

# 3-Dimensional Diffusion-Reaction Model for DNA Hybridization on Microarrays

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## ABSTRACT

DNA microarrays have, since their introduction around 1995, gained wide use in bio-analytical chemistry. Yet the hybridization is limited by molecular diffusion, leading to overnight waiting times. During the last couple of years a large number of methods were developed to overcome this diffusion-limitation and to enhance the speed and detection limits of the microarray experiments. The generation of a convective flow increases the transport rate of the sample molecules beyond their normal diffusion rate. Considering the existence of different kinetic regimes in diffusion-driven systems, it should be obvious that a sound mathematical framework is needed to interpret the kinetic measurements of microarray experiments. There are however few fundamental reports on the mathematical modeling of the hybridization on microarrays. Here we present an analytical expression for the hybridization on microarrays. This expression was obtained by solving the 3D diffusion equation and looking at the influence of a number of different parameters. We were able to determine the influence of each parameter in the creation of a depletion zone above the spot surface and we were also able to compare the hybridization rate in diffusion- and convection-driven systems. It clearly demonstrates that convective systems are able to enhance the hybridization rate, leading to higher signal intensities and faster hybridization times. This finding enables us to accurately design convection-driven hybridization systems.

## 1. INTRODUCTION

DNA microarrays have, since their introduction around 1995<sup>(1)</sup>, gained wide use in bio-analytical chemistry, with applications in important areas such as gene identification<sup>(2)</sup>, genetic expression analysis<sup>(3)</sup>, DNA sequencing<sup>(4)</sup> and clinical diagnostics<sup>(5-6)</sup>. The hybridization on the microarrays occurs in a passive mode, by pure molecular diffusion. However, this slow process limits the rate of the hybridization assay, leading to overnight waiting times and to a very poor detection efficiency and detection limit. During the last couple of years a large number of different methods and devices were developed to overcome the diffusion-limitation problems and to enhance the speed and the detection limits of DNA hybridization. These methods<sup>(7-10)</sup> generally use a convective flow to increase the transport rate of the sample DNA strands beyond their normal (extremely slow) diffusion transport rate. Making a

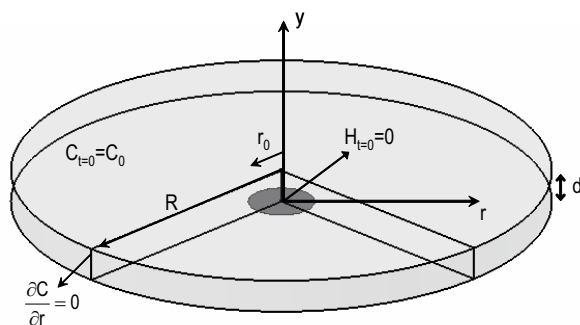
comparison between the different systems is relatively difficult since the available data relate to widely differing experimental conditions (probe spot density, length and complexity of the sample target strands, size and concentration of the applied samples...).

Considering the expected existence of different kinetic regimes in diffusion driven systems and the great variety of convection-driven systems, it should be obvious that a sound mathematical framework is needed to interpret the kinetic measurements of microarray hybridization experiments. Only few fundamental reports are available on the mathematical modeling of the binding kinetics on DNA biochips. These reports however do not allow to represent the time-dependent depletion effects inevitably occurring after a given time span<sup>(11)</sup>. Gadgil *et al.*<sup>(12)</sup> (2004) simulates the hybridization reaction on microarray spots but the simulations do not cover the influence of a large number of different parameters in the hybridization process.

In a previous publication<sup>(13)</sup> we have already addressed the modelling of the DNA microarray hybridization kinetics by solving the unsteady-state reaction-diffusion mass balance for the case of a 1-D diffusion and binding process occurring on a line piece with finite length  $h$ . This 1-D assumption is correct during the first phase of a diffusion-driven hybridization assay, i.e., as long as the amount of probes coming from the regions aside of the target spot is negligible. The generated expression for the rate of the 1-D heterogeneous DNA hybridization process covers the entire range of reaction and the diffusion limited regimes occurring in a liquid layer with finite height. It predicts the  $t^{1/2}$ -regime observed experimentally by Sadana & Vo-Dinh<sup>(14)</sup> (1998) and Vontel *et al.*<sup>(15)</sup> (2000). For the 1-D case the dynamics of the hybridization process can be fully interpreted in terms of the Damkhöler number. When  $Da > 10$ , the process clearly behaves as a diffusion-limited process, whereas when  $Da < 0.1$ , the process clearly is reaction-limited.

Here we present an analytical expression for the rate of DNA hybridization on the surface of DNA microarrays for the case of a finite 3-D diffusion space (Figure 1). This expression is obtained by solving the 3-D diffusion equation and inserting four major dimensionless constant factors. We were able to simulate the influence of seven major parameters that are important in the microarray hybridization process: the kinetic parameters  $k_{on}$  and  $k_{off}$ , the microarray spot size ( $r_0$ ) and maximal spot capacity

( $H_{\max}$ ), the height of the hybridization system ( $h$ ), the diffusion coefficient ( $D_{\text{mol}}$ ) of the sample molecules and the concentration of the sample ( $C_0$ ).



**Figure 1.** Cylindrical domain in which the diffusion equation [1] is solved.

The hybridization rate in diffusion- and convection-driven systems is compared and analysed. This finding enables us directly to accurately design convection-driven hybridization systems, immunoassay screenings and the measurement of affinity binding kinetics. The data also hint at the importance of the liquid layer height in the conventional, diffusion-driven hybridization assays. As this is a parameter which is ill-controlled in the current biochip protocols, a more accurate control of this parameter might lead to more accurate and repeatable results (e.g., by using a system of micro-machined spacers and/or by using flatter chip substrates).

## 2. THEORETICAL SECTION

The preferred modelling approach that is suited to yield a general reaction rate expression is the establishment of the continuous mass balance. For the diffusion process governing the transport across the liquid layer, this mass balance corresponds to the well-established radial 3-D diffusion equation:

$$\frac{\partial C}{\partial t} = D_{\text{mol}} \left[ \frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} + \frac{\partial^2 C}{\partial y^2} \right] \quad (1)$$

This formula describes the concentration change in time in a cylindrical domain around a circular probe spot (figure 1). The cylinder has a specific height  $d$ , radius  $R$  and a spot radius  $r_0$ . For the starting conditions we can assume that initially the concentration in the entire cylindrical volume is constant and equal to  $C_0$  and that the number of bound targets to the probe spot is 0. Boundary conditions were chosen so that a regime of total reflection was present at the edges of the domain and a reversible hybridization reaction occurred on the spot surface.

The obtained formulas contain a large number of different parameters making it very hard to look at the influence of each parameter individually. Therefore the number of parameters was reduced by writing the equations in a dimensionless form and by introducing four major dimensionless parameters.

$$Da = \frac{k_{\text{on}} \cdot H_{\max} \cdot d}{D_{\text{mol}}} = \frac{\text{Forward Reaction Speed}}{\text{Normal Diffusion Speed}}$$

$$\beta = \frac{k_{\text{off}} \cdot d}{k_{\text{on}} \cdot H_{\max}} = \frac{\text{Driving Force (off)}}{\text{Driving Force (on)}}$$

$$C'_0 = C_0 \cdot \frac{d}{H_{\max}} = \frac{\text{\# of molecules above the spot at } t = 0}{\text{Spot Capacity}}$$

$$\text{And } \alpha = \frac{\text{Spot Radius}}{d}$$

Now that the equations have been rewritten into a form where four major parameters ( $\alpha$ ,  $\beta$ ,  $Da$  and  $C'_{y=0}$ ) describe the entire microarray system, a large number of simulations were performed to look at the influence of each parameter individually. The equations were numerically solved with a self-written C++ program and two major cases were studied. We considered diffusion driven as well as convection-driven hybridization. An important aspect of the last case is that fresh target molecules are continuously fed to the system. This was included in the model by feeding the system continuously at a distance  $d$  above the surface.

Realistic values for these parameters would be  $Da=3$ ,  $\beta=0.3$ ,  $\alpha=1.67$  and  $C'_0=3 \cdot 10^{-3}$ . These values are however no fixed parameters and for each type of microarray system other values will be obtained. The height of the system for instance has a major influence on all four parameters and this height is different in most hybridization systems and sometimes even ill controlled.

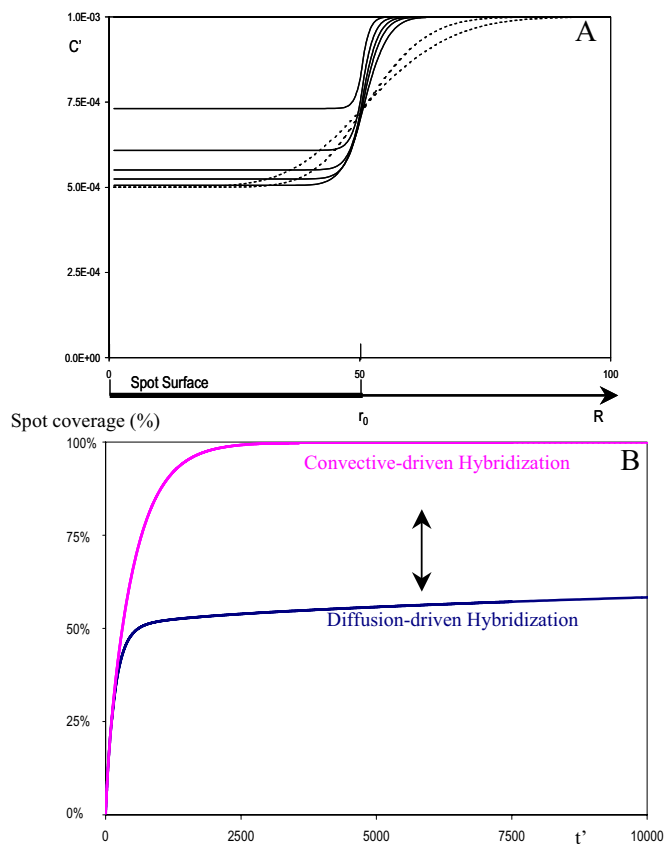
## 3. RESULTS

Two major sets of data were obtained from the simulations. At different time points snapshots of the concentration profile were made along the radial axis. Figure 2a shows how the concentration profile changes in time. The creation of a depletion zone (a zone where the target concentration is low) right above the spot surface can be observed. This depletion zone develops itself in time until it reaches the equilibrium. The time needed for the depletion zone to develop, can be seen as a reaction-limited regime because no diffusion limitation is present. When the depletion zone is established, the hybridization reaction turns to a diffusion-limited regime. In order to hybridize, target strands have to diffuse from the regions next to the spot surface (lateral diffusion). These two regimes can also be distinguished when observing the rate at which target strands bind to the spot surface. By plotting the number of hybridized strands in function of time, the difference between the two kinetic regimes is very clear (figure 2b). During the first time period the number of hybridized strands enhances very rapidly but when the depletion zone is established the hybridization rate drops dramatically. This is not the case in a convection-driven system where only the first regime (reaction limitation) is present.

By comparing how fast the depletion zone is created and to which extent it develops and by looking at the hybridization rates for the diffusion- and convection-driven systems the parameters for an ideal convective system can be obtained.

### 3.1 Influence of the Damkhöler Number

The Damkhöler number is a major factor if we look at microarray hybridization. In the 1D-model<sup>(13)</sup> the Da-number was the factor that determined whether the hybridization reaction was diffusion-limited (where  $h \sim t^{1/2}$ ) or reaction-limited (where  $h \sim t$ ). The transition between the two regimes was observed between  $Da=1$  and  $Da=10$ .

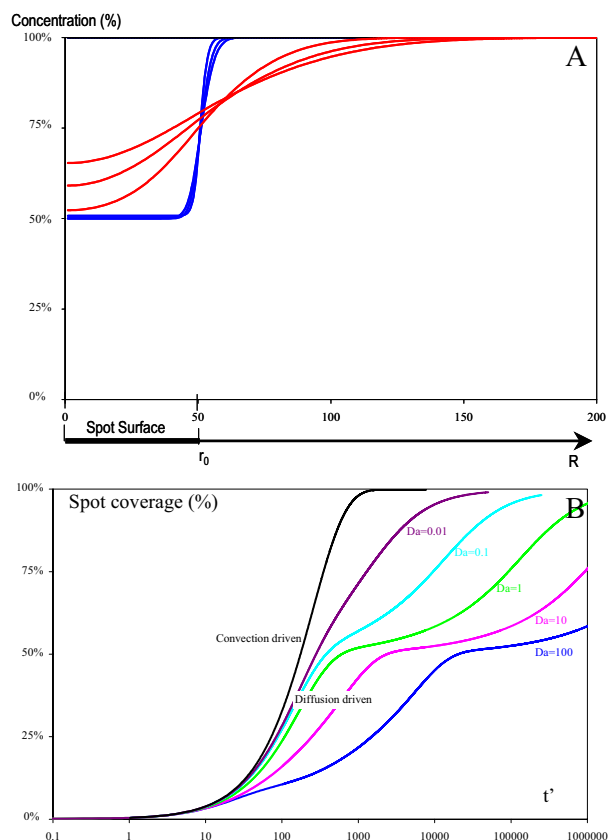


**Figure 2.** Overview of the obtained results from a numerical simulation ( $Da=1$ ,  $\beta=1$ ,  $\alpha=5$  and  $C'_0=1 \cdot 10^{-3}$ ). The results show the establishment of the depletion zone above the spot surface (A). The black lines show that the depletion zone is established in a short time period ( $t'=0$  to  $t'=500$ ). After this diffusion from the regions next to the spot surface (dotted lines) takes place over a longer time period ( $t'=3750$  and  $t'=7500$ ). The second graph (B) shows the hybridization rate for diffusion (blue) and a convection (magenta) driven system. Both are equal during the first phase of the reaction, in a second phase the convective system enhances the hybridization compared to the pure diffusion system.

3D-simulations for  $Da > 1$  show the creation of a depletion zone above the spot surface (Figure 3A) due to a higher reaction rate of the system compared to the diffusion rate. When  $Da < 1$  a depletion zone is also created but to a lesser extent (Figure 3A). The fast diffusion rate sees to a constant supply of fresh targets towards the spot area.

A plot (figure 3B) of the hybridization rates for the different Da-numbers clearly shows the main difference. As Da increases, so does the time it takes to reach the saturation level of the spot. For  $Da=0.01$  only one regime is

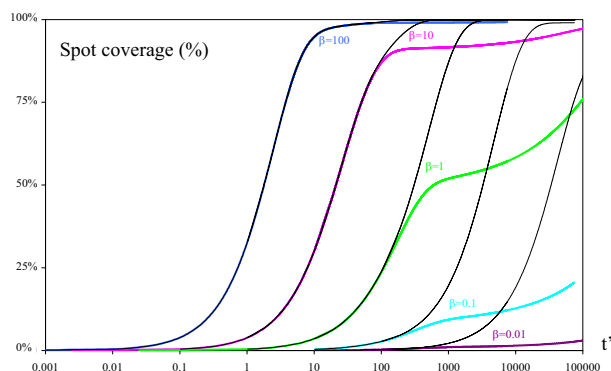
present, but as Da enhances, two hybridization regimes, reaction-limited and diffusion-limited can be distinguished. For the convection-driven simulations only one regime (reaction limited) is present. No or almost no depletion zone is noticed so no diffusion limitation can be observed.



**Figure 3.** (A) Concentration profiles taken at different time points for  $Da=0.1$  (red) and  $Da=10$  (blue). (B) Hybridization rates in the case of diffusion- (coloured) and convection-driven (black) systems. The curves were obtained for different Da-values (100, 10, 1, 0.1 and 0.01). ( $\beta=1$ ,  $\alpha=5$  and  $C'_0=1 \cdot 10^{-3}$ )

### 3.2 Influence of $\beta$

While the Damkhöler number played a major role in the diffusion of the targets to the probe spot area,  $\beta$  is the factor that describes the reaction of the target molecules to the probe strands.  $\beta$  has an influence on the equilibrium state to which the reaction is pushed. The maximal value that is reached during the reaction is low when  $\beta$  is high and will be high for a small  $\beta$ -value. This factor has also a major influence on the speed at which the hybridization occurs. When  $\beta$  is low, the driving force for the on-reaction is higher than the off-reaction. This will favour the creation of a depletion zone and a diffusion-limited regime will be created. Figure 4 shows that for high values of  $\beta$  only one regime is present, while when  $\beta$  has a value lower than 1, two different kinetic regimes can be distinguished. Again the convection-driven system circumvents this limitation and makes sure the process is completely reaction-limited.



**Figure 4.** Hybridization rates in the case of diffusion-driven (coloured) and the corresponding convection-driven systems (black). The curves were obtained for different  $\beta$ -values (100, 10, 1, 0.1 and 0.01). ( $Da=1$ ,  $\alpha=5$  and  $C'_0=1.10^{-3}$ )

### 3.3 Influence of $C'_0$

While  $Da$  and  $\beta$  are factors that difficult to be controlled by user,  $C'_0$ , which can be defined as the total number of molecules above the spot surface at the beginning of the simulations over the maximal spot capacity, is a factor that can be manipulated by the user.

The initial concentration ( $C_0$ ) and the height ( $d$ ) have a major influence on  $C'_0$ . The simulations showed that when  $C'_0$  is larger then 1 there is a high enough target concentration above the spot surface to cover it and no depletion will occur. When it drops below 1 the concentration is low enough for a depletion zone to be created. It is important to notice that as the height of the system increases so will the liquid volume that is needed to cover the microarray. It will be very hard to increase the system height and at the same time maintain a concentration. Microarray experiments performed under identical conditions lead to similar trends as showed by the simulations.

### 3.4 Influence of $\alpha$

$\alpha$  is the only factor that is determined by the dimensions of the system. It is the ratio of the two main geometrical parameters of the system: the spot radius and the height of the system.

The spot radius is a relatively fixed dimension although different spot sizes can be obtained from different microarray spotters, the general dimensions of the spots do not vary much. The height of the system is the major variable that has an influence on  $\alpha$ . Simulations for different values of  $\alpha$  show that larger values tend to create depletion zones (due to the smaller height), while smaller  $\alpha$ -values do not have that problem. However  $\alpha$  has no influence on the hybridization rate for convection-driven systems and no difference is noticed between convection-driven systems and diffusion-driven systems with low  $\alpha$ -values (bigger heights). It is however also important to note that diffusion-driven systems with a higher height also require a larger sample volume.

## 4. DISCUSSION

In this study a 3-dimensional diffusion-reaction model was developed for hybridization on microarrays. The process could be described by four major parameters: the delivery speed versus the reaction speed ( $Da$ ), the reaction on the spot surface ( $\beta$ ), the initial concentration versus the capacity of the spot ( $C'_0$ ) and the spot radius over the height of the system ( $\alpha$ ). This study shows the influence of each of these parameters on the hybridization reaction rate and this was also compared to the hybridization rate in convection-driven systems. It clearly demonstrates that convective systems are able to enhance the hybridization rate, leading to higher signal intensities and faster analysis times. These finding enables us directly to accurately design convective hybridization systems. The data also hint at the importance of the liquid layer height in the conventional, diffusion-driven hybridization assays. At Nanotech 2005 we will be able to present an even more widespread study.

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