BIOCHEMICAL BINDING IN MICROSPHERE-BASED ASSAYS

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ABSTRACT

Microspheres (referred commonly as beads) are being extensively used in biochemical assays as supports for proteins, DNA, etc. We have successfully developed computational models for simulating sample detection using flowing beads. The model *fully integrates* Lagrangian transport of the beads, convective-diffusive transport of analyte and biomolecular binding reactions on the bead surfaces. The Eulerian-Lagrangian transport equations that govern the entire process are presented. Model inputs are the geometry, bead and sample transport properties, and surface binding kinetics along with assay protocol such as flow rates etc. with surface coverages on the beads as output. Quantitative capabilities of the model are demonstrated using an immunoassay [IL-2 binding with IL-2Rα] in a typical Y-junction.

Keywords*: Microsphere, Bead, Immunoassay, Binding, Sensor, Model*

1 INTRODUCTION

Qualitative and quantitative understanding of the interactions between biological macromolecules is of critical importance in the emerging bio-tech industry and its impact on society. Bioagent detection [1] , food/airborne toxin quantification [2] etc. nowadays rely on quantifiable, repeatable, interactions between antigen-antibody, protein-DNA, DNA-DNA, cell-receptor etc. Microspheres are extensively used in a variety of these applications, as support for the antibodies, proteins and DNA. Coupled with microfluidics to transport and contact sample with beads bearing reagents, and flow cytometry to measure individual bead coverage signals, microsphere based assays provide vastly superior performance over conventional surface-derivatized assays in critical areas:

1. Improved mixing and contacting
2. Ease of processing, such as cytometry, elution, etc.
3. Ready multiplexing
4. Continuous monitoring (air/food quality)
5. Functionality with magnetic/electric fields

2 MODEL FORMULATION

Accurate characterization of interactions between bulk and bead-surface immobilized biomolecules necessitates the fundamental understanding of (a) Coupled fluid and bead motion (Fluid-Solid Momentum Transfer) and (b) Analyte transport and binding (Fluid-Solid Mass Transfer). Each of these will be treated separately in the section to follow.

2.1 Two-Phase Flow Model Classification

Two-phase modeling strategies can be broadly classified as follows [3]

Two-Fluid (Eulerian Fluid – Eulerian Solid): In this method, the dispersed phase is regarded as a continuum and the mixture is treated as the flow of two immiscible fluids within an Eulerian-Eulerian framework[3]. It assumes the presence of a statistical number of particles within each computational cell. Its strength is in the robust and stable formulation of (including volume exclusion) of the particle-fluid interactions. However, there are substantial difficulties associated with this approach, namely in prescribing continuum properties for the particle phase, boundary conditions (say at walls) and the high numerical diffusion effects smearing results over the computational domain. Other limitations, related to bio-kinetics, include

- Extension to multi-cell binding on different specially coated beads (i.e. N-Fluid Model) is not currently possible
- Identity of individual beads is lost and modeling of particle-particle interactions such as collisions is not straightforward.
- Modeling of polydispersity, binding non-uniformities in binding is difficult

For these reasons, this method is not recommended for high-fidelity computations.

Trajectory (Eulerian, Single-Phase Fluid – Lagrangian Solid): Here microsphere motion is computed on a Lagrangian framework (with no artificial diffusion effects). Multiple, polydisperse beads, binding heterogeneity etc. are readily accounted for. This approach is chosen for this study. One limitation of this traditional method is that it neglects volume exclusion by the beads (uses point-particle approximations), which restricts its use to *very small beads and dilute loadings only.* Adaptations to overcome these limitations are possible but are beyond the scope of this paper.

2.2 Fluid & Analyte Flow (Eulerian)

Mass transport of bioanalytes in the bulk (buffer) solution due to both convective (governed by convective flow rate) and diffusive transport (determined by mass

diffusivity of analyte). Electrokinetic motion can be superimposed in this framework, but is not considered presently for the sake of simplicity. The Navier-Stokes equations governing bulk transport are given as:

$$\frac{\partial u_i}{\partial x_i} = 0 \tag{1}$$

$$\frac{\partial \rho_c u_i}{\partial t} + \frac{\partial \rho_c u_j u_i}{\partial x_j} = -\frac{\partial P}{\partial x_i} + \frac{\partial \tau_{ij}}{\partial x_j} + \rho_c g_i \tag{2}$$

where u, ρ_c, P and g are the fluid velocity, density, pressure and gravity respectively

The mass conservation of analyte is written as

$$\frac{\partial C}{\partial t} + \frac{\partial u_j C}{\partial x_j} = D\frac{\partial^2 C}{\partial x_i \partial x_j} + S \tag{3}$$

where C, D and S are the antigen concentration, diffusivity and source/sink term (due to binding), respectively

2.3 Bead Tracking (Lagrangian)

Unlike the analyte, the beads are represented in a Lagrangian fashion (i.e. we are following the path of individual microspheres in the system). For small beads, the equation of motion can be represented as [4]

$$\frac{\partial v_i}{\partial t} = \frac{f}{\tau_v}(u_i - v_i) + g_i \tag{4}$$

where v, f, τ_v are the bead velocity, friction factor and response time, respectively.

2.4 Biochemical Surface Reactions

Analyte association/dissociation with the receptor occurs at the bead surface and is often successfully modeled using a pseudo first-order reaction [5]

$$\frac{d\theta}{dt} = K_{on} C_s (1-\theta) - K_{off}\theta \tag{5}$$

where θ is the surface coverage fraction and K_{on} and K_{off} are the adsorption kinetic rate constants.

At the bead surface we impose a mass balance between the amount of analyte that is being diffused and convected to the bead surface and the amount of analyte that is being bound/released. This method can be readily extended to more complicated surface reaction models involving denaturation, higher-order adsorption/desorption steps etc.

3 MODEL VALIDATION

Preliminary validation was carried out using Stokes flow & binding on a single microsphere in 2D, uniform flow. 50 micron beads were released from a stationary position in uniform flow carrying antigen (flow rate of 1 μL/s/m) in a channel 1cm long and 1mm high. The antigen concentration was maintained at 0.1M and the adsorption rate set to 10 [1/Ms]. The trajectory (along with the change in surface coverage of the bead indicated in color) is shown in Figure 1. (The bead size is exaggerated for ease of visualization). The velocity speed-up and surface binding curves exhibit expected exponential behavior (see Figure 2).

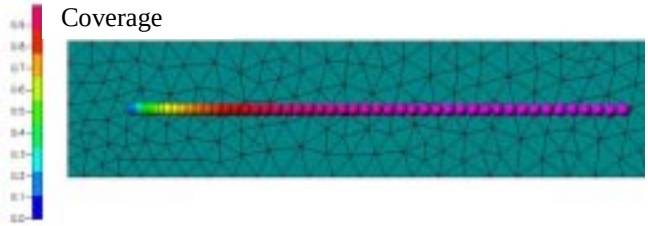

Figure 1. Coverage on a bead undergoing Stokes flow

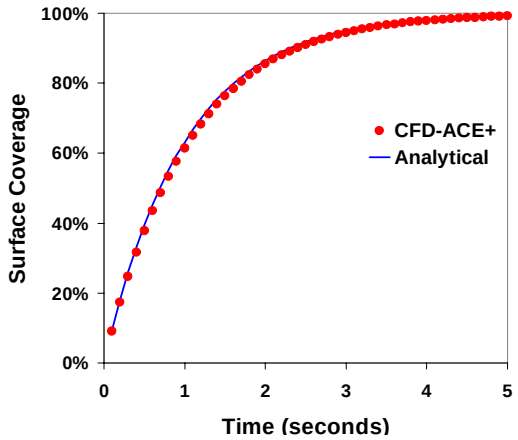

Figure 2. Coverage comparison with analytical solution

In addition the model was used to reproduce a static incubation experiment with antigen-coated beads exposed to antibody (at Lawrence Livermore National Laboratory by Dr. Rich Langlois). The solution was continuously stirred to provide a well-mixed state. At regular time intervals, beads were analyzed for antibody coating by measuring the fluorescence intensity in the flow cytometer. The surface coverage of the bead with the antibody was monitored over 40 minutes (as in experiments). Figure 3 shows the simulation and experimental data where I_{max}, I_0 and I are the maximum intensity (corresponding to full coverage), intensity at the start of experiment and the intensity at any given time, respectively

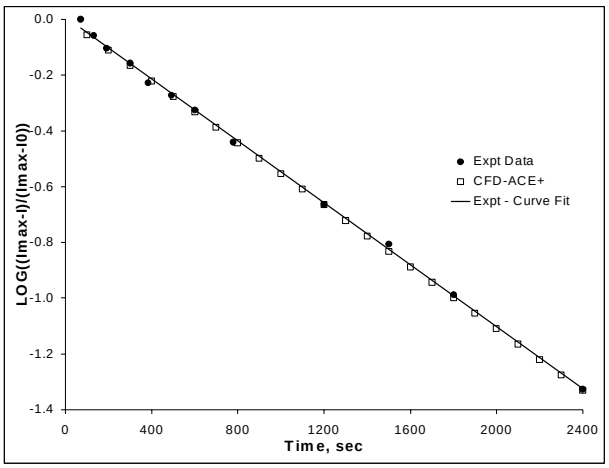

Figure 3. Comparison with static incubation experiment

4 RESULTS AND DISCUSSION

The goal of all biochemical assays is to yield the maximum signal in the shortest time possible. The signal, however, depends upon a multitude of variables such as geometry, flow rates, concentrations etc. in a very complex and intertwined manner. Availability of a high-fidelity model, such as the one described here, allows easy exploration of detection dependence on key parameters. Here, we have chosen to study the effect of two of the most important and easily controllable parameters i.e. (a) antigen and bead flow rates and (b) channel geometry.

4.1 Model System

The capabilities of the model are demonstrated using a microsphere based immunoassay for interleukin, specifically IL-2 binding to IL2-Rα derivatized on the beads. The geometry considered is a Y-junction employed in many microfluidic systems. The length of the arm of channel is 0.5 mm and its width is 0.05 mm. The length of the straight channel is 2 mm and its width is 0.1 mm. The IL-2 (plug) and buffer enters from upper arm of the channel and beads in a buffer solution enter from lower arm of channel. The diffusivity of IL-2 is set at $5x10^{-10}$ m^2/s. The bead fluorescence (coverage) is measured at the exit (detector location) of the channel. IL-2 concentration used in all the cases is 10^{-6} M. 5 μm beads, coated with IL-2Rα receptors, are released in the lower arm. A forward adsorption constant of $5x10^6$ $[M\text{-}s]^{-1}$ and a desorption rate of 1e-4 s^{-1} are prescribed, for this calculation.

4.2 Effect of Flow Rate

For the baseline case, we consider equal flow rates of IL-2 and bead solutions entering the channel (Figure 4). Note that IL-2 solution and bead each occupy half the channel with not much mixing. The suboptimal mixing and presentation of IL-2 to the bead, causes a low level of surface coverage. Considering that the threshold of detection in many cases requires around 30% coverage, it can safely be concluded that more contacting time must be provided to minimize false positives.

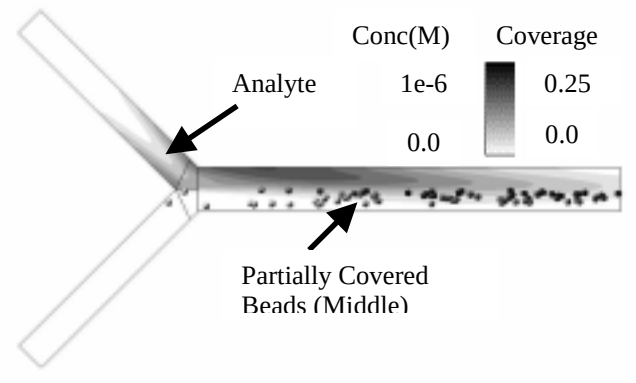

Figure 4. Analyte concentrations and bead coverages for a equiflow ratio of 5x(IL-2):5x(Bead) at t=2seconds.

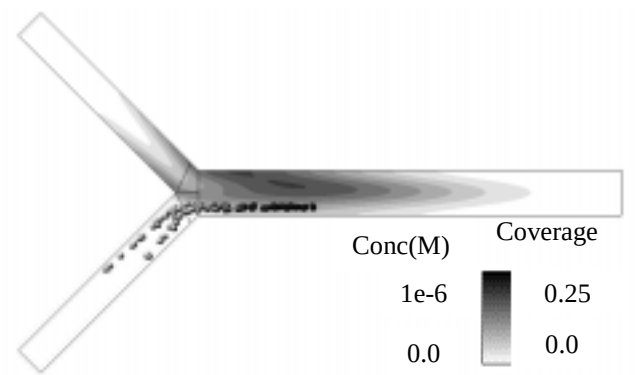

Figure 5. Analyte concentrations and bead coverages for a slow bead flow ratio of 5x(IL-2):1x(Bead) at t=4 seconds

Decreasing the bead flow rate can be intuitively expected to increase the residence/contact times between the IL-2 and IL-2Rα (on the surface of the beads) and thereby increase coverage/detection. (IL-2 plug injection was delayed by 2 seconds, timed so as to arrive at the junction at the same time as beads). This is indeed seen to happen as seen in Figure 5. Moreover, the hydrodynamic effect of focusing the beads in the low-speed fluid near the wall, also enhances the signal via increased diffusive transport of IL-2 to the surface of the beads

The overall signal evolution with time at the detection point (at the exit of the channel) is shown in Figure 6. Firstly, as expected, the time to detect is shortest in the equal flow (5x:5x) case, as the beads travel fastest through the system and reach the detection point. However, as seen earlier, the coverage levels are low with the possibilities of false negatives, high. The 5x:1x case, where the beads travel slowly, on the other hand exhibits a higher coverage, albeit with longer times. The sharp rise in the signal indicates that bead residence time is large enough for saturation to occur (even for the fastest beads).

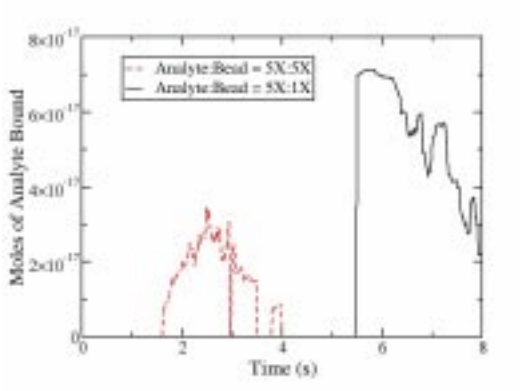

Figure 6. Moles captured (signal detected) at channel exit

4.3 Effect of Geometry

Channel geometries are another easily controlled factor in trying to bring about fast and robust detection. Optimally chosen channel geometry (tailored based on flow rates and bead sizes/inertia) can also facilitate the accomplishment of sample mixing and bead separation all on-chip.

Here, we repeated the IL-2 and IL-2Ra immunoassay efficiently, however with T junctions (90 degree angle). Beads, due to their large size (as compared to biomolecules) are intuitively expected to penetrate into the analyte stream when injected perpendicular to the flow. Two separate simulations were performed with the beads entering via the vertical channel in one and horizontally in the other [Note: Beads are neutrally buoyant]. All other parameters were maintained identical to the equal flow rate (5x:5x) study presented earlier.

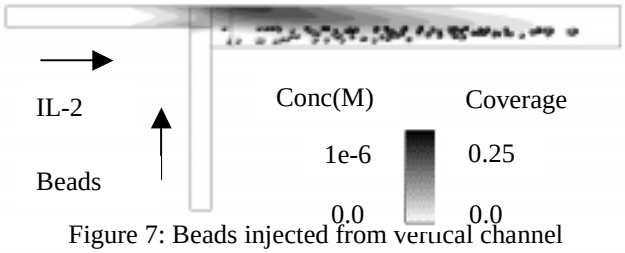

Figure 7: Beads injected from vertical channel

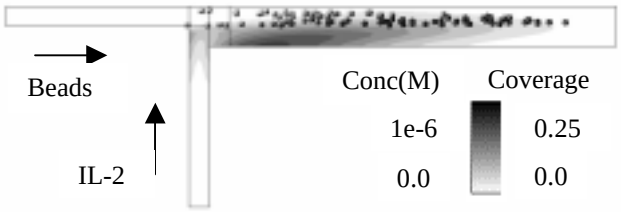

Figure 8. Beads injected from horizontal channel

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Snapshots of the bead and analyte flow for these two cases, at times identical to Figures 4 & 5 are shown in Figures 7 & 8. The bead-averaged coverages, measured at the detector location at the exit, are shown in Figure 9. T-junctions are seen to promote better contacting and hence higher signals. Vertical bead system was expected to show higher coverages on account of bead penetration into the analyte stream but this effect is minimized by (a) small bead sizes (fast response times) and (b) fast flow rates. Nevertheless, bead-turning designs may perform significantly better with other protocols.

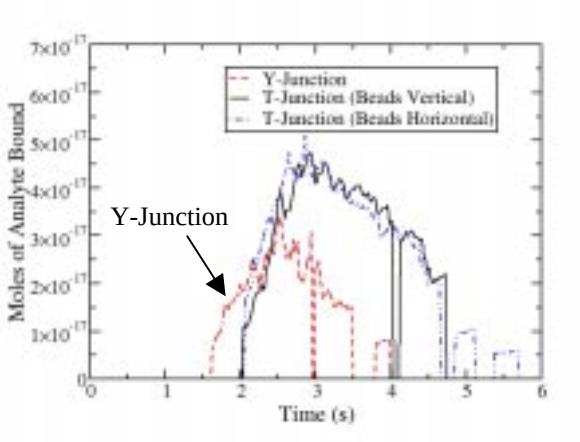

Figure 9. Beads injected from horizontal channel

5 SUMMARY

CFDRC has successfully developed high-fidelity, coupled computational models for simulating biochemical reactions on flowing beads. This multi-physics model is capable of simulating time-dependent, multiplexed detection (binding of multiple analytes on multiple sets of coated beads) in flow-based (microfluidic or flow/sequential injection analysis) or static environments. The effect of key parameters such as flow rates of analytes vs. beads and bead size were studied.

Such models can be used then, with great impact, to

- Develop a fundamental understanding of the biochemical / transport processes involved
- Optimize study protocols (size, flow rates, concentrations, etc.) or device geometry
- Evaluate specificity and cross-contamination effects
- Screen new assays/concepts for improvement

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