Temperature-dependence of electrostatic DNA melting

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Abstract

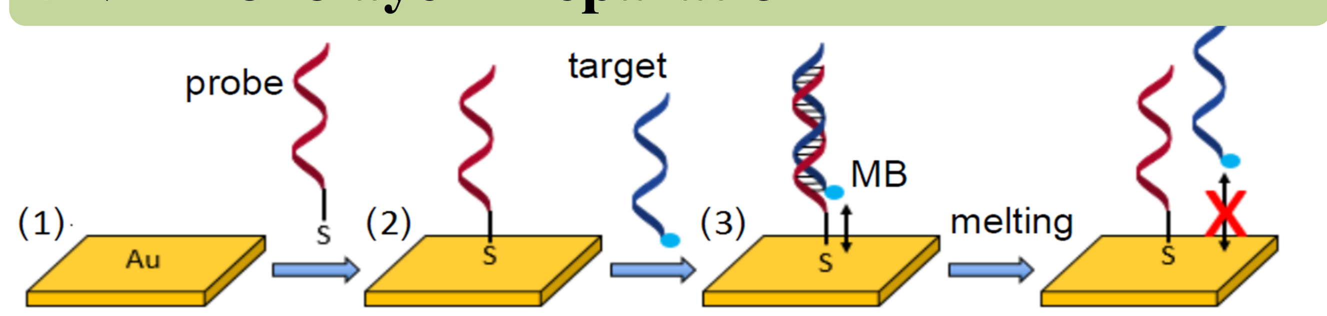
- Electrostatic melting is a relatively new tool used to interrogate DNA stability, different sequences of double-stranded DNA (dsDNA),the presence of mismatches, deletions and other mutations (1).
- We explore the dependence of temperature and potential on the kinetics of electrostatic melting in an effort to better understand the mechanism.
- Previous studies, by another group, have shown that the potential at which electrostatic melting occurs decreases with temperature in the range 10 – 18 °C, i.e. the melting becomes easier at higher temperatures up to 18 °C.
- Previous authors found no effect of temperature above 18 ºC (2). Unlike previous studies, the work presented here examines the kinetics of electrostatic melting at relatively low melting potentials (300 – 500 mV vs Ag/AgCl reference electrode).
- Current research shows that the temperature increases, the extent of melting and rate of melting increases in the range 20-50 ºC. Notably, the kinetics follow Arrhenius behaviour, thus allowing us to determine the electric-field modulated activation energies of melting as a function of melting potential. Based on these measurements, we hope to determine optimal temperature and potential ranges for discrimination of dsDNA.

Experimental

DNA sequences used in this work

18-bp Duplex (MB on target)	Probe: 5'HS-C6-TTG ATC GGC GTT TTA TTC 3' Target: 3' (MB)-AAC TAG CCG CAA AAT AAG 5'

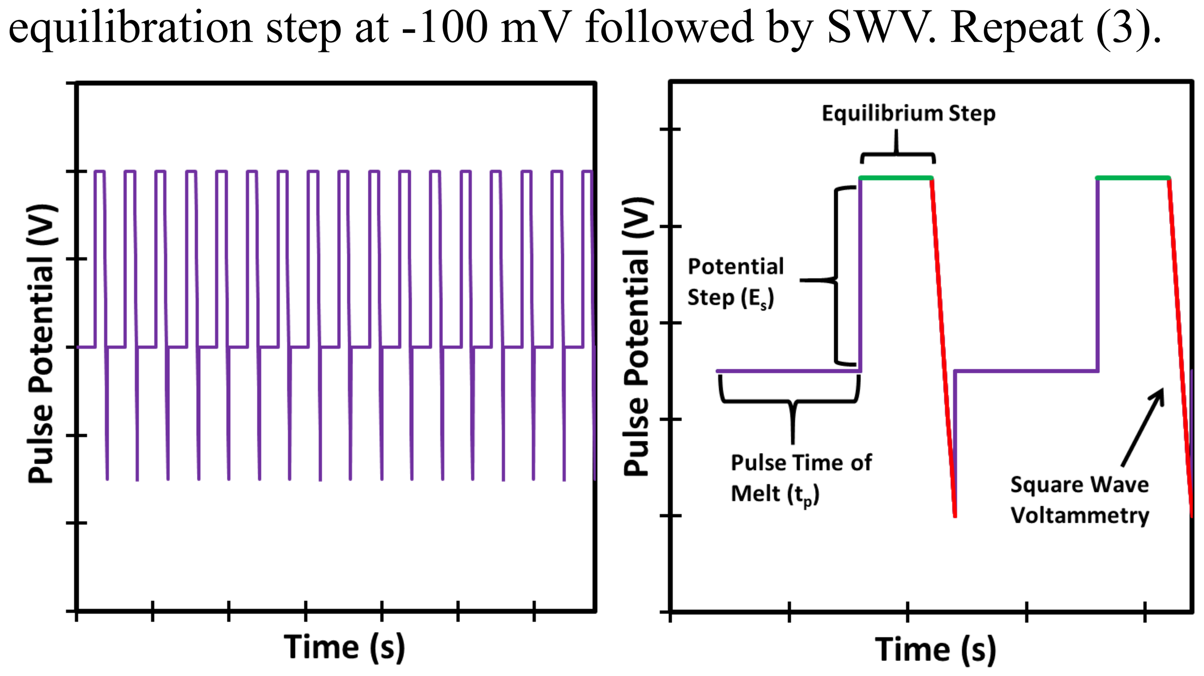
DNA Monolayer Preparation



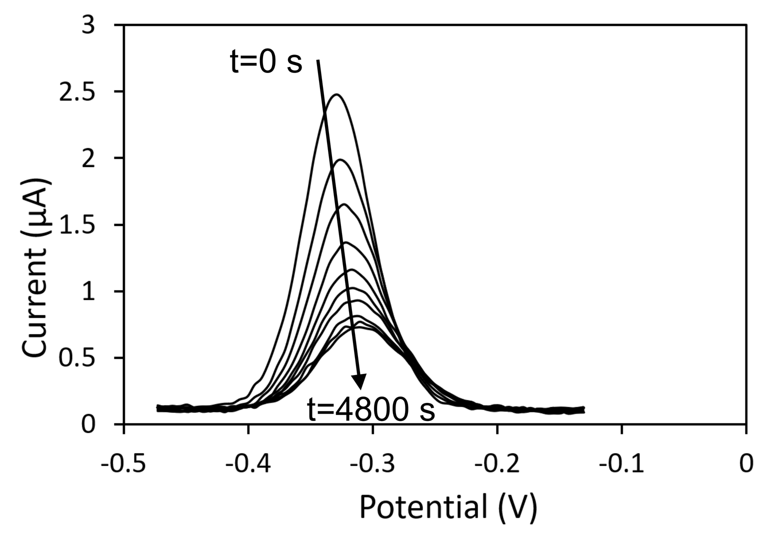
1. The mixed monolayers of DNA and mercaptohexanol (MCH) are prepared by potential pulsing from -100 mV to -500 mV at 10 ms each for 15 minutes in a 0.5 µM , followed by overnight incubation in MCH at a concentration 9 mM
2. Modified electrodes are incubated in a solution of the target DNA at room temperature for 60 minutes. (4)

Electrostatic Melting

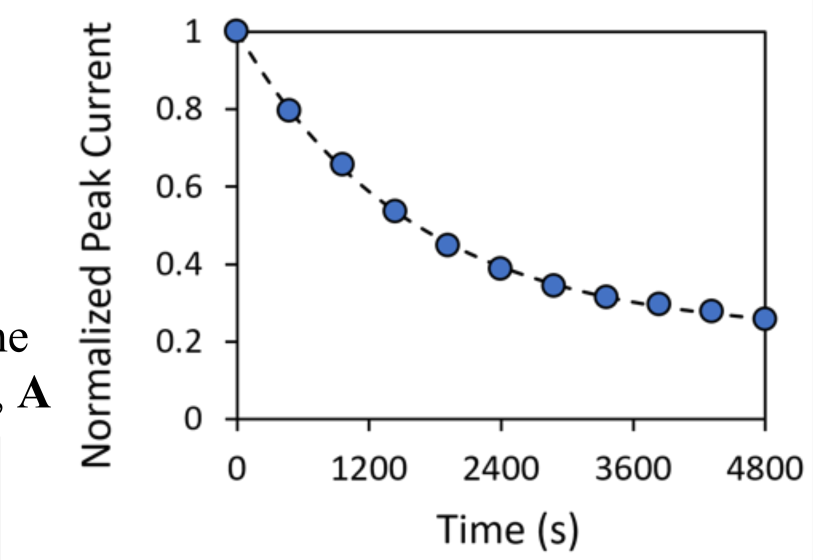
A duplex is formed on modified gold electrodes by using it as working electrode in a 3-electrode cell. The electrolyte is 10 mM Tris buffer (pH 7.2) 8 min at -500 mV followed by 10 s equilibration step at -100 mV followed by SWV. Repeat (3).



Melting curves and data analysis



- The voltammograms (left) show a cathodic peak due to the reduction of the MB on the target strands.
- After each step of the melt, this peak decreases due to loss of the target DNA during melting.

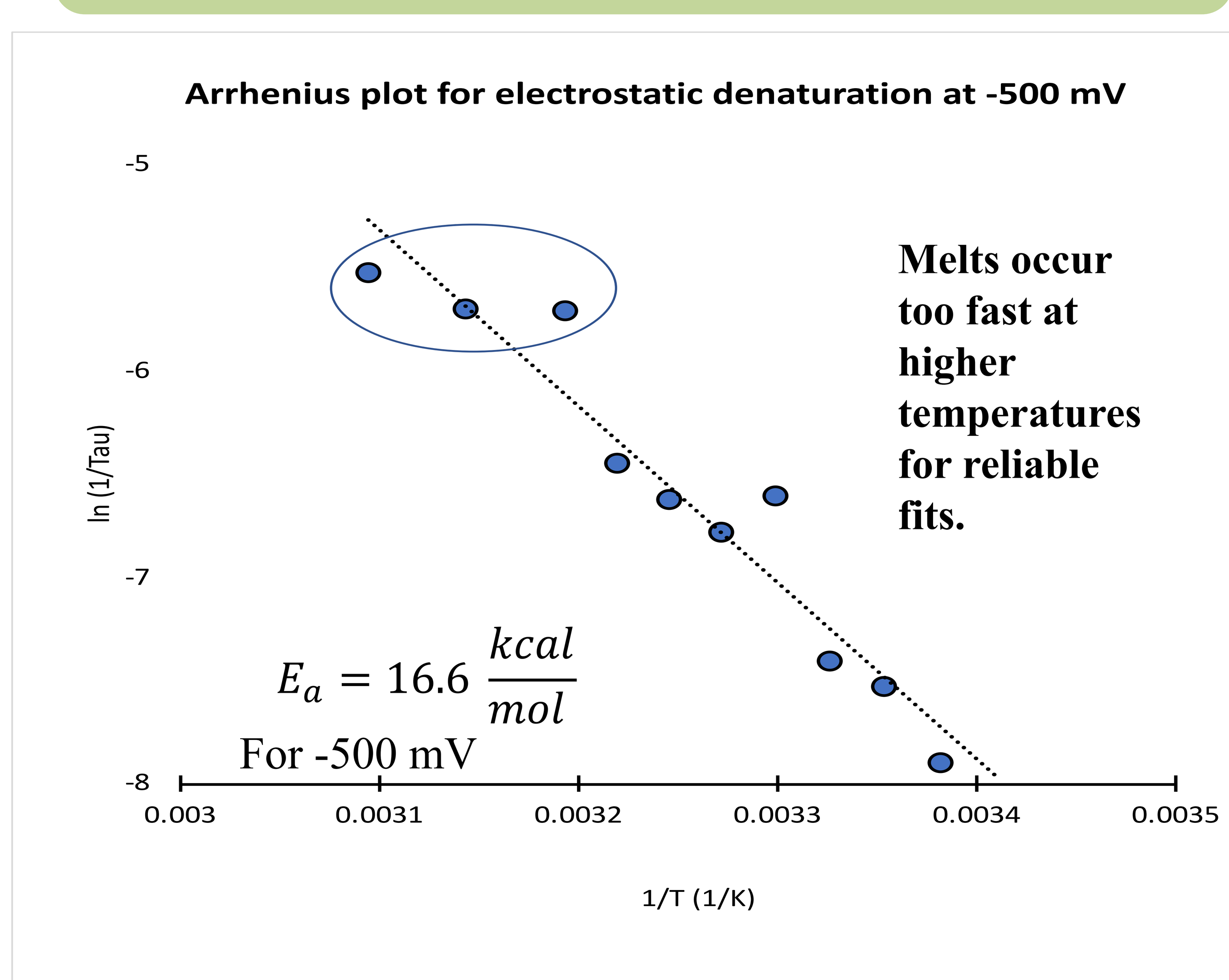


- The baseline-subtracted peak currents are plotted versus time (right)
- Each curve is fit with an exponential function to obtain the time constant, **τ**, and signal loss, **A**

$$i_p(t) = \boldsymbol{A}e^{-t/\tau} + (1 - \boldsymbol{A})$$

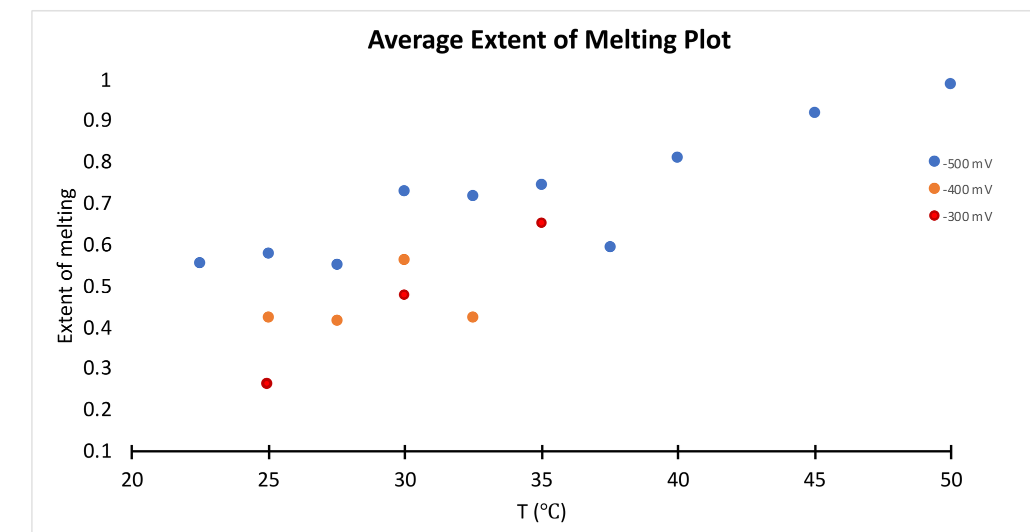
Results and Discussion

Arrhenius Behavior of electrochemical Denaturation

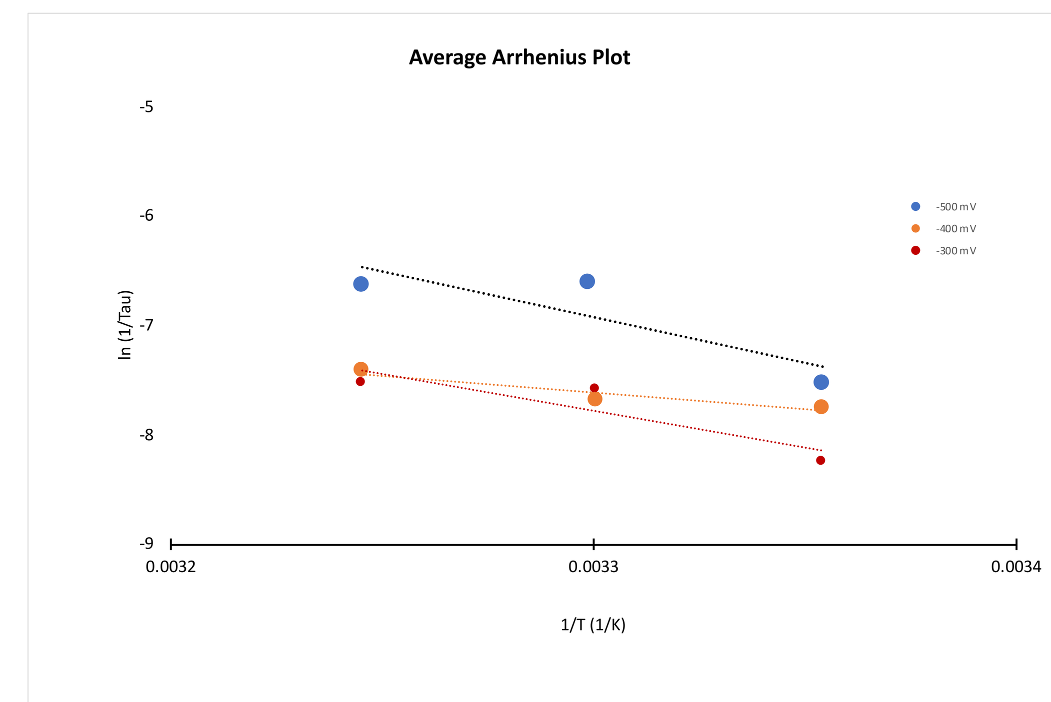


- This Arrhenius plot demonstrates how as temperature is increases , so does the extent of melting.
- The three points in blue outline that the melts are occurring to fast so data can not be adequately collected at those points.
- Arrhenius Equation: $\ln\left(\frac{1}{\tau}\right) = \ln(A) - \frac{E_a}{R}\left(\frac{1}{T}\right)$
- Where A= pre-exponential factor,τ= time constant, R= gas constant = 8.314 $\frac{kJ}{K*mol}$, T= temperature in Kelvin, E_a= activation Energy

Effect of melting potential on Arrhenius Behavior



- The average extent of melting increases as temperature increases.



- The general trend is seen as temperature increases the extent of melting is the same for many point as -300 and -400 mV respectively.

-500 mV	-400 mV	-300 mV
$16.6 \frac{kcal}{mol}$	$6.6 \frac{kcal}{mol}$	$13.2 \frac{kcal}{mol}$

Conclusions

- Overall, the current results show that electrostatic melts are temperature dependent
- The extent of melting increases with temperature.
- Time constant decreases with increasing temperature and 1/tau follows Arrhenius' behavior.
- Furthermore, the melting potential influences the extent and rate of melting as well, although the relationship between activation energy and potential is not linear.
- The next steps are to continue evaluating the relationship between potential and activation energy and to determine the optimal temperature to detect mismatched DNA sequences.
- Understanding the interdependence of potential and temperature will provide greater insight into the mechanism of electrochemical denaturation.

Acknowledgments

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References

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