Optimization of electrochemical biosensors of the transcription factor c-Myc for point-of care cancer diagnosis

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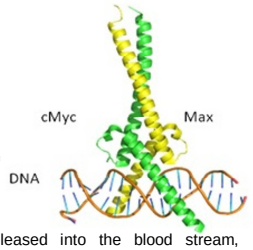
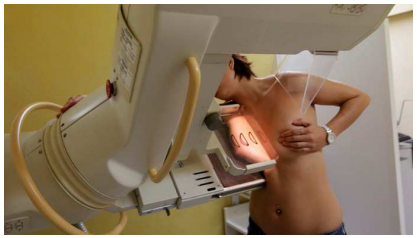
Introduction

Breast cancers are prolific diseases, which both directly and indirectly affect many people during their lifetime. Detection of breast cancer is currently a lengthy process, which is often painful and costly. This project is aimed at producing a point of care diagnostic to detect breast cancer that is quick, cost effective, minimally invasive, and can be done at any doctor's office.

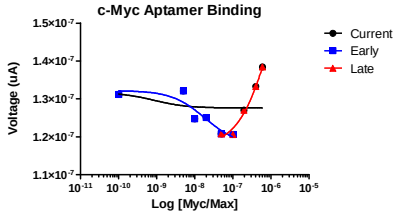
A common marker for breast cancer is the c-Myc/Max complex, which is up-regulated between 2% and 15% in several breast and kidney cancers.[1] Many cancers feature high rates of apoptosis and lysis. When this occurs, the c-Myc/Max complex can be released into the blood stream, increasing the blood concentration of c-Myc/Max. We use a DNA based biosensor that binds to the c-Myc/Max complex. Upon binding, the sensor elicits a quick, measurable current change that can be translated into the transcription factor concentration. The concentration of the c-Myc/Max complex could thus be used to as a general indication of cellular health, and high concentrations could indicate that additional screening may be warranted.



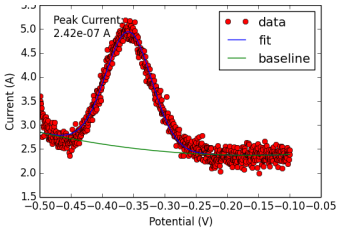


Results

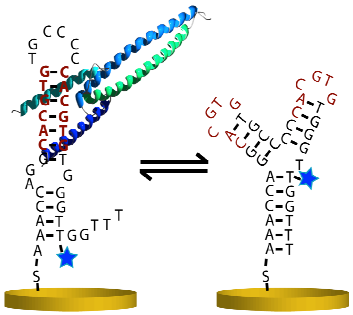
The E-DNA biosensor was originally designed to utilize a conformationally-based mechanism that would give "off" signal when protein is not bound, and an "on" signal when protein is bound. Due to the steric bulk of the protein, we observed reduced flexibility of the bound complex which resulted in an "off" signal when protein was bound, and an "on" signal when protein was not bound.[6]



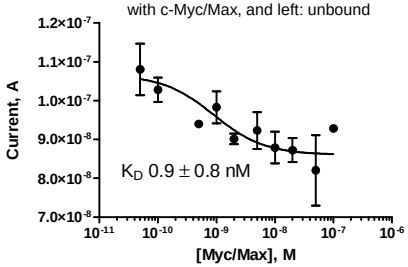
Additionally, the biosensor is not well-behaved in response at high concentrations. We suspect that this will not be a severe issue, as c-Myc/Max concentrations in the blood are on the nanomolar level, even in the elevated levels found in disease states. More testing is needed to verify this behavior.



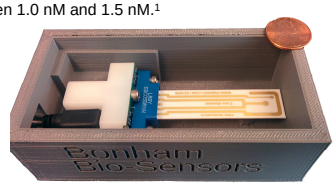
Our raw data was fit using a custom fitting algorithm.[5] The peak fit of 0.01 nM of c-Myc/Max binding to sensor is shown. The peak current is observed at the expected redox potential for methylene blue.



C-Myc Max Biosensor, right: bound with c-Myc/Max, and left: unbound



As protein is titrated into our sample, we observe a signal decrease. This decrease fits to an apparent K_D of 0.9 ±0.8 nM, which is consistent with current literature affinity values of between 1.0 nM and 1.5 nM.[1]



Gold patterned microelectrode in Custom sensor box, penny for scale

Conclusions

The current E-DNA biosensor gives a robust response with an apparent K_D close to values found in literature under optimal conditions, which include purified protein in phosphate buffered saline buffer and at low protein concentrations. However, these are not the conditions that it must function in to be used in a medical point-of-care. Our next step is to optimize the E-DNA biosensor under more complex conditions, such as in serum, blood, and from tissue samples, as well as with extensive negative controls. Once the sensor has been shown to elicit a robust response under multiple conditions, we will begin the process of quantifying healthy levels of c-Myc/Max in the blood, and the range of unhealthy, or cancerous, levels. That information is not currently available and will provide valuable information on the feasibility of using these biosensors as medical diagnostics for cancer screening. Ultimately, we show here the multiple stages a biosensor must undergo to ensure sensitivity, specificity, and suitable reactivity for clinical use.

Literature cited

1. Farina, A., Faiola, F., & Martinez, E. (2004). Reconstitution of an E box-binding Myc:Max complex with recombinant full-length proteins expressed in Escherichia coli. Protein Expression and Purification, 34(2), 215–222.
2. Schaffner, S. R., Norquest, K., Baravik, E., Stephens, J., Fetter, L., Masterson, R. M., … Bonham, A. J. (2014). Conformational design optimization of transcription factor beacon DNA biosensors. Sensing and Bio-Sensing Research, 2, 49–54.
3. Bonham, A. J., Kuangwan, H., Ferguson, B.S., Vallée-Bélisle, A., Ricci, F., Soh, H. T., Plaxco, K. W. (2012) Quantification of Transcription Factor Binding in Cell Extracts Using an Electrochemical, Structure-Switching Biosensor. Journal of the American Chemical Society, 134(7), 3346-3348
4. Markham, N. R.; Zuker, M. UNAFold: Software for Nucleic Acid Folding and Hybridization. Methods Mol Biol 2008, 453, 3–31
5. Ferguson, B. S., Hoggarth, D. a, Maliniak, D., Ploense, K., White, R. J., Woodward, N., … Soh, H. T. (2013). Real-time, aptamer-based tracking of circulating therapeutic agents in living animals. Science Translational Medicine, 5(213), 213ra165.
6. Xiao, Y., Uzawa, T., White, R. J., Demartini, D., & Plaxco, K. W. (2009). On the Signaling of Electrochemical Aptamer-Based Sensors: Collision- and Folding-Based Mechanisms. Electroanalysis, 21(11), 1267–1271.

Materials and methods

We obtained recombinant, his-tagged c-Myc and Max proteins via purification from *E. coli* BL21(DE3).[1] Protein was purified via nickel-affinity chromatography with purity confirmed via SDS-PAGE and UV/Vis. Our electrochemical DNA (E-DNA) biosensor was based on the E-Box binding element.[1] Around this core, a conformation-switching structure was designed[3] and verified with UNAFold secondary structure server[4] and a custom biosensor prediction algorithm, Fealden.[2] The biosensor was synthesized with a 5' disulfide and an internal methylene blue attached to a thymine. The biosensor was coupled to a gold patterned electrode with a silver/silver chloride reference, with mercaptohexanol added to passivate unreacted gold surface. The biosensor was interrogated using square wave voltammetry. Electrochemical measurements were taken using a Wave Nano potentiostat and analyzed via a custom baseline subtracting algorithm.[5]

Acknowledgments

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Further information

Bonham Research Lab
http://BonhamLab.com

Fealden Biosensor Prediction Server
http://Fealden.BonhamLab.com