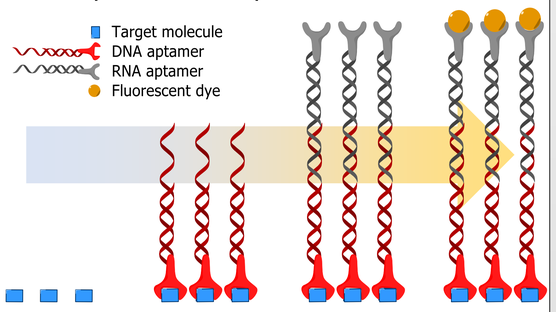
Fluorescence Enhancing Aptamers in Biosensing Applications

Volition La, Thorsten Dieckmann

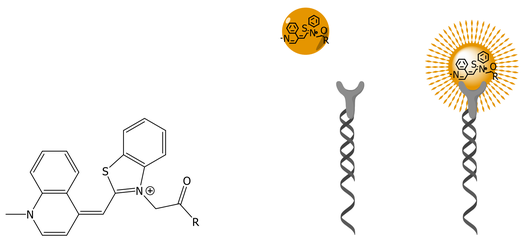
University of Waterloo, 200 University Ave W, Waterloo, ON N2L 3G1, Canada

Introduction

Aptamers, nucleic acid sequences that are selected *in vitro* to recognize and bind to target molecules, are diverse and can be used as a key component in biosensors. Here we explore the use of a two aptamer system to provide both selectivity and sensitivity in this biosensor.



The mango RNA aptamer is able to recognize and bind to derivatives of the dye thiazole orange. This dye exhibits a slight florescence signal at 535 nm which is enhanced up to 1200 fold when bound to the mango aptamer. This large increase in fluorescence is used to provide high sensitivity in this biosensor.



IgE is the desired target for this biosensor. The selectivity is provided by a DNA aptamer that was selected against IgE. In order to utilize the selectivity and sensitivity of both the RNA and DNA aptamers, the sequences were modified to incorporate a complimentary stretch of adenine and thymine. A challenge that is still present is that both binding events are uncoupled.

Results

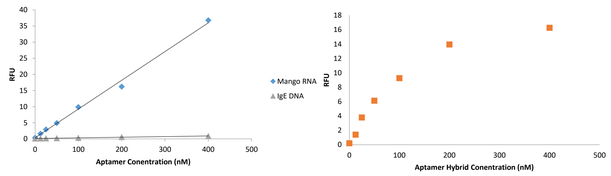


Figure 1:
The selectivity of binding of the mango RNA aptamer to the target can be seen with the graph above. Fluorescence enhancement can only be observed with the mango aptamer present, however saturation is reach faster with the addition of the IgE DNA aptamer.

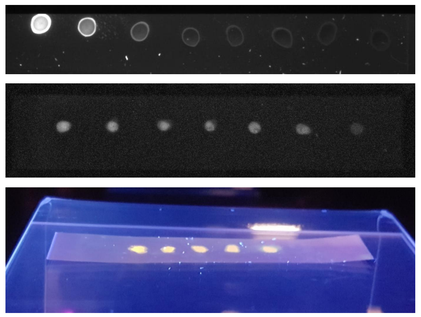


Figure 2:
Fluorescence enhancement is observed for the RNA aptamer in solution state and when absorbed into a membrane. The perceived limit of detection is in the nanomole range.

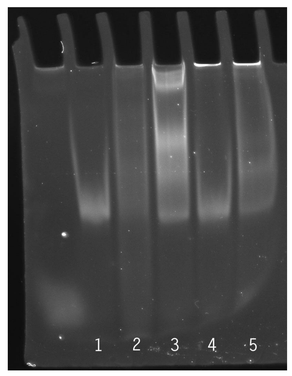


Figure 3:
An Electrophoresis Mobility Shift Assay (EMSA) is performed to show a that the DNA/RNA duplex interacts with the IgE protein.

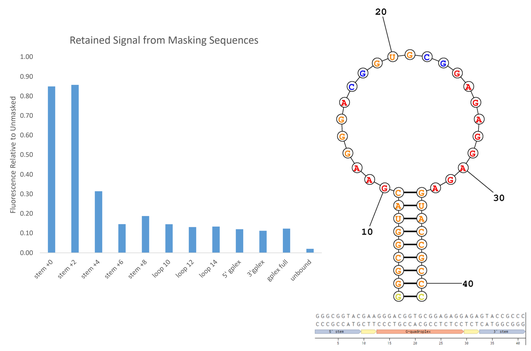


Figure 4:
Sequences that are designed to hybridize with certain regions of the aptamer to couple the two binding events.

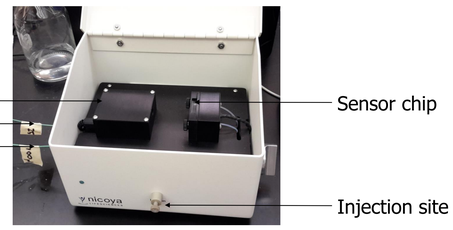


Figure 5:
This SPR system is the be used to determine kinetic parameters of the aptamers to better understand biophysical characteristics under different solution conditions.

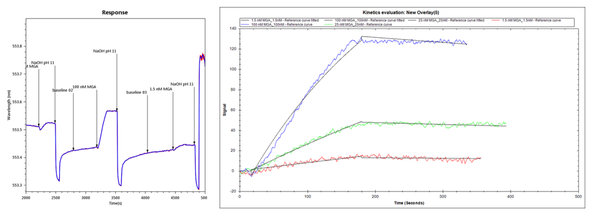


Figure 6:
A typical response curve given from an experiment and the analyzed kinetics of binding for the aptamer/target complex. This experiment is done using the malachite green aptamer as a model system for RNA aptamer binding.

Conclusions

A proof of concept using two aptamers to provide selectivity and sensitivity in a biosensor by linkage though hybridization has been shown. The study of the physical characteristics of aptamers can help rational design of novel solutions to current problems.
The coupling of the aptamer binding events is desired because the fluorescent signal is only wanted when bound to the target to reduce the signal to noise ratio. Masking the fluorescence signal until the DNA aptamer binds to the target is one such approach.

Future Work

The kinetics and thermodynamics of the aptamers systems would be interesting to study when under the coupled and uncoupled conditions. This could give insight into the rational design of future hybrid aptamers. Exploration of current aptamer systems would be relevant for future applications such as in biosensing.

Works Cited & Acknowledgements

Dolgosheina E V, Jeng SCY, Panchapakesan SSS, Cojocaru R, Chen PSK, Wilson PD, Hawkins N, Wiggins PA, Unrau PJ: **RNA Mango Aptamer-Fluorophore: A Bright, High- Affinity Complex for RNA Labeling and Tracking**. *ACS Chem. Biol.* 2014, 9:2412–2420.

Da Costa JB, Andreiev AI, Dieckmann T: **Thermodynamics and Kinetics of Adaptive Binding in the Malachite Green RNA Aptamer**. *Biochemistry* 2013, 52:6575–6583.

Bernard Da Costa J, Dieckmann T: **Entropy and Mg(2+) control ligand affinity and specificity in the malachite green binding RNA aptamer**. *Mol Biosyst* 2011, 7:2156–2163.

NSERC CRSNG EXVIVO nicoya LIFESCIENCES