Multiscale (Nano-to-Micro) Design of Integrated Nanobio Systems

J. W. Jenkins[1], M. Hagan[2], A. Chakraborty[2], S. Sundaram[1]
[1]CFD Research Corporation, 215 Wynn Drive, Huntsville, AL 35805
[2]Department of Chemical Engineering, 209 Gilman Hall, UC Berkeley, CA 94720

ABSTRACT

The design of devices that incorporate both nanoscale and continuum scale physics is challenging due to the nature of simulation techniques required for each scale. This paper presents a hierarchical approach where micro/mesoscopic models of molecular phenomena are coupled to continuum device descriptions. This method is developed and demonstrated with reference to DNA hybridization on a microcantilever platform. A macroscopic (Langmuir) model of DNA hybridization is coupled to a mesoscopic description that captures the effect of critical parameters such as salt concentration and graft density. Bridging of the two models was achieved by way of a stochastic Master Equation which was transformed into a Fokker-Planck equivalent that can then be solved in a PDE framework.

Keywords: Microfluidics, nanoscale modeling, DNA hybridization.

1 INTRODUCTION

Biological systems can often be thought of as nanoscale systems that leverage molecular interactions to perform specific tasks. Integrated nano-bio systems have emerged as strong candidates for single molecule level detection, genomic sequencing, and the harnessing of naturally occurring biomotors [1]. Research efforts directed at the problem of integrating nanotechnology and biology to form integrated nano-bio systems is rapidly becoming a priority.

In this context, there is a growing need for novel design tools that can incorporate sufficiently detailed models of the nanoscale molecular phenomena with overarching transport and related effects in a microfluidic devices, in a computationally feasible framework. Currently no models are available that can perform this task. This void is sought to be filled by the proposed research, whose innovative aspects include

- Development of a novel multi-scale simulation tool for integrated nanosystem design, analysis and optimization
- Multi-tiered modeling approach consisting of (a) microscopic/mesoscopic models with stochastic nature and (b) device scale, deterministic continuum models

Our overall objective is to develop a generalized, multi-scale, multiphysics CFD (continuum)-based design software where nanoscale effects of biosystems are accurately, efficiently and seamlessly integrated with transport-based models carrying device-level information

1.1 DNA Hybridization on Microcantilevers

The general techniques applicable to multi-scale modeling of nano-bio systems is developed and demonstrated in the context of a sample problem: the design and characterization of a DNA sensing microcantilever system. Recent experiments show that the adsorption of biomolecules on one surface of a microcantilever generates surface stresses that cause the cantilever to deflect [2]. These experiments are performed by immobilizing a group of probe molecules on one side of the microcantilever beam. A solution of target molecules that can bind to the immobilized probes is then introduced. The experiments reveal that when the target binds to the probe a surface stress is generated and the tip of the microcantilever is deflected. The deflection can be monitored using an optical technique. One of the most interesting result from these experiments is that the identity and concentration of the target molecule can be related to the deflection of the microcantilever.

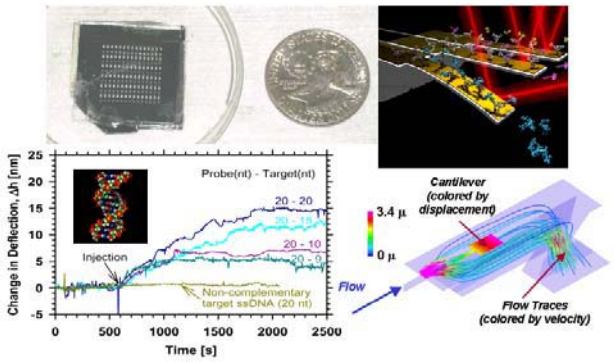

Figure 1: Model of a chemomechanical cantilever-based sensor. The figure shows several aspects of the project; the experimental cantilever array, some experimental data from DNA hybridization, and simulation results to date [2].

As shown in Figure 1, the microcantilevers can be created in an array with a different probe molecule immobilized on the target molecules. A fluid sample can then be screened for various target molecules based on the deflection of each cantilever. The ultimate goal is to create devices based on this technology that can be used in medical diagnostics and screening. The design of medical devices based on microcantilevers poses a challenge because of the scales (molecular to continuum) involved in the problem. Design of devices containing integrated nano-bio components can benefit from the help of accurate

simulation tools in much the same way as the design of microfluidic devices have benefited. Currently a large stumbling block in the development of simulation tools for integrated nano-bio devices is the lack of adequate simulation methods capable of handling nanoscale physics, device level physics, and the coupling of the two scales.

2 MACROSCALE DNA BINDING MODEL

In many cases the hybridization of DNA from solution onto a surface with a complimentary strand is modeled using Langmuir kinetics. Using this model the kinetics are modeled as a second order binding rate with a first order debinding rate.

$$\frac{dC_{PT}}{dt} = k_a C_P^{Bulk}\left(\sigma - C_{PT}\right) - k_d C_{PT} \qquad (1)$$

where C_{PT} is the surface concentration of hybridized probe/target pairs, C_P^{Bulk} is the concentration of probe DNA in solution, σ is the graft density of probes on the surface, and the rate constants are given by k_a and k_d. This type of a rate equation can be easily incorporated into a CFD modeling framework, making it an attractive choice for use in hybridization modeling. Using this model, quantitative estimates of DNA hybridization in the microcantilever system have been obtained and reported elsewhere [2]. Figure 2 illustrates an example simulation of DNA hybridization onto a paddle cantilever.

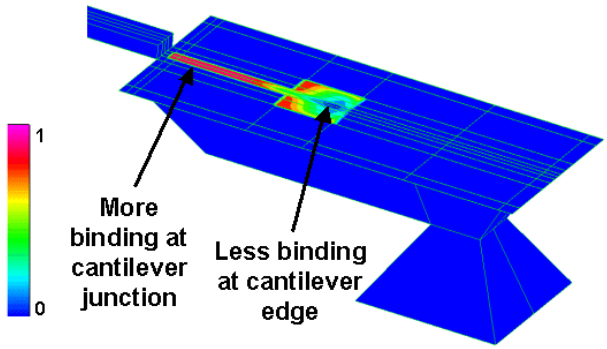

Figure 2: 3D model of the binding of DNA to a paddle type cantilever. The legend is a normalized amount of binding.

However this macroscopic model has serious shortcomings such as the fact that it does not allow for the variation in binding rate with salt concentration, graft density, and DNA chain length. All of these parameters are known to have a strong impact on the hybridization kinetics. A microscopic models that more accurately describes the functional dependence of the hybridization rate on these fundamental parameters is needed.

3 MICROSCALE DNA BINDING MODEL

We consider the system of single stranded DNA hybridizing with a complimentary DNA strand immobilized on the microcantilever surface (termed the "brush" layer). This system can be modeled by appealing to methods used for characterizing surface immobilized polyelectrolyte polymer systems. A brief overview of the theory given below (more details may be found in [3]). The main goal of the polyelectrolyte theory is to predict the free volume distribution of the monomers in the direction normal to the immobilized surface. The free volume distribution arises due to the conformations taken on by the surface immobilized polymers. This theory is valid under the conditions of sufficiently high immobilized probe density (>0.01 polymers/nm^2).

A description of the effective binding rate begins with the calculation of electrostatic potential within the DNA brush layer. In order to obtain the electrostatic potential we will need to solve the Poisson equation for the system. The solution to the Poisson equation can be broken into two contributions, one for inside of the brush layer and one for outside of the brush layer in the bulk solution. These equations are given below in equations 2 and 3. In the equations below, y is the direction normal to the surface.

$$\frac{d^2\Psi^i}{dy^2} = -\frac{4\pi}{\varepsilon}\left[-2C_{salt}Ze\sinh\left(\frac{Ze}{kT}\Psi^i\right) + \frac{fZ_s e}{v_s}\Phi(y)\right] \qquad (2)$$

$$\frac{d^2\Psi^o}{dy^2} = -\frac{4\pi}{\varepsilon}\left[-2C_{salt}Ze\sinh\left(\frac{Ze}{kT}\Psi^o\right)\right] \qquad (3)$$

Where the subscripts "i" and "o" denote the electrostatic potential inside and outside of the brush layer. Also, C_{salt} is the electrolyte concentration, v_s is the volume of a polymer segment. The potentials are subject to the following continuity boundary conditions at the edge of the brush layer (denoted by y*):

$$\Psi^i(y^*) = \Psi^o(y^*)$$
$$\frac{d\Psi^i(y^*)}{dy} = \frac{d\Psi^o(y^*)}{dy} \qquad (4)$$
$$\Psi^o(\infty) \to 0$$

In order to obtain an equation for the free volume distribution, Φ(y), we first need to define an expression for the total potential energy for the system of immobilized polyelectrolyte polymer strands. The reader is referred to reference [3] for details. From the potential energy expression, a differential equation for the free volume distribution can be derived and is given below.

$$\frac{d\Phi}{dy} = \frac{r\left(\frac{d\Psi^i}{dx}\right) - 2\beta y}{(1-\Phi)^{-1} - 2\chi} \qquad (5)$$

Where r is a dimensionless number for the average segment charge, and β is a potential energy coupling parameter. The other boundary conditions for equations 2-3 and 5 are given below:

$$\Phi(y^*) = 0$$
$$\frac{d\Psi^i}{dx}(0) = -\frac{\kappa q_{surf}}{2ZeC_{salt}} \quad (6)$$
$$\frac{d\Psi^i}{dx}(y^*) = -2\sinh\left(\frac{Ze}{2kT}\Psi^i(y^*)\right)$$

Where y^* is the ultimate brush height, κ is the Debye length, q_{surf} is the surface charge distribution. The first condition is to ensure that the volume fraction distribution goes to zero at y^*. The second condition is to account for the affect of a charged surface on the electrostatic potential. The final condition is to ensure that the value and derivative of the electrostatic potential match at y^*.

The main problem with solving equations this system of equations subject to 4 and 6 is that y^* is not known ahead of time, and must be determined by a constraint equation. The constraint equation is a balance equation on the number of segments contained in the brush volume given below in equation 7.

$$\sigma N v_s = \left(\frac{1}{\kappa}\right)\int_0^{y^*}\Phi(t)dt \quad (7)$$

Where N is the number of segments in the polymer chain, σ is the density of chains (chains/nm^2), and the rest of the symbols have already been defined.

The solution to the above equations gives the free volume distribution, which can be used to compute the effective molecular binding rate in the brush layer, as has been shown in [4],

$$k_{eff}(t) = \frac{k_a^{Bulk}\sqrt{L}}{\sigma C_{Bulk}}\int_0^{y^*} C_{Target}(y,t)\Phi(y,t)f_{max}\,dy \quad (8)$$

where L is the total chain length, k_a^{Bulk} is the bulk hybridization rate, C_{Target} is the target molecule concentration, and f_{max} is the maximum binding fraction defined by physical constraints of the polymer molecules.

The above expression provides a microscopic model that captures the impact of parameters such as salt concentration, brush density etc. This detail can be directly communicated up to the continuum level by means of the Langmuir model described earlier in equation 1. However, this communication is better handled by a "bridging equation" based on a master equation description of the hybridization process. More details on the exact nature of this multi-scale information cascade can be found in [5].

4 MESOSCALE BINDING MODEL

In general the binding rate will be a function of time, since during the binding process DNA chains are being integrated into he brush layer changing the free volume distribution. The hybridization process inside of the brush layer can be described using a Master Equation such as.

$$\frac{dP(n,t)}{dt} = k_{eff}(n-1,t)P(n-1,t) + k_{rev}P(n+1,t) - \left[k_{eff}(n,t) + k_{rev}\right]P(n,t) \quad (9)$$

Here P denotes the probability of finding "n" DNA chains hybridized in the brush layer at time "t". In a well characterized limit [6], the Master Equation can be cast in a Fokker-Planck form to give the same probability function as output. The FPE is a partial differential equation with a continuous variable, unlike the Master Equation which describes probability in discrete states. The Fokker-Planck Equation offers a unique advantage of being based on partial differential equation, which facilitates integration with the continuum approach. At CFDRC we have successfully coupled a FPE-based description of biomolecular events with a continuum-based convective-diffusive-reactive treatment of biosystems (CFD-ACE+) [5]. Our main goal is to be able to simulate nano-resolved, biomolecular reactions in complex, spatially inhomogeneous systems with convective-diffusive transport.

The master equation (equation 9) can be converted into a Fokker-Planck Equation by taking the immobilized area, A, as the largeness parameter. The FPE is used to obtain solutions for the probability distribution function P(n,t) in a continuous sense by describing the rate of change of P(n,t) in terms of a gradient of probability flux and source terms S. The flux term consists of a drift D_1 and diffusion coefficient D_2. In general the drift and diffusion coefficients are functions of the local conditions in the simulation and must be solved simultaneously with the momentum and mass transport equations. The implementation in the CFD-ACE+ software package breaks the flux of probability into a real space term and an auxiliary variable, making the probability space 4 dimensional as given in equation 10.

$$\frac{\partial f}{\partial t} + \nabla_x\cdot\left[uf - \nabla_x(Df)\right] + \nabla_n\cdot\left[D_1 f - \nabla_n(D_2 f)\right] = S \quad (10)$$

The drift and diffusion coefficients for the particular problem are relatively simple expressions given below.

$$D_s\left(\frac{n}{A},t\right) = k_{eff}\left(\frac{n}{A},t\right) + (-1)^n k_{rev} \quad (11)$$

Equations 10 and 11 assumes that the effective binding rate is a function of the amount of DNA that has already hybridized. This will change the free volume distribution and ultimately change the binding rate. This equation also assumes that the debinding rate k_{rev} is constant, and does not vary with time.

The solution of the Fokker-Planck equation using the drift and diffusion coefficients given in Equation 14 can be solved using CFD-ACE+ FPE solver. The solutions of the FPE above give the distribution of bound DNA molecules as a function of time. The first moment of the distribution is the number of hybridized DNA molecules. The temporal change in the first moment is the local binding rate. Solutions to this equation will give binding rates that use the molecular binding rates taken from polyelectrolyte brush theory, and can be incorporated into the surface chemistry solver in CFD-ACE+.

5 EXAMPLE RESULTS

In the example calculations presented here, the ratio of the effective binding rate given in equation 8 to the bulk solution binding rate are given as a function of probe density. The rate ratio is given for two DNA chain lengths of 20 and 40 base pairs from a chain density of 0.01 to 0.15 chains/nm^2. The scaling of the rate ratio is seen to approach $\sigma^{-1.8}$ as the grafted probe density increases. This is in agreement with [4]. For this calculation, the background electrolyte concentration was held constant at 0.50 mol/L. This result is shown in Figure 3.

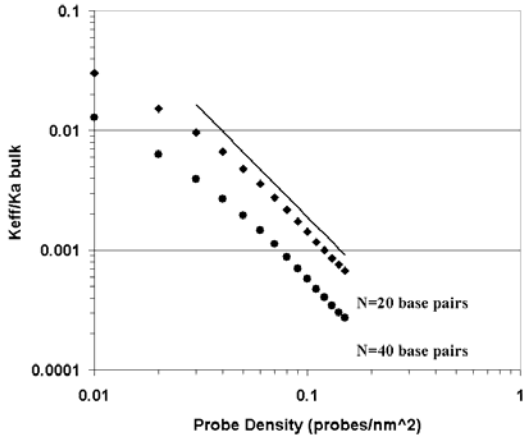

Figure 3: Functional dependence of the effective hybridization rate relative to the free solution binding rate with probe density. The calculations were performed for chains of length 20 and 40 base pairs. The line in the figure is to illustrate the $\sigma^{-1.8}$ scaling with probe density.

Figure 4 shows the equilibrium coverage of immobilized probe DNA molecules. The equilibrium coverage is the fraction of immobilized DNA molecules that have hybridized with a DNA chain out of solution. Figure 4 shows that the equilibrium coverage decreases dramatically with an increase in probe density, and that the equilibrium coverage is dependent on the chain length.

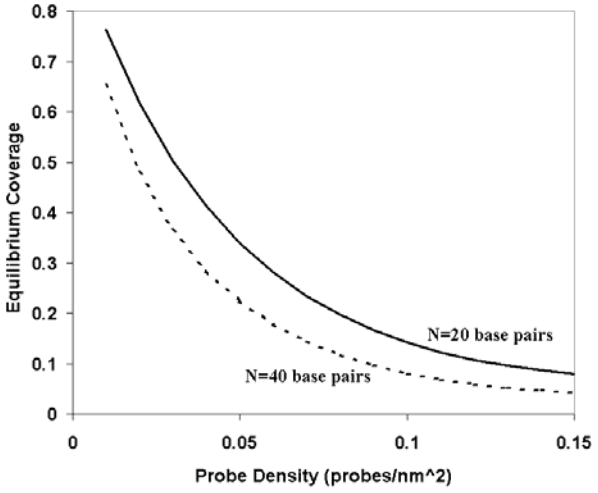

Figure 4: Functional dependence of the equilibrium coverage with probe density. The calculations were performed for chains of length 20 and 40 base pairs.

6 SUMMARY

Progress in our effort to incorporate nanoscale models into continuum level design simulations of microfluidics devices has been summarized. A macroscopic (Langmuir) model of DNA hybridization on a microcantilever was described and demonstrated. Following the approach of [4], a mesoscopic model that captures the effect of critical parameters (such as salt concentration and probe density) was adapted. The model showed consistent behavior in the variation of the effective binding rate with the probe density and in the calculated coverage. Information from the mesoscopic model was communicated to deterministic, continuum models by way of a stochastic Master Equation. The Master Equation was transformed into a Fokker-Planck equivalent that can then be solved in four-dimensional space (along with transport of DNA in the physical domain). Fully coupled, device level calculations with the multi-scale approach are underway and will be reported in a future communication.

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