Microarray Analysis Using Competitive Hybridization

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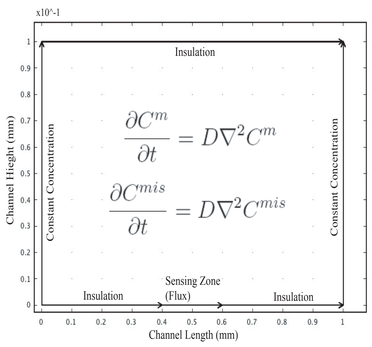
Introduction

In this work we explore the effects of wild-type and single nucleotide polymorphism (SNP) target specie concentrations, temperature, and the time of hybridization on sensing specificity in two component systems. A finite element method is used to simulate the diffusion of DNA through a microfluidic chamber to the sensing surface of bound oligonucleotide probes where hybridization of DNA is modeled using the corresponding chemical reaction equation assuming low grafting density. The association rate constant for the 20-mer oligonucleotides is obtained from experimental data, and is used with a thermodynamic model to determine dissociation rate constants at different temperatures. Our results show the observed dynamic range between the wild-type target sequence and the SNP target sequence, hybridizing to wild-type complimentary probe, increases with temperature because of enhanced dissociation of the SNP target, assuming thermodynamic equilibrium is not reached. In addition, competitive hybridization can be enhanced by decreasing the immobilized probe concentration on the surface of the substrate. Using the two observations above we propose a novel analysis method for hybridization experiments which could be used during real-time experiments. The method uses a novel label-less detection mechanism for multi-analyte samples. By introducing a label target that is known to be of a lower affinity than the targets to be investigated we can watch the dissociation of the lower affinity species and predict the concentration of the complement.

Mathematical Model



Hybridization Equations

$$\frac{dB^m}{dt} = k_a^m C^m (R_t - B^m - B^{mis}) - k_d^m B^m$$

$$\frac{dB^{mis}}{dt} = k_a^{mis} C^{mis} (R_t - B^m - B^{mis}) - k_d^{mis} B^{mis}$$

$$\frac{\partial C^m}{\partial t} = D\nabla^2 C^m$$

$$\frac{\partial C^{mis}}{\partial t} = D\nabla^2 C^{mis}$$

Thermodynamic Equilibrium

$$B_{eq}^m = \frac{R_t k_a^m C^m k_d^{mis}}{k_a^m C^m k_d^{mis} + k_a^{mis} C^{mis} k_d^m + k_d^m k_d^{mis}}$$

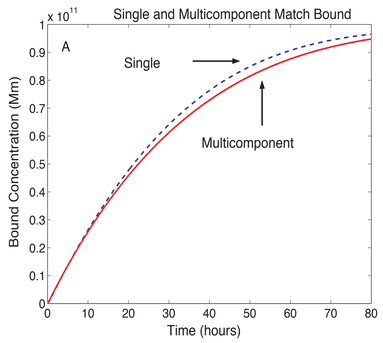
Theoretical Dynamic Range

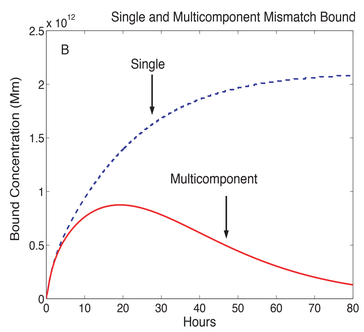
$$DR_{max} = \frac{B_{eq}^m}{B_{eq}^{mis}} = \frac{k_a^m C^m k_d^{mis}}{k_a^{mis} C^{mis} k_d^m}$$

2-D channel design with the boundary conditions for each wall and mass transport equations.

B is bound target, C is target concentration, R_t is initial probe density, k_a association rate constant, k_d is dissociation rate constant, and "mis" and "m" superscripts represent SNP and wild-type targets respectively.

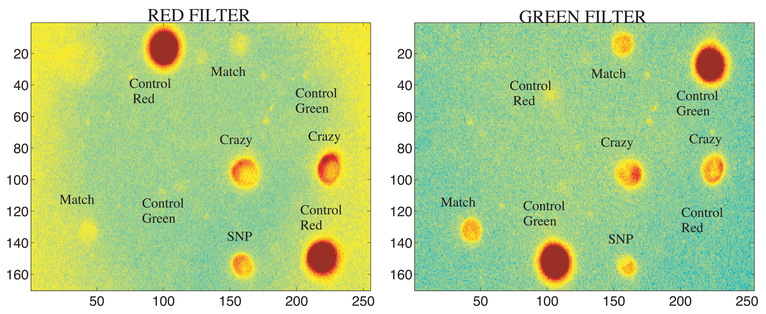
Single Component vs Multicomponent





Hybridization curves simulating target concentrations at 100 pM at a temperature of 330 K. A) wild-type targets simulated in a single, dashed line, and two component, solid line, system, B) SNP targets simulated in a single, dashed line, and two component, solid line, system.

Experimental Probe Layout (shown with target hybridizing)

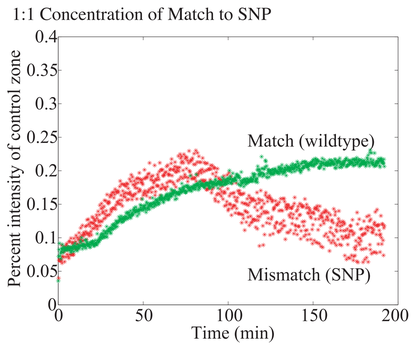


Cy5, red, and Cy3, green, intensity figures created by reading pixel intensities from CCD images using an in house matlab program. The figures shown represent a typical two component hybridization toward the end of an experiment. In this example the match spots show that there is more Cy3 labeled target than there is Cy5 labeled target which is expected because the match was labeled with Cy3 while the SNP was labeled with Cy3. Also notice that the control spots on the intensity figures confirm that we do not have Cy5 intensity coming through the Cy3 filter and the case same with Cy3 going through the Cy5 filter.

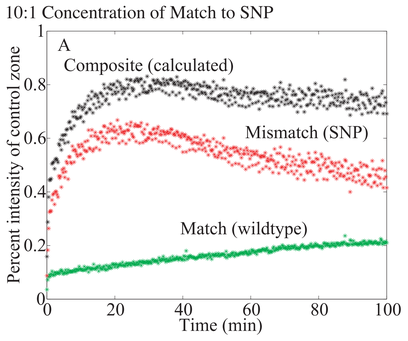
Competitive Hybridization

Experimental

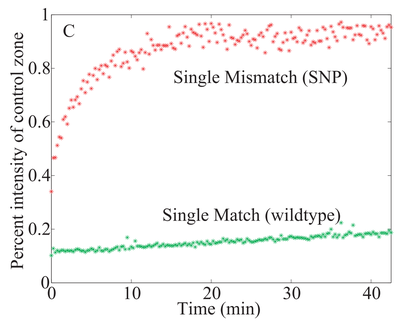
1:1 Concentration of Match to SNP



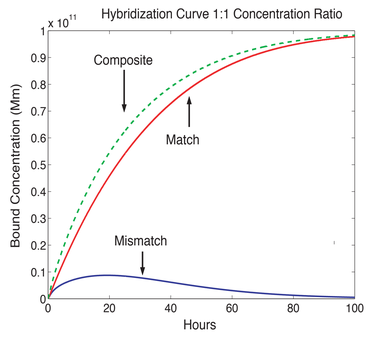
10:1 Concentration of Match to SNP

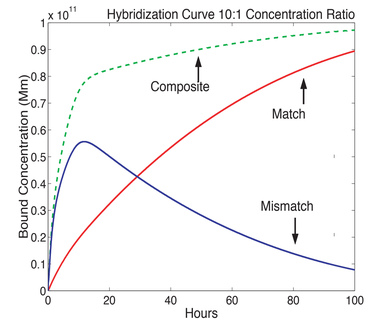


Single Component Experiment with 10:1 Concentrations



Simulation

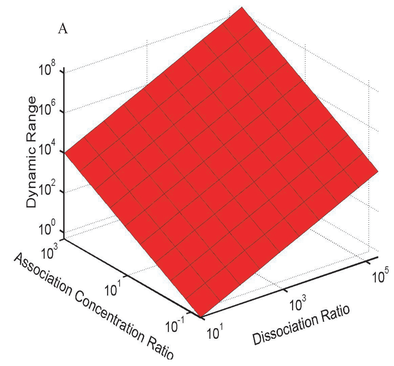


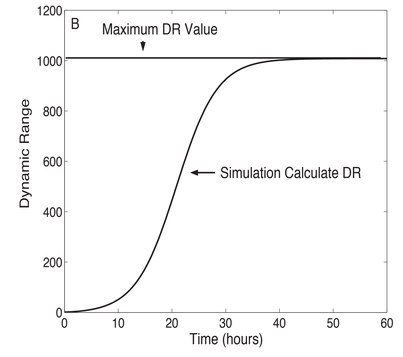


Comparisons between simulation and experimental results. Note simulation results, x-axis, are in hours while experimental results are in minutes due to simulation results (1,2) using picomolar concentrations and experimental results (3) using nanomolar concentrations.

1. Justin Bishop, Steve Blair, and Alexander M Chagovetz, " A competitive model of nucleic acid surface hybridization in the presence of point mutants," Biophysical Journal 90, 831-840 (2006)
2. Justin Bishop, Steve Blair, and Alex M. Chagovetz, "Convective flow effects on DNA biosensors," Biosensors and Bioelectronics (Accepted 2006)
3. Justin Bishop, Colby Wilson, Alex M Chagovetz, and Steve Blair, "Competitive displacement of DNA during surface hybridization," Biophysical Journal-BioFAST (Accepted 2006)

Dynamic Range





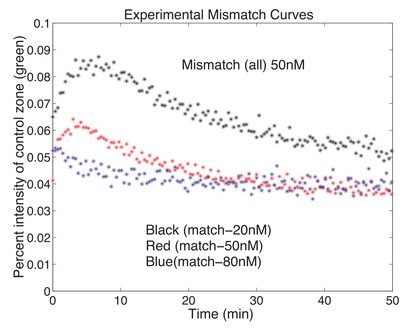
A) Dynamic range achievable at thermodynamic equilibrium using different values of dissociation constant ratios and association constant concentration ratios. B) Dynamic range calculated using simulation results for equal concentrations of target,100 pM, and dynamic range maximum from panel A.

Temperature (K)	Predicted DR	Simulation DR
325	1,650	10
327	1,350	12
330	1,010	35
333	759	160
335	628	310

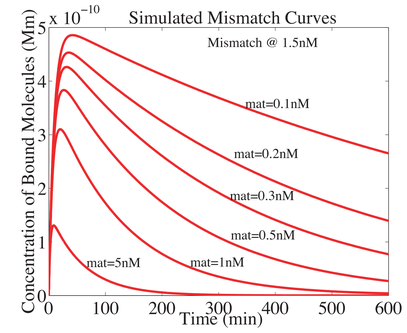
Comparison of predicted dynamic range at equilibrium and dynamic range, from simulation data with the wild-typ and SNP at 100 pM concentration, at 90% match equilibrium for different temperatures.

Label-less Detection

Experimental



Simulation



Experimental, left, and simulation, right, results showing a novel label-less detection scheme. By labeling and adding a competitor species, in this case a species with a single nucleotide change from the match target, to a solution in which a match target is of interest we can watch the displacement of the competitor by the match target. As shown in the experimental and simulation results, we can detect a change in match concentration of less than a half. Note, that the reason the time axis are different is due to the use of different concentrations of match and competitor species.

Conclusion

Using a two component model we have presented results describing kinetic behaviors of wild-type and SNP targets at a sensing zone. Even though the model does not simulate a complete array, the effects of competitive binding would increase as the simulated size of the array increases. In addition to simulated results we have also shown with experimental traces of a wild-type and SNP sequences that displacement of a low affinitiy species is possible if another species has a higher affinity for the same sensing zone. This knowledge has lead to a novel label-less detection mechanism that uses the displacement of the low affinity species, in this case a SNP, to determine the concentration of a higher affinitiy species. We have demonstrated using experimental and simulation results that a change in wild-type concentration of a half when comparing two concentrations is distinguishable.