Numerical Analysis of Deoxynucleotide Hybridization Characteristics using Single Reaction Cell Model

Heng-Chuan Kan

National Center for High-performance Computing, Taiwan, R.O.C.

Abstract—**Due to the progress of Human Genome Project, microfluidic microarray has become an effective tool for genomic analysis applications such as gene expression profiling in recent years. In this setting, each single strand oligonucleotide (oDNA) that serves as a probe attached on the microarray surface can bind with its genomic counterpart with specific designed sequences in solution phase. The binding mechanism between single strand DNA probe and its target known as DNA hybridization reaction usually takes from 6 to 24 hours depending upon the testing biological samples of interest as well as the experimental conditions. In addition, hybridization efficiency is linked to binding energy barrier that is hindered by the sequence affinity. Hence, overcoming the duration and efficiency issues of DNA hybridization presents a great challenge to microarray experiments. In this study, a computational model which consists of an inlet channel, outlet channel and single reaction cell is constructed to characterize the effects of vital experimental parameters such as infusion sample flow rate, sample size and reaction kinetic constants on the efficiency and duration of oDNA hybridization. Simulation results reveal that higher infusion flow rate as well as smaller sample fragment can accelerate the hybridization process. And the reaction kinetic constants determine the binding affinity under the equilibrium condition.**

Keywords—**DNA Hybridization, Microarray, Computational Modeling, Reaction Cell.**

I. Introduction

Recent advances in microarray technology have generated significant impacts in world of chemistry, medicine, and life sciences. Microfluidic systems have been shown to various applications including bio-molecular separation and detection [1-2], biological cell manipulation [3-4], and DNA amplification [5]. Such systems have also been demonstrated to perform hybridization assays efficiently on small DNA samples.

Hybridization analysis by microarray experiments with immobilized oligonucleotides (oDNA) as DNA probes is being developed for genomic applications including gene expression profiling, and genetic disease detection. DNA probes are attached on the surface of a microfluidic device and interact with infusing sample with specific counterpart sequences. Detection of DNA hybridization reaction involves the covalent labeling of samples with fluorescent labels prior to hybridization. Hybridization affinity can be determined using a fluorescent dye and fluorescence microscopy with CCD imaging [6]. DNA samples and dye could be transported to the hybridization sites by continuously supplied of DNA samples. Concentrations of biological sample as low as 50 nM can be detected by this method.

However, it is a rather difficult task to improve hybridization reaction efficiency strictly from try-and-error approach of laboratory work. In fact there are so many experimental parameters which play essential roles to alter the hybridization rate and stringency. In this study, a simplified computational model is constructed to elucidate the DNA hybridization characteristics and a commercial computational fluid dynamics (CFD) software from CFDRC [7] is employed to investigate the effects of vital experimental parameters such as infusing sample flow rate and sample size on the hybridization efficiency by numerical simulation method.

II. Problem description and mathematical formulation

In this study, we intend to investigate the effects of vital experimental parameters on the DNA hybridization rate and affinity by numerical simulation method.

Consider the 3-dimentional transient, incompressible, reactive Navior-Stokes flow. The governing equations in conversation forms can be expressed as following:

(i) Continuity equations

$$\frac{\partial \rho}{\partial t} + \nabla \bullet \left(\rho \bullet \vec{U}\right) = 0 \quad (1)$$

(ii) Momentum equations

$$\rho\left(\frac{\partial \vec{U}}{\partial t} + \vec{U} \bullet \nabla \vec{U}\right) = -\nabla P + \mu \nabla^2 \vec{U} \quad (2)$$

(iii) Species transport equations

$$\frac{\partial C_i}{\partial t} + \vec{U} \bullet \nabla C_i = D_i \nabla^2 \vec{U} + S_i \quad (3)$$

where ρ is the fluid density, U is the fluid velocity, P is pressure. C presents the analyte/sample concentration, S is

the source term and subscript i indicates the ith species in the sample mixture.

The boundary conditions as well as initial conditions to be applied on the reactive surface of single reaction cell are listed below.

(i) Surface reaction

$$\frac{\partial x_i}{\partial t} = K_i^a C_{iw}(1-x) - K_i^d x_i \ ; \ x = \sum_i x_i \tag{4}$$

(ii) Surface flux

$$-D_i \frac{\partial C_i}{\partial t} = K_i^a C_{iw} P_s (1-x) - K_i^d P_s x_i \tag{5}$$

(iii) Initial conditions

$$x_i = 0,\ C_i = C_{iw} = C_{io} \tag{6}$$

where x is the fraction of available surface covered by reversibly absorbed species, C_{iw} is the initial surface concentration of single reaction cell, K^a and K^d indicate the association and disassociation reaction constants, respectively. P_s represents the maximum surface species concentration or total receptor density and C_{io} is the initial analyte concentration at inlet channel.

III. Results and discussion

A computational model as shown in Fig. 1 is constructed to characterize the effects of vital experimental parameters on DNA hybridization efficiency. The 3-D simulation model consists of an inlet channel, outlet channel, and single reaction cell with total 77,295 nodes and 68,208 mesh elements. The sample mixture with initial analyte concentration of 2×10^{-8} mole is infused continuously at the inlet channel of single reaction model to mimic the recirculation of biological sample during the hybridization experiment. All surfaces of single reaction model except inlet and outlet channels are designated as reactive surface with maximum surface concentration density of 2×10^{-7} mole/m^2.

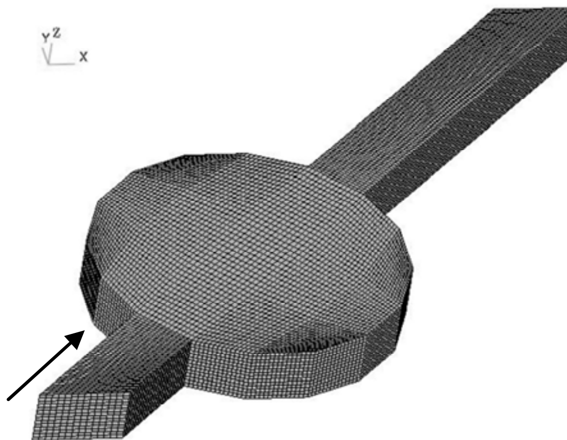

Figure 1. Schematic of the computational model. The model consists of an inlet, outlet, and single reaction cell with total 77,295 nodes and 68,208 mesh elements. Arrow head indicates the flow direction of infusing sample.

A. Sample infusing flow rate

Three different infusing sample flow rates Q_{in} (e.g., Q_{in} = 10μl/min, 100μl/min, 1ml/min) are applied in this study. Figure 1 demonstrates the effects of infusing flow rate on the DNA hybridization reaction time. It is clearly seen that as the flow rate increases 100-fold (from 10μl/min to 1ml/min) the reaction time shortens to 28.5% (from 7,000 sec to 2,000 sec). However, the non-linearity behavior becomes more profound for the flow rate of 1ml/min case.

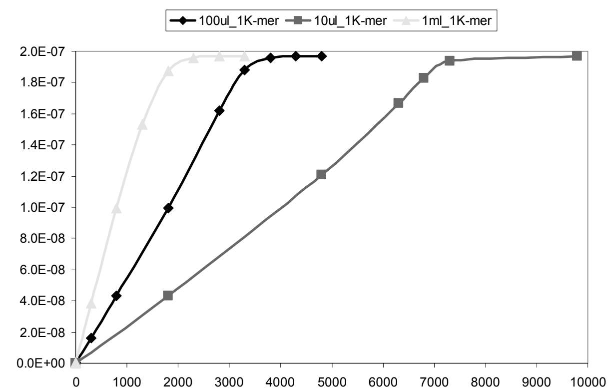

Figure 2. Effects of sample infusing flow rate on DNA hydridization efficiency.

B. Sample size/Diffusivity

The effects of sample DNA size on the DNA hybridization efficiency can be examined by the diffusivity of the biological sample. We investigate three different sample sizes (e.g., 1000-mer, 200-mer, and 15-mer) corresponding to three different diffusion coefficients based on the empirical formulation proposed by [8]. It is shown in Fig. 3 that as the sample size becomes larger (e.g., the diffusion coefficient is smaller), the longer it takes to complete the hybridization reaction. The 15-mer case takes only about 1/3 time compared with that of 1000-mer case.

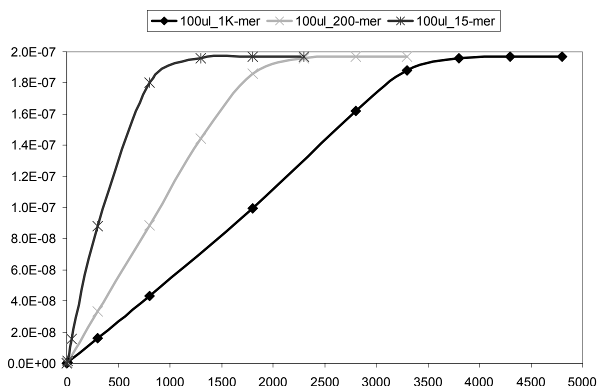

Figure 3. Effects of sample DNA size on DNA hydridization efficiency.

C. Kinetic reaction constants

The affinity of probe-sample interaction can be quantitatively determined by the kinetic reaction constants. We vary the association (K^a = 3x10^5/mol-s and 3x10^8/mol-s) and disassociation (K^d = 1x10^{-2}/s and 1x10^{-4}/s) constants corresponding to degrees of specific and non-specific binding of sample DNA fragments to oDNA probes. It is seen in Fig. 4 that the hybridization profiles are diffusion-dominated at the beginning and return to reaction-limited regime afterwards. The maximum values of surface concentration density change with the reaction equilibrium constants ($K^{eq} = K^a/K^d$) and match fairly well with the analytical solutions.

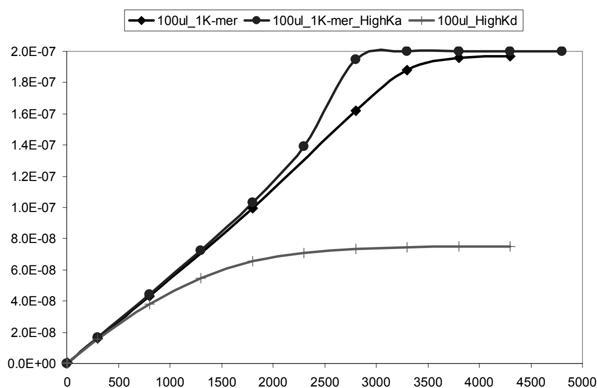

Figure 4. Effects of kinetic reaction constants on DNA hybridization efficiency.

IV. Conclusions

In the present paper, a computational model is constructed to characterize the features of DNA hybridization reaction by numerical simulation method. We utilize the commercial CFD software to investigate the effects of vital experimental parameters such as infusing sample DNA flow rate, sample DNA size as well kinetic reaction constants on the hybridization efficiency. The findings of this study can be summarized as following:

(i) The higher the infusing flow rate, the faster for the analyte/sample to reach maximum surface concentration density values.

(ii) The larger of sample DNA size, the slower to complete the hybridization reaction.

(iii) The transient surface concentration density profiles are diffusion-limited at the beginning and become reaction-limited in the later stage. Note that maximum surface concentration density values vary with reaction equilibrium constants ($K^{eq} = K^a/K^d$) and match well with analytic solutions.

Acknowledgment

This work was supported by the Ministry of Economic Affairs, Department of Industrial Technology of Taiwan under the contract no. 91-EC-17-A-05-S1-0017 and the National Center for High Performance Computing in Hsinchu, Taiwan.

References

1. Harrison DJ, Fluri K, Seiler K et al. (1993) Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. Science 261: 895-897
2. Weigl BP, Yager P (1999) Microfluidic diffusion-based separation and detection. Science 283: 346-347
3. Thorsen T, Maerkl, SJ, Quake, SR (2002) Microfluidic large-scale integration, Science 298: 580-584
4. Paul C, Li H, Harrison DJ (1997) Transport, manipulation, and reaction of biological cells on-chip using electrokinetic effects, Analy. Chem., 69: 1564-1568
5. Kopp MU, de Mello AJ, Manz A (1998) Chemical amplification: continuous-flow PCR on a chip, Science 280: 1046-1048
6. DeRisi J, Penland PO, Brown ML et al. (1996) Use of microarray to analyze gene expression patterns in human cancer. Nat. Genet. 14: 457-460
7. CFD-ACE+ 2003, CFD Research Corporation, Hontsville, Alabama, USA.
8. Borden JR, Paredes CJ, Papoutsakis ET (2004) Diffusion, mixing, and association dye effects in DNA microarray hybridizations, Biophys. J. 89: 3277-3284

Address of the corresponding author:

Author: Heng-Chuan Kan
Institute: National Center for High-performance Computing
Street: No. 28, Nan-Ke 3rd Road
City: Hsin-Shi, Tainan County
Country: Taiwan, R.O.C.
Email: hckan@nchc.org.tw