Supporting Information for

Anti-DNA:RNA Antibodies and Silicon Photonic Microring Resonator Arrays Enable the Ultrasensitive, Multiplexed Detection of microRNAs

*Abraham J. Qavi[1,‡], Jared T. Kindt[1,‡], Martin A. Gleeson[2], Ryan C. Bailey[1]**

[1]Department of Chemistry, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave, Illinois, 61801 and [2]Genalyte, Inc., 11099 North Torrey Pines Road, Suite 170, La Jolla, California, 92037.

*Email: baileyrc@illinois.edu

Table of Contents

Specificity of S9.6 Binding Only to DNA:RNA Heteroduplexes

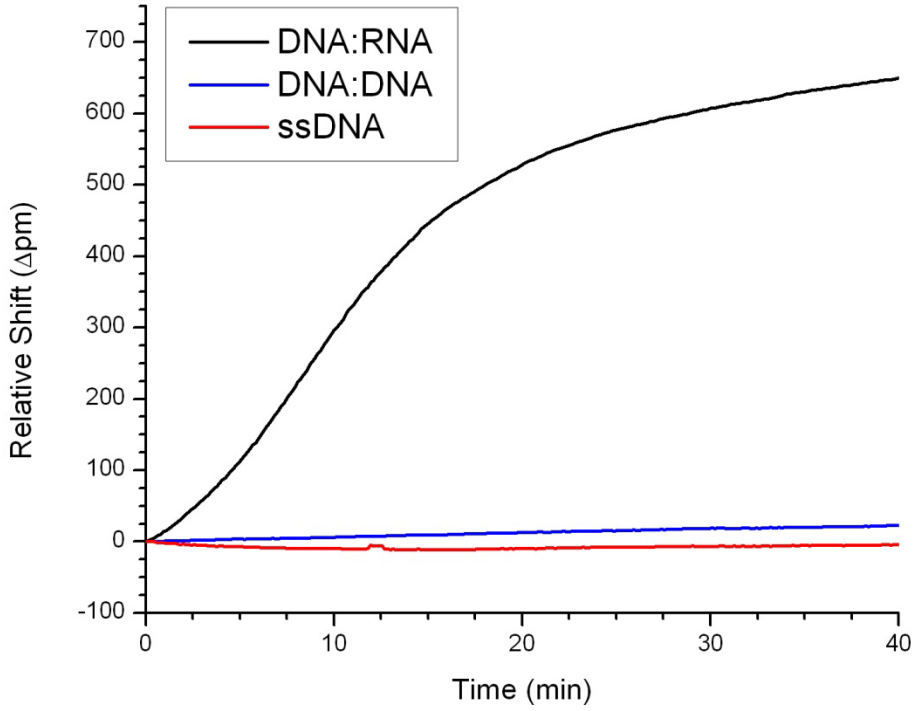

Figure S1. Sensor rings were functionalized with cDNA complementary to miR-16, and incubated with one of the following: 40nM miR-16, 40 nM DNA analogue of miR-16, or buffer only. Real time response of S9.6 amplification towards the resulting single stranded DNA (red), a DNA:DNA duplex (blue), and a DNA:RNA heteroduplex (black) illustrate the minimal nonspecific adsorption properties of the antibody.

Binding Response and S9.6 Amplification of a 1 nM miR-24-1 Target

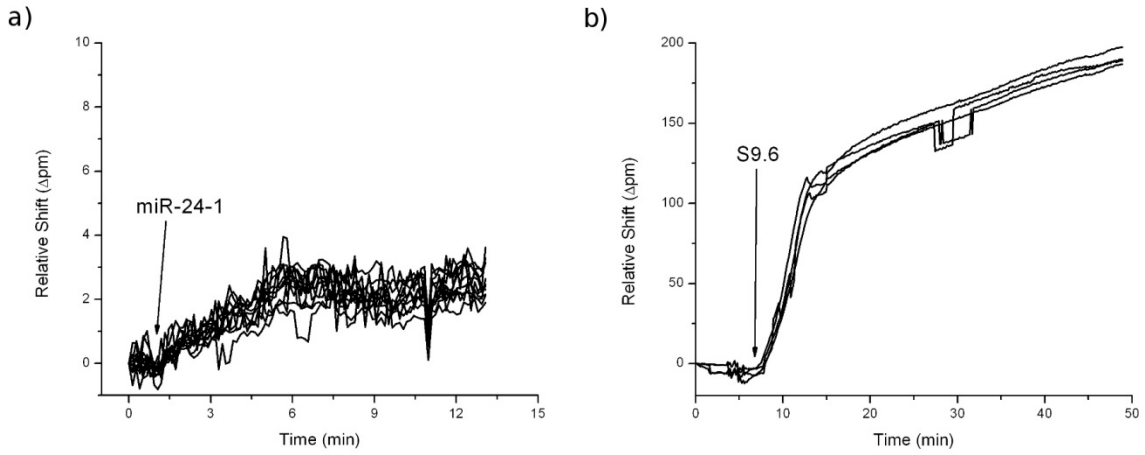

Figure S2. a) A 1 nM solution containing miR-24-1 is flowed across sensor rings functionalized with a perfectly complementary DNA capture probe giving a measureable signal, but one that is approaching the noise floor of the assay. b) A solution containing 2 µg/mL of the S9.6 antibody is then flowed across the bound heteroduplexes and a much larger and more easily measured response. Although the S9.6 response is not yet at equilibrium after 40 minutes of binding, it is clear that the amount of amplification is significantly larger than that expected based upon a 1:1 binding interaction. The bound miRNA (~7 kDa) is approximately 21 times smaller than the S9.6 antibody (~150 kDa), but the observed amplification factor is at least a factor of 60, suggesting that multiple S9.6 antibodies can bind to each surface-bound heteroduplex.

Length Dependent Response of S9.6 Amplification

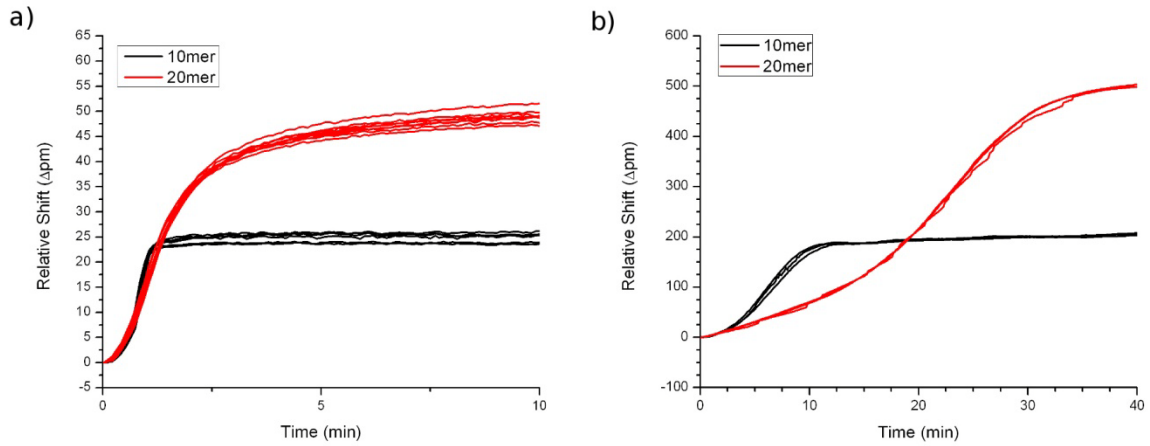

Figure S3. a) Microrings functionalized with a 40-mer ssDNA capture probe were incubated with 100 nM solutions of 10-mer and 20-mer RNA targets complementary to the 3' end of the capture probe, revealing a length dependent signal response. As expected, the hybridization of the 20-mer results in a signal that is approximately two times larger than for the 10-mer. b) The subsequent S9.6 amplification response on the DNA:RNA heteroduplexes consisting of the 20-mer RNA also shows a larger response than the 10-mer heteroduplex, further supporting the notion that multiple (2-3) S9.6 antibodies can bind to a single bound miRNA. Experiments performed with a 40-mer test RNA sequence (not shown) confirm that multiple S9.6 antibodies can bind to single heteroduplexes, and also reveal that longer strand responses are accompanied by more complex steric binding considerations.

Binding Response of S9.6 to Mouse Brain Total RNA

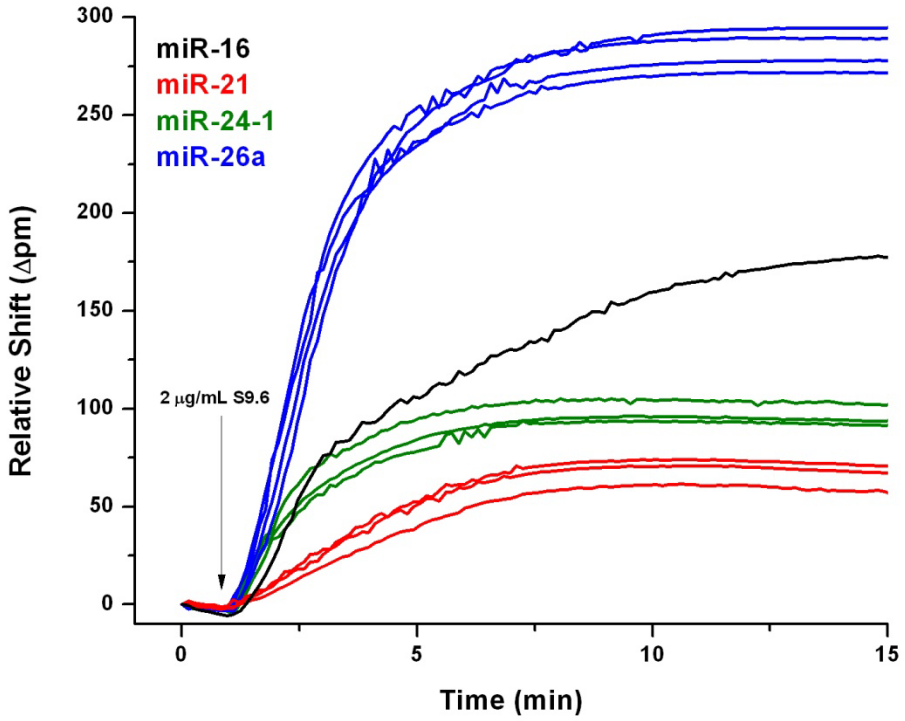

Figure S4. Microrings previously functionalized with 4 different capture probes complementary to different miRNA of interest were incubated with mouse brain total RNA overnight. After a blocking step the microrings are subsequently exposed to S9.6 in buffer. The resulting shift is then quantitated via calibration plots for each miRNA.

Comparison of S9.6 Binding Towards RNA:DNA and RNA:LNA Heteroduplexes

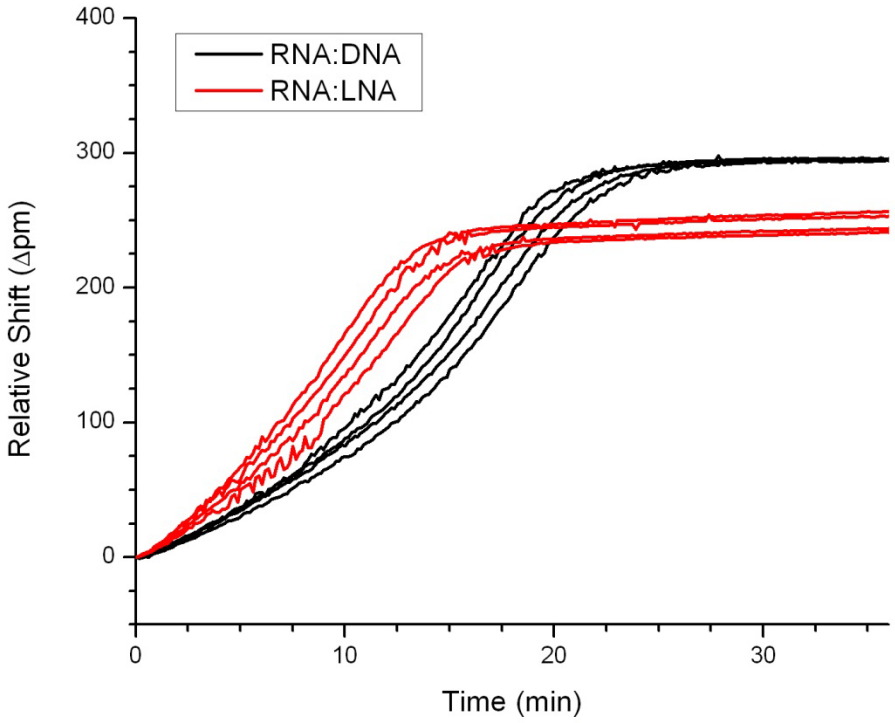

Figure S5. Sensor rings were functionalized with either cDNA complementary to miR-24-1 or an LNA analogue of the DNA capture probe. A solution of 40 nM miR-24-1 was flowed over the entire chip. The real time response of the S9.6 amplification towards each of the heteroduplex pairs is shown above. While the LNA:RNA heteroduplex (red) elicits a response to the S9.6 amplification, the response is lower than seen with an DNA:RNA heteroduplex (black). However, the fact that S9.6 can recognize LNA:RNA heteroduplexes should prove to be quite useful as LNAs have previously been demonstrated to be higher affinity capture probes for miRNA detection applications, compared to DNA.