A Step toward Cost-efficient DNA Sequencing

Creating an Abasic Map of ssDNA Held in an α-hemolysin Nanopore

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Background

- Goal: Detect single nucleotide differences between individual strands of DNA with a nanopore
- Toward this end, we explore sensitivity of the nanopore, varying voltage and the locations of a single abasic* residue within a homopolymer (poly C)
- Specific Task: Identify voltages at which the differences are observable

*Abasic: Nucleotide removed, sugar-phosphate backbone left intact

Motivation

- Determine the current changes for DNA with single abasic residues
- Find the optimal voltage such that Exonuclease I (ExoI) will bind and single nucleotide differences can be detected
- Clarify the dynamics of the pore through voltage titrations

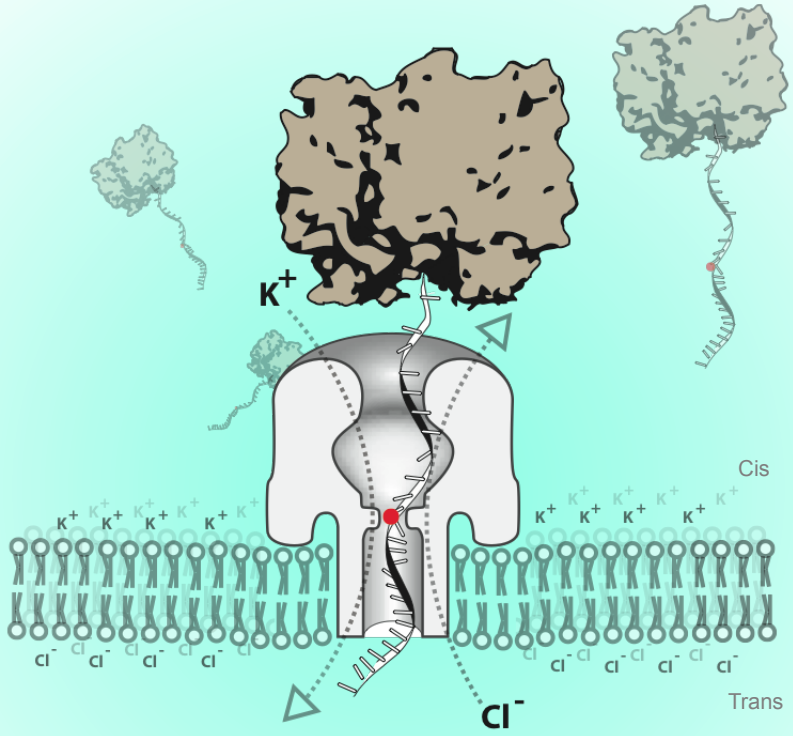
End Goal

- An abasic map will reveal the optimal voltages for observable hydrolysis on the pore by ExoI

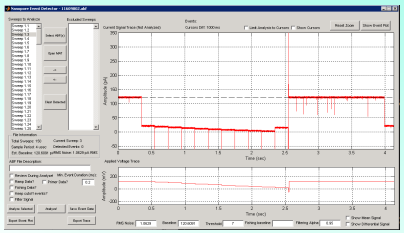
References

D Stoddart, AJ Heron, J Klingelhoefer, EMikhailova, G Maglia, and H Bayley. Nucleobase recognition in ssDNA at the central constriction of the alpha-hemolysin pore. Nano Lett., 10(9):3633–7, Sep 2010.

"This work was sponsored by the National Science Foundation, SURF-IT (surf-it.soe.ucsc.edu) Research Experience for Undergraduates Program. NSF grant Award No. CNS-0852099. In addition, thanks to the University of California, Santa Cruz."

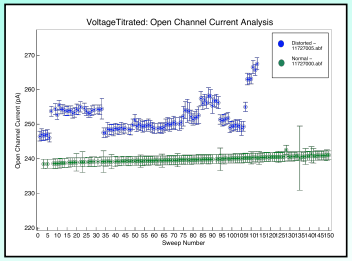


Event Detector



Event Detector: software used to isolate events from sweeps of the data. The upper screen displays current and the lower screen voltage. The flat red lines represent open channel current with a constant voltage. The stepwise decreasing line represents an event: DNA held in the pore with voltage titrated down.

Data Analysis



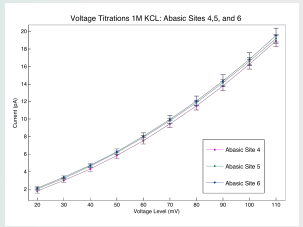
The above plot shows, in green, the expected trend in open channel current as the experiment progresses. The blue data points are taken from a file that had a great deal of variability in open channel current and was thrown out, as our results are highly dependent on keeping this variable stable.

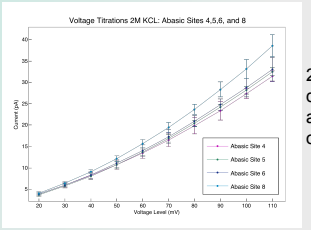
Methods

- Heptameric α-hemolysin (αHL) forms a nanopore ~1.5 nm in diameter in a lipid bilayer
- Biotinylated DNA with single abasic residues and Streptavidin are added to the cis side of the membrane in bulk
- A 120 mV current is applied across the membrane. Voltage titrates down to 20 mV in increments of 10mV. Eject voltage of -50mV is applied, DNA escapes the pore if it has not

Results

1M KCL voltage titrated data with abasic residues at positions 4,5, and 6 on the DNA strand.





2M KCl voltage titrated data with abasic residues at positions 4, 5, 6, and 8 on the DNA strand.

Conclusion

At 2M KCL, voltages at 80,90,100, and 110 mV show 1 abasic resolution that is detectable (SNR>=2) when the abasic residue passes from position 6 to 8. At 1M KCL more data will be collected with templates containing other abasic residue locations in order to allow for detection at 1 abasic resolution.