Engineering FRET Biosensors for microRNA Presence/Absence Analysis

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Introduction: MicroRNAs (MiRs) are small regulatory RNAs. Several studies have found a connection between changes in miR expression and disease progression, but limitations in current sensors and techniques have hindered validation of these links. Current miR detection techniques such as RNAseq can identify global changes in microRNA expression, but cannot easily distinguish true differences between single cells as the cells must be combined and lysed prior to analysis. Current *in situ* sensors like molecular beacons offer intracellular analysis, but suffer from false positives when nucleases cleave off the dyes. To combat these issues, we developed a reporter+probe (R+P) biosensor that uses a conformational change in the reporter to bring two dyes together to produce a signal change, decreasing susceptibility to false positive signal generation. The biosensor works in both a signal-off and signal-on manner using both quenching and Förster Resonance Energy Transfer (FRET) enhancement, respectively. For the FRET dye pair, Fluorescein and ATTO 633 served as the donor and acceptor dyes, respectively.[2] Measurement of the decrease in donor signal provides the ability to measure the absence of miR, and an increase in the acceptor signal can be used to measure the presence of miR. Here we analyzed the biosensor's sensitivity and selectivity for miR-146a-5p, a potential biomarker for the development of TNBC (triple-negative breast cancer).[1]

Materials and Methods: All nucleic acids were ordered from Integrated DNA Technologies (Coralville, IA) and used as received. DNA analogues were used instead of RNA during testing because DNA is more stable. Reporter and probe were hybridized at 5 nM at 37 °C for 3 hours. Then miR-146a-5p or off-analytes were added and hybridized for 3 hours at 37 °C before analysis. Calibration curve solutions contained increasing miR-146a-5p concentrations from 0 to 5 nM in 1 nM increments. For off-analyte experiments, a constant concentration of 5 nM off-analytes miR-146a-3p or miR-146b-5p (differ from miR-146a-5p by as little as two bases) were tested with 5 nM R+P. Fluorescence measurements were made with a MaiTai ultrafast laser (Spectra-Physics, Santa Clara, CA) and an Acton spectrometer equipped with a 300 groove / mm grating blazed at 500 nm and an EMCCD camera (Princeton Instruments, Trenton, NJ). The grating was centered at 520 nm and 660 nm for the emission from Fluorescein donor dye and ATTO 633 acceptor dye, respectively. Samples were excited with 250 mW of 935 nm laser light and fluorescence emission was collected for 2500 ms / frame.

Results and Discussion: When R+P was converted to R-hairpin in the presence of miR, the Fluorescein signal decreased and the ATTO 633 signal increased. When comparing equimolar R+P to R, the Fluorescein signal decreased by 1010 ± 40 % and the ATTO 633 signal increased by 98 ± 5 % (Figure 1). From the calibration curves with 5 nM R+P, a limit of detection (LOD) was found to be 218 pM (± 54 % RSD) for signal-off and 276 pM (± 25 % RSD) for signal-on. The sensor also showed better selectivity for miR-146a-5p over off-analytes miR-146a-3p and miR-146b-5p.

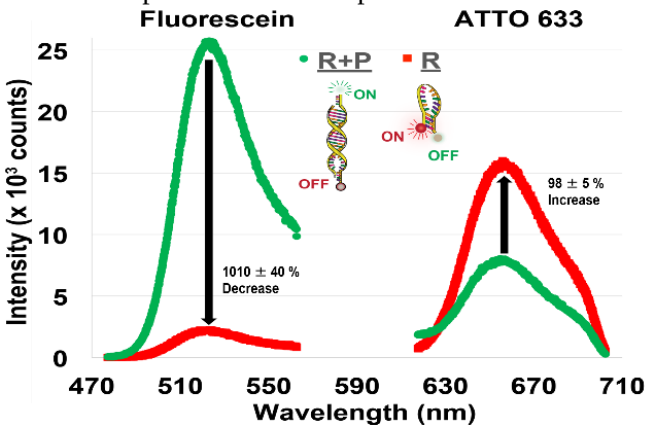

Figure 1. Emission spectra of 5 nM R+P complex compared to 5 nM R when excited at 935 nm. FRET enhancement caused an increase in ATTO 633 signal and a decrease in Fluorescein signal for R-hairpin versus R+P. Signal for each dye was summed over 100 pixels around the peak emission.

Conclusions: The reporter+probe (R+P) biosensor for miR-146a-5p showed picomolar LODs for both signal-on and signal-off methodologies using a Fluorescein|ATTO 633 FRET dye pair. The biosensor also showed resistance to off-analyte binding. Future work will study how the addition of off-analytes to calibration curves will affect the sensitivity for miR-146a-5p, and test whether newer R+P designs can further increase the selectivity for miR-146a-5p. Future work will also focus around intracellular miR analysis with these sensors.

References: 1. Li Y., *Cancer Biomarkers*, 2015, 15, 881–887. **2.** Larkey N. E., *Analyst*, 2016, 141, 6239-6250.