Effect of Salt Concentration on Electrochemical Detection of DNA

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Introduction

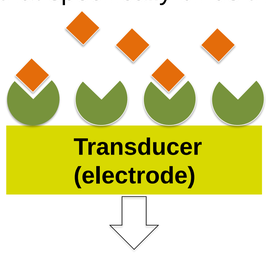
What is a Biosensor?

A device that detects
- living organism or product derived from living systems (**biorecognition element**) *via*
- **transducer** to provide an indication, signal or other form of recognition of the presence of a specific substance in the environment.

Applications include:
- environmental monitoring
- medical diagnostics
- drug discovery
- process control
- defense and national security

Electrochemical Biosensors

- Detection is based on potential or current change from the target molecule.
- The electrode surface is modified with a biorecognition element that specifically binds the target molecule.



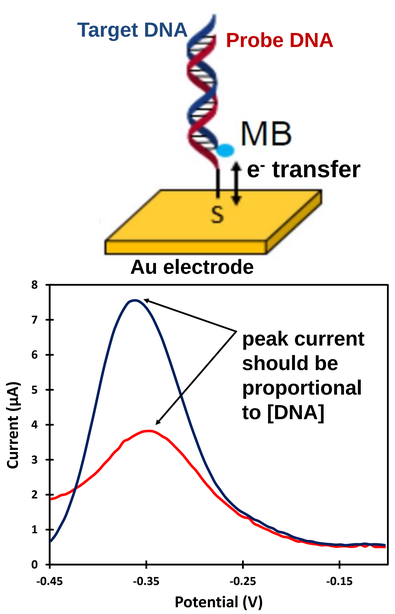
Ideally, signal output is:

1. proportional to concentration of analyte
2. free of interference from other species in solution.
3. **free of matrix effects**

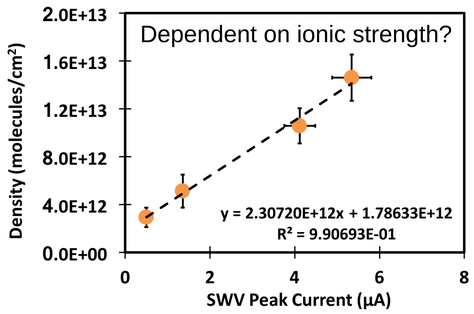
Background

Why is this important?

- In this work we explore how the presence of NaCl in our electrolyte effects the measured signal from DNA.
- We will show that the concentration of NaCl effects not only the stability of the DNA duplex but also the electrochemical response, i.e. the signal.
- Since NaCl, and possibly other ions in solution, effect the measured signal, the presence of these ions should be treated as a matrix effect.
- The concentration of salts like NaCl must be taken into account when when using electrochemical methods to detect DNA

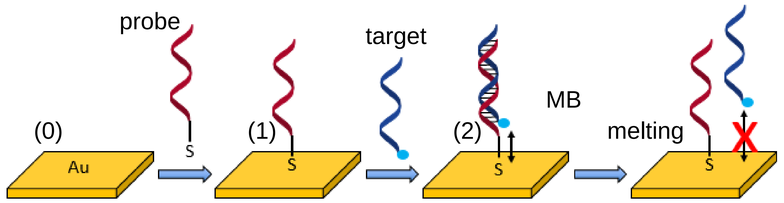


Linear Response



- Electrochemical signal is linearly proportional to surface density of DNA in 0 mM NaCl.
- **QUESTIONS: Is this calibration curve still valid at other concentrations of NaCl?**

Experimental



0. Polishing and Cleaning

Reliable attachment and detection of DNA requires thorough cleaning.
1. Mechanical polishing using alumina slurry
2. Exposure to Piranha solution for removal of residual organic matter
3. Electrochemical polishing by pulsing and cycling in 0.5 M H_2SO_4

1. Monolayer Formation

- Probe DNA is attached to a cleaned electrode via a fast-pulsing step
- The electrodes are then placed in a solution of 6-mercaptohexanol to passivate the exposed gold surface and orient the probe DNA so they can more efficiently hybridize with the target DNA containing methylene blue.

2. Hybridization

- The electrodes are incubated in a solution of the target DNA for one hour to allow hybridization.

DNA Sequence

- Short oligomers (18 base. Pairs)
- 100% complementary sequences
- Probe strand is modified with a thiol bound to the 5' end via a 6 carbon linker
- Target is modified with an electroactive methylene blue at the 3' end
- Upon hybridization, the MB is within close proximity to the electrode surface.

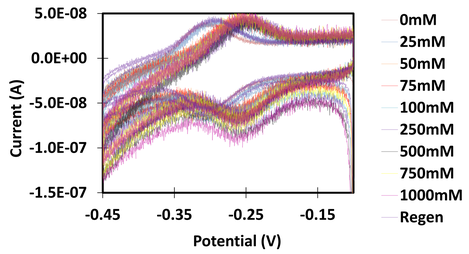
DNA Sequences:

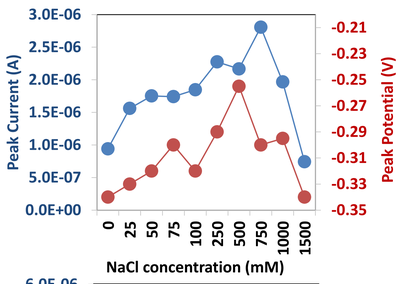
18-bp Duplex (MB on target)	Probe: 5'HS-C6-TTG ATC GGC GTT TTA TTC 3' Target: 3' (MB)-AAC TAG CCG CAA AAT AAG 5'

Results

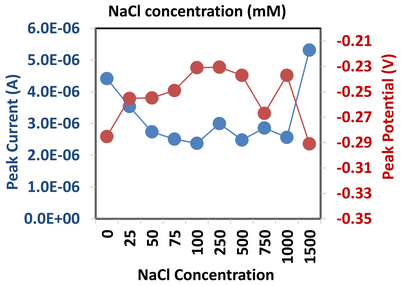
Comparison of signal in various methods

Cyclic Voltammetry (CV)
- High noise
- Low sensitivity
- Not much dependence on [NaCl]
- Not suitable for the measurement of DNA signal under our conditions.





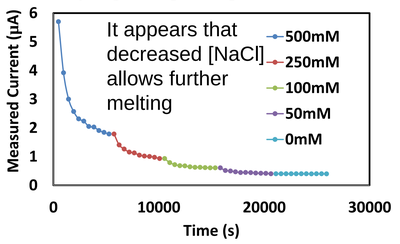
Alternating Current Voltammetry (ACV)
- Higher NaCl concentration results in more noise and fluctuations in the detection of DNA (not shown)
- Peak current increases with [NaCl] until 750 nM.
- Peak shifts positive

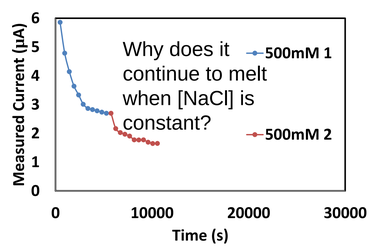


Square Wave Voltammetry (SWV)
- Most sensitive of the three methods.
- Smaller effect of [NaCl]
- 1500 mM represents the "regeneration" trial.

Effect on Melting Behavior

- An electric field can influence the hybridization of surface-bound DNA.
- Negative potentials destabilize the duplex and lead to dehybridization (melting) [2].
- Interestingly, the melt can be repeated on the electrode, even when keeping the [NaCl] constant.





Results and Outlook

- Between ACV, CV, and SWV, SWV was the most sensitive.
- Calibration curves of DNA density vs. signal will depend on [NaCl]
- The presence of cations in electrolyte shield the DNA duplex from the electric fields and makes melting more difficult.
- "re-melting" an electrode results in additional melting. Why?
- The effect of other salts should be studied.

Acknowledgments

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References
[1] I. Wong, N. Melosh, Nano Lett., 2009, 9 (10), pp 3521–3526
[2] S. Majahan, J. Richardson, T. Brown, P. Bartlett, J. Am. Chem. Soc., 2008, 130 (46), pp 15589–15601
[3] R. Johnson, N. Gale, J. Richardson, T. Brown, P. Bartlett, Chem. Sci., 2013, 4, 1625-1632