Hybridization Enhancement Studied Using Real-Time Detection

Justin A. Bishop[1] S. Blair[1] N. Adey[2]

[1]Univeristy of Utah Department of Electrical Engineering, 50 S Central Campus Drive, Salt Lake City, Utah, 84112
[2]BioMicro Systems, Inc., 1290 W 2320 S, Suite D, Salt Lake City Utah, 84119

Abstract

The performance of most hybridization arrays is determined by how fast target in solution can be interrogated by probe tethered to the surface of the array. In most cases, where target concentrations and volumes are low, this will be determined by the diffusion time of the target, unless other mechanisms are used to decrease the effects of diffusion. Researchers are investigating the use of mixing techniques during the hybridization experiments. However, reported results used post hybridization analysis tools instead of real-time detection to determine the hybridization enhancement due to mixing. Using real-time detection data, kinetic curves can be generated and studied to understand where mixing is affecting the diffusion process. Using the MAUI platform, real-time detection was used to study the increase in hybridization due to mixing. It was found that mixing did not affect the kinetic curves the same throughout the whole experiment and most of the benefits came within the first thirty-five minutes of hybridization.

Keywords: Hybridization, Real-Time Detection, Mixing, Microarray

1. Introduction

Microarrays are becoming a staple in research labs around the world. Arrays are being used for studying diseases [1], conducting genetic and drug screening [2], and DNA sequencing [3]. However, using the typical methods for hybridization and analysis have their limitations. One such limitation is the time it takes to do a hybridization experiment using low target concentrations and volumes, generally 12-24 hours [4]. In addition, analysis of the hybridization experiments is generally done post hybridization using an array scanner. The intensity information from the scanner is then used to decide whether a target was present in the solution. In some cases, ratiometric analysis is used to try to quantify concentrations in solution but this requires an additional labeled target for each target in solution.

To decrease the hybridization times some are looking into the idea of laminar mixing. Using this idea, different channel geometries for delivery of target to the probe site [5-6] and microfluidic planetary centrifugal mixing [7] have both shown to decrease hybridization times and increase the usable signal from an array after hybridization. Another design, created by BioMicro Systems and used in this study, is an air bladder setup that pushes the solution back and forth within a chamber. This system has also been shown to increase sensitivity [8].

The majority of these studies reported improvement in sensitivity at the end point rather than increase in hybridization. Using a real-time detection system would allow the complete analysis of the enhancement of hybridization because one would be able to observe the real-time hybridization data and determine what was causing the increase in signals and whether there was a point at which mixing was no longer accomplishing measurable results.

2. Experimental

Experiments were carried out using a modified MAUI platform, created by BioMicro Systems, that allowed for real-time detection of the affinity interactions using fluorescently labeled target DNA. The mixing mechanism for the MAUI platform has previously been described [8]. The array of probes were covalently bound using an amino linker to microscope slides that were coated with 3-glycidoxypropyltrimethoxysilane (GPS) by Full Moon BioSystems and then spotted by Microarray Inc. The slides were blocked by submerging them in a bovine serum albumin (BSA) solution for five minutes at 50° C, washed with DI water, and then dried under a nitrogen flow. The sensing surface was enclosed using the air bladder coverslip provided for use with the MAUI system. The platform modification allowed for light from a 635 nm diode laser to be endfire

coupled into the microscope slide which acted as the waveguide and produced the evanescent field used to excite the Cy5 labeled to the target DNA. Three substantially different 60- mer target DNA sequences were used so that heteroduplex formation due to target probe mismatch would be negligible. The chamber created by the coverslip was filled using personalized fluidic connections, designed by BioMicro Systems, as well as a syringe pump that pushed 0.40 ml of target solution, 4X SSC, into the chamber at 0.10 ml/min. During the mixing experiments, the mixing mechanism was turned on after 0.40mL of solution had flowed into the chamber. The fluorescence intensity from the Cy5 was captured using a Santa Barbra CCD and the intensity data was then analyzed using a program written in MatLab.

3. Results & Discussion

Hybridization was done at 40° C for 90 minutes. A time lapse of collected intensity is shown in figure 1. Control experiments were run using three different targets all at different concentrations, 10 nM, 5 nM, and 1 nM, without the mixing mechanism turned on. The results are shown in figure 2. It can be seen that at the beginning of hybridization there is a quick rise in intensity, especially for the 10 nM concentration, which indicates that kinetics are dominated by the reaction rate. However, after the initial increase the curve seems to level off. This leveling off is an indication that diffusion of DNA to the surface is starting to drive the kinetics.

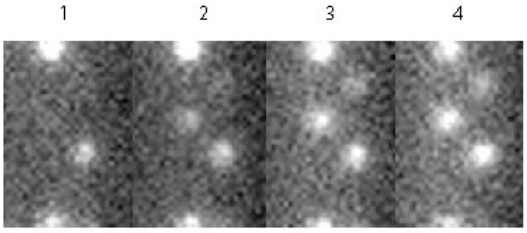

Fig 1: Intensity pictures of zones as time progresses from 10 min to 90 min during a no mix experiment. Top spot is a hybridized reference spot and the bottom spot, that can barely be seen, is a reference spot of bound probes with Cy5 attached. Starting from the top of picture 4, the 2nd spot is where 1nM target binds, 3rd spot is where 5nM target binds, and the 4th spot is where 10nM target binds.

Mixing experiments were then preformed using the same concentrations as were used for the control experiments. Figure 3 shows, similar to the control experiment, there is an initial rise in hybridization at the beginning of the experiment, but toward the end of the run the curve seems to level off to the same slope as in the control experiment. However, after the initial growth, the lower concentrations do seem to prolong a higher rate of reaction for the first thirty-five minutes of hybridization before rolling off to the slope of the corresponding control experiment.

In addition, figure 3 shows that during the mixing experiment the 5 nM concentration was lower in intensity than the 1 nM concentration of target. This result was unexpected, however; it highlights a problem with many arrays, good repeatability. It is thought that the reason for this unexpected result is due to the probe density on the surface of the array, which is not specifically known. Results suggest that the density of the probes used for the 5 nM target is lower than the density of the 1 nM target which would lead to the observed results.

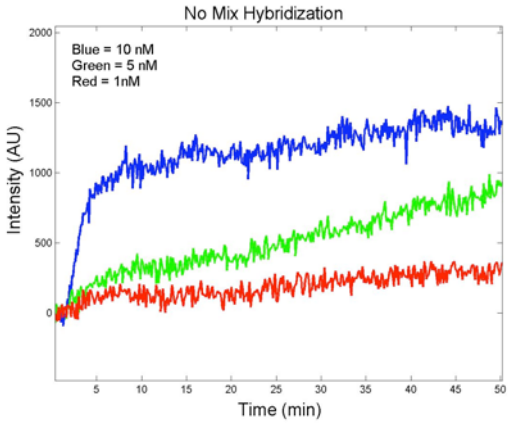

Fig. 2: Data from control experiment three different concentrations of target.

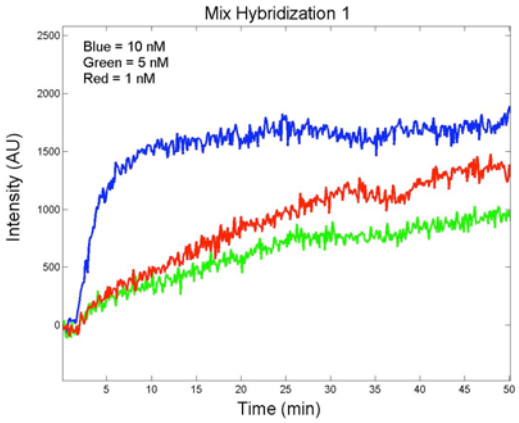

Fig. 3: Data from mixing experiment using three different concentrations of target.

Figure 4 shows a comparison of the control experiment and the mixing experiment and the reason for a higher sensitivity in the system can be seen. The initial rise for the mixing experiments is much greater than that for the control experiment. However, it

should also be pointed out that after this initial upsurge, the mixing does not seem to further improve the hybridization for the higher-concentration target. The curves once again seem to be driven by diffusion to the surface. There are two possible reasons for this. First, the probe sites could be used up and therefore continued growth in intensity will only happen as fast as dissociation, which at the temperatures used is almost zero. This is most likely the reason for the high concentrations, where there is an excess of target at the surface. Another reason for the decrease in slope is the fact that the mixing is not occurring out of the plane, which is the probable reason for the decrease in slope for the low concentrations. The bladder action causes the boundary layer at the surface to move back and forth, but once this boundary layer is depleted of target the kinetics then become limited by diffusion in the vertical direction.

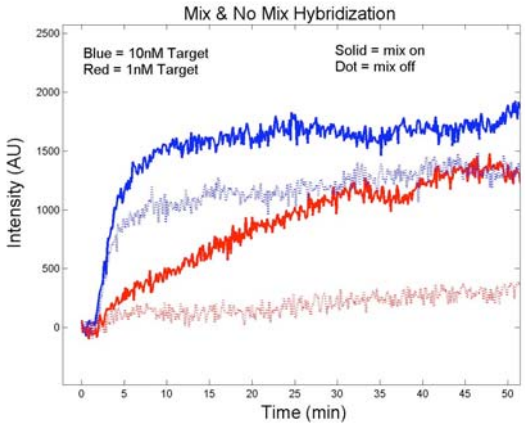

Fig. 4: Comparison of control and mixing experiments using 2 target concentrations.

4. Conclusion

It has been shown that the platform used can increase hybridization. However, using the method of real-time detection, it was seen that mixing did not affect hybridization kinetics the same over the complete span for which it was being used. It was observed that if one mixed for the first thirty-five minutes of the experiment then most of the benefit from this platform would have been used. However, due to the nature of the sensing mechanism, longer-term sensing was not plausible. Therefore, one cannot say whether or not allowing the mixing to continue for a long period of time would actually help the hybridization. Real-time detection has also shown to be a beneficial tool if used to evaluate the mixing ability of a hybridization setup. Using the data from the real-time detection, the kinetic data is not lost therefore; it can be analyzed, as it was here, to determine how well the mixing is actually helping the affinity interactions.

5. References

[1] V. Chan, D. J. Graves, P. Fortina, and S. E. McKenzie, "Absorption and Surface Diffusion of DNA Oligonucleotides at Liquid/Solid Interfaces," Langmuir, Vol. 13, pp. 320-329, 1997.

[2] K. Pappaert, P. V. Hummelen, J. Vanderhoeven, G.V. Baron, and G. Desmet, "Diffusion-reaction modeling of DNA hybridization kinetics on biochips," Chemical Engineering Science, Vol. 58, pp. 4921-4930, 2003.

[3] R. Lenigk, M. Carles, N.Y. Ip, and N. J. Sucher, "Surface characterization of a silicon-chip-based DNA microarray," Langmuir, Vol. 17, pp. 2497 – 2501, 2001.

[4] M. K. McQuain, K. Seale, J. Peek, T. S. Fisher, S. Levy, M. A. Stremler, and F. R. Haselton, "Chaotic mixer inmproves microarray hybridization," Analytical Biochemistry, Vol. 325, pp. 215 – 226, 2004.

[5] P. K. Yuen, G. Li, Y. Bao, and U. R. Muller, "Microfluidic devices for fluidic circulation and mixing improve hybridization signal intensity on DNA arrays," Lab on a Chip, Vol. 3, pp. 46 – 50, 2003.

[6] R. A. Vijayendran, K. M. Motsegood, D. J. Beebe, and D.E. Leckband, "Evalution of a three-dimensional micromixer in a surface-based biosensor," Langmuir, Vol. 19, pp. 1824 – 1828, 2003.

[7] M. A. Bynum and G. B. Gordon, "Hybridization enhancement using microfluidic planetary centrifugal mixing," Analytical Chemistry, Vol. 76, pp. 7039 – 7044, 2004.

[8] N. B. Adey, M. Lei, M. T. Howard, J. D. Jensen, D. A. Mayo, D. L. Butel, S. C. Coffin, T. C. Moyer, D. E. Slade, M. K. Spute, A. M. Hancock, G. T. Eisenhoffer, B. K. Dalley, and M. R. McNeely, "Gains in sensitivity with a device that mixes microarray hybridization solution in a 25-µm-thick chamber," Analytical Chemistry, Vol. 74, pp. 6413 – 6417, 2002.