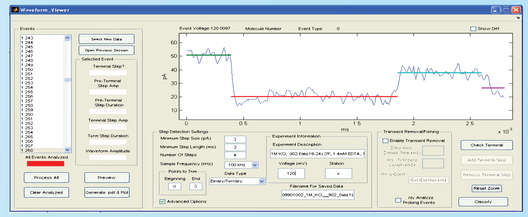
Experiments were run with various DNA templates and under various concentrations of Exo, magnesium, and calcium.

Using in-house software developed within Matlab, we were able to identify different current steps in the DNA translocation events.

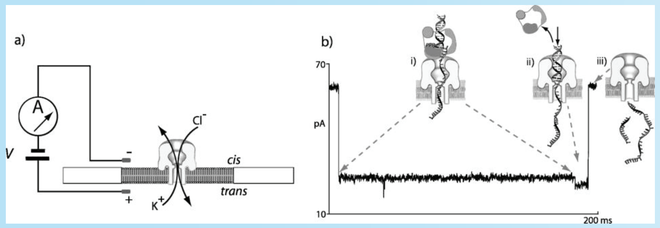
We were able to get statistics on durations, amplitudes, and step patterns of translocation events.



An event in EventDetector



Steps in an event on Waveform_Viewer

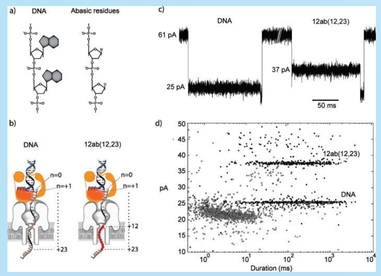


Current trace for DNA-Exo complex[1]

Motivation:

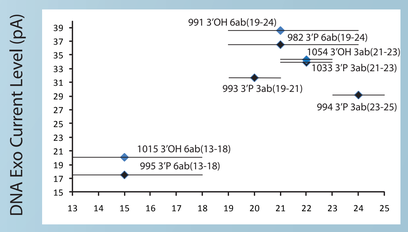
To develop tools and methods for analyzing and manipulating single molecules.

A more specific goal was to develop an abasic map for exonuclease. DNA with abasic residues lacks the nucleotide component but retains the sugar and phosphate backbone. These abasic residues impede current less than normal DNA residues, resulting in a higher current.



Current trace for abasic and non-abasic residues[1]
Abasic residues have a higher current trace than non abasic residues.

Results: Abasic Mapping of Exonuclease 1



Midpoint of Abasic Segment

The convention Nab(x,y) is used to denote the number of abasic residues N, and their beginning and ending position along the DNA segment from the enzyme's catalytic site.

The graph shows the 6ab(19-24) abasic region impedes current less than does the 6ab(13-18) region. The graph also shows that templates bearing a 3′OH end impede current slightly less than do similar templates bearing a 3′P end.

Acknowledgements

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References:

[1] Brett Gyarfas, Felix Olasagasti, Seico Benner, Daniel Geralde, Kate R. Lieberman and Mark Akeson. Mapping the Position of DNA Polymerase-Bound DNA Templates in a Nanopore at 5Å Resolution. American Chemical Society Vol. 3 No. 6. (2009): 1457-1466. Print.

[2] http://visualscience.ru/en/illustrations/visualization/alpha-hemolysin/alfa-hemolysin_top.jpg