Electrothermal stirring for heterogeneous immunoassays

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A technique is proposed to enhance microfluidic immuno-sensors, for example, immunoassays, in which a ligand immobilized on a microchannel wall specifically binds analyte flowing through the channel. These sensors can be limited in both response time and sensitivity by the diffusion of analyte to the sensing surface. In certain applications, the sensitivity and response of these heterogeneous immunoassays may be improved by using AC electrokinetically-driven microscale fluid motion to enhance antigen motion towards immobilized ligands. Specifically, the electrothermal effect is used to micro-stir analyte near the binding surface. Numerical simulations of antigen in a microchannel flow subjected to the electrothermal effect show that 6 Vrms applied to electrodes near a binding region can increase binding in the first few minutes by a factor of seven. The effectiveness of electrothermal stirring is a strong function of the Damköhler number. The greatest binding enhancement is possible for high Damköhler numbers, where the reaction is limited by diffusion. Based on these results, the utility of this technique for diffusion-limited microfluidic sensor applications is demonstrated.

1 Introduction

1.1 Immunoassays

Immunoassays, which rely on specific antigen–antibody binding for identification of proteins in a sample, have applications in both clinical laboratories for medical diagnostics and treatment monitoring, and in research laboratories for highly multiplexed testing, such as for biomarker identification. Immunoassays are also used for environmental and food monitoring. In these cases, throughput is a key consideration. One factor that can limit test duration is diffusion rate of analyte to the reporter. This is particularly true for high sensitivity ELISA tests. An incubation step of minutes to hours allows diffusion-limited binding to reach detectable levels. Tests are often performed at central labs where, despite a long test duration, high throughput is achieved through robotics and highly parallel assays. However, if the assay could be moved from the centralized lab to the point of care, real-time immunoassay-based diagnostics could be performed. In order for this to be achieved, the test must be faster, as well as more portable, while maintaining high sensitivity.

In response to the needs for increasing throughput, portability, and sensitivity, new formats for miniaturized immunoassays have developed dramatically in recent years. These include spotted microarrays, common for “gene chips”, and now used for “protein chips” (e.g. the ProtoArray from Invitrogen, Carlsbad, CA), and various forms of lab-on-a-chip devices which can perform fluid processing and detection steps on a single chip. Small length scales permit small sample sizes and shorter test times; on-chip sample preparation reduces fluid handling steps. Though greatly aided by their small length scales, these assays can still be limited in response by diffusion of the analyte to an immobilized ligand. In this paper, we present a method where AC electrokinetics is used to enhance the performance of heterogeneous assays. Here electrothermal forces are used to micro-stir the analyte near a functionalized surface, increasing the rate of transport to the surface. This addresses the need for faster assays by offering a tool that is adaptable to a wide variety of assay configurations and reduces the incubation time required to perform a specific test while maintaining its sensitivity.

1.2 AC electrokinetic phenomena

AC electrokinetics refers to induced particle or fluid motion resulting from externally applied AC electric fields. DC electrokinetics has been widely used for lab-on-a-chip applications such as electroosmotic pumping and capillary gel electrophoresis for DNA fractionation. In contrast, AC electrokinetics has been widely used for lab-on-a-chip applications such as electroosmotic pumping and capillary gel electrophoresis for DNA fractionation. In contrast, AC electrokinetics has received significantly less attention. AC electrokinetics has advantages of (1) largely avoiding electrolysis, and (2) operating at lower voltages (1–20 V), which is important for portable systems. AC electrokinetics can be classified into three broad areas: dielectrophoresis (DEP), electrothermal forces and AC electroosmosis.

Dielectrophoresis takes advantage of a force arising from differences in polarizability between the particle and the fluid medium in the presence of a non-uniform electric field. DEP has been used to separate blood cells and to capture DNA molecules. DEP has been used to separate blood cells and to capture DNA molecules. DEP has been used to separate blood cells and to capture DNA molecules. The DEP force scales with the cube of particle radius, its magnitude increases as the particle size decreases. In addition, DEP has been used to separate blood cells and to capture DNA molecules.
AC electroosmosis arises when the tangential component of the electric field interacts with a field-induced double layer along a surface. A bioprocessor has been developed at UCLA\textsuperscript{12} for the concentration of bioparticles including bacteria and λ-phage DNA. This device relies on the balance between electroosmotic flow and DEP force on suspended particles. In another application, Bazant and Squires\textsuperscript{13} developed AC electroosmosis for pumping by inducing a double layer around metal posts that are asymmetric either in shape or in surface properties. AC electroosmosis is only effective when there is a substantially deep induced double layer, that is, for low conductivity solutions and low applied field frequencies. For example, in an aqueous saline solution with an electrical conductivity of $\sigma = 2 \times 10^{-3}$ S m$^{-1}$, it is predicted that AC electroosmosis is not important for frequencies $f > 100$ kHz.\textsuperscript{14}

Transport enhancement of small proteins may be most successful through electrothermally driven flow (ETF). A non-uniform electric field produces uneven Joule heating of the fluid, which gives rise to nonuniformities in conductivity and permittivity. These interact with the electric field to generate flow, often in circulating patterns. Unintentionally-induced electrothermal flow in a traveling wave DEP cell-sorter was studied numerically,\textsuperscript{15} and found to be quite sensitive to thermal boundary conditions such as channel material, and applied temperature differential boundary conditions. Characteristic swirling flow patterns can be used to circulate suspended molecules past the binding region, thereby providing more binding opportunity for the suspended molecules.

2 Experimental measurements

With the objective of exploring two-dimensional fluid and particle motion in a non-uniform electric field, a cavity was fabricated with sidewall electrodes (Fig. 1). The device was sandwiched between glass slides and filled with a solution of 0.05 M KCl and fluorescent polystyrene tracer particles. When the electrodes were driven at $V = 7$ V$_{\text{rms}}$ and $f = 200$ kHz, a fluid circulation pattern was observed. The two-color micron-resolution particle image velocimetry (μ-PIV) technique presented in Wang et al.\textsuperscript{16} was used to measure fluid motion. These experiments are described in detail in ref. 17 and are summarized here to provide motivation for the numerical simulations. The measured fluid velocity field is shown in Fig. 2. The applied field induces a circulating flow, with a characteristic velocity of about 100 μm s$^{-1}$. When the driving voltage is varied between 3–7 V$_{\text{rms}}$, the flow pattern remains self similar, but the velocity magnitude varies with voltage to the 4th power, which is characteristic of electrothermal flow\textsuperscript{6} (data not shown).

3 Numerical simulation

The electrothermally-driven fluid motion was simulated with the finite element simulation software Femlab\textsuperscript{®} (Comsol; Stockholm, Sweden). First, the two-dimensional quasi-static potential field is calculated, according to Laplace’s equation, $\nabla^2 V = 0$. The resulting electric field, given by $\mathbf{E} = -\nabla V$, gives rise to a non-uniform temperature field through Joule heating. Ignoring unsteady effects and convection, and balancing thermal diffusion with Joule heating yields

$$k \nabla^2 T + \sigma \mathbf{E}^2 = 0 \quad (1)$$

where $T$ is temperature, $E$ is the magnitude of the electric field, and $k$ and $\sigma$ are the thermal and electrical conductivities. The temperature field is estimated for the experimental conditions shown in section 2. The numerically-simulated temperature field is shown in Fig. 3, with a maximum temperature rise of about 2.9 K, for the high conductivity solution used in the experiments ($\sigma_w = 0.66$ S m$^{-1}$ for 0.05 M KCl). Boundary conditions are insulating except at the metal electrodes, which are assumed to be isothermal with the environment. For this length scale, convective flux is negligible.\textsuperscript{6} The treatment of electrodes as isothermal is appropriate for electrodes of sufficient thickness relative to length.
Gradients in temperature produce gradients in electrical permittivity and conductivity in the fluid. For water, the dependence of electrical conductivity on temperature is \((1/\sigma)(\partial \sigma/\partial T) = 0.9\%\) and the dependence of dielectric permittivity on temperature is \((1/\varepsilon)(\partial \varepsilon/\partial T) = -0.4\%\) K\(^{-1}\). These variations in electric properties produce gradients in charge density and perturb the electric field. Assuming the perturbed electric field is much smaller than the applied electric field, and that advection of electric charge is small compared to conduction, the time-averaged electrothermal force per unit volume for a non-dispersive fluid can be written as:

\[
\vec{F}_{ET} = -0.5 \left[ \nabla \sigma \frac{\partial \varepsilon}{\partial T} \cdot \nabla \varepsilon - \nabla \varepsilon \frac{\partial \sigma}{\partial T} \cdot \nabla \sigma \right] + 0.5 |\nabla \varepsilon|^2 \varepsilon \nabla E \tag{2}
\]

where \(\tau = \omega \sigma\) is the charge relaxation time of the fluid medium, and the incremental temperature-dependent changes are

\[
\nabla \varepsilon = \left( \frac{\partial \varepsilon}{\partial T} \right) \nabla T, \quad \nabla \sigma = \left( \frac{\partial \sigma}{\partial T} \right) \nabla T \tag{3}
\]

The first term on the right hand side of eqn (2) is the Coulomb force, and is dominant at low frequencies, \(\omega = 2\pi f\). The second term is the dielectric force, and is dominant at high frequencies. The crossover frequency scales inversely with the charge relaxation time of the fluid.\(^5\) For example, an aqueous solution with conductivity \(\sigma = 10^{-2}\) S m\(^{-1}\) has a crossover frequency around \(f = 10\) MHz.

The electrothermal force shown in eqn (2) is a body force on the fluid. The motion of the fluid can be determined by solving the Stokes’ equation for zero Reynolds number fluid flow, such that

\[
0 = -\nabla p + \mu \nabla^2 \vec{u} + \vec{F}_{ET} \tag{4}
\]

\[
\nabla \cdot \vec{u} = 0
\]

where \(\vec{u}\) is the fluid velocity, \(p\) is the pressure in the fluid, and \(\mu\) is the dynamic viscosity of the fluid. Fig. 4 shows the resulting velocity field. With an applied voltage of \(V = 5\ V_{rms}\), the velocity of the electrothermally-driven flow is on the order of 100 \(\mu\)m s\(^{-1}\) and is characterized by a pair of counter rotating vortices. This velocity field, simulated for \(V = 5\ V_{rms}\), closely matches experimentally measured velocity for \(V = 7\ V_{rms}\) (Fig. 2). Out of plane heat transfer, unique to the thin experimental device, is not accounted for in the 2D model, and may cause this difference. The model was run with several grid resolutions, and determined to be grid-independent.

The total power consumption was calculated by integrating the Joule heating term, \(\sigma E^2\), over the flow domain. For this particular model (\(\sigma_w = 0.66\) S m\(^{-1}\); applied potential \(V = 5\ V_{rms}\) at \(f = 200\) kHz), and for an out of plane cavity depth \(d = 200\) \(\mu\)m, the predicted power dissipated is 4.3 mW.

### 4 Effect on binding: simulation

#### 4.1 Microcavity simulation

The highest flow velocities are predicted directly over the inside edges of the electrodes. Because of the high transport rate here, we expect this to be an optimum sensor location. The gold electrode surface provides a convenient surface for antibody immobilization through thiol linkers. Therefore, we investigate the effect of this flow pattern on the binding response of an assay in which antibody has been immobilized along a short length of the electrode near the electrode gap (Fig. 5b). For these microstirring enhanced binding simulations, a slightly higher potential is applied, 6 \(V_{rms}\), than in the previous simulations (5 \(V_{rms}\)), for faster stirring. The convective scalar equation describes the suspended concentration \(c(x,y,t)\) of antigen within the microchannel:

\[
\frac{\partial c}{\partial t} + \vec{u} \cdot \nabla c = D \nabla^2 c \tag{5}
\]
where \( \vec{u} \) is the fluid velocity and \( D \) the diffusivity of the antigen. An initial concentration in the cavity \( c_0 \) is depleted through binding at the wall. Following the model given by Myszka et al., the rate of binding at the wall for a first order reaction is 

\[
k_{\text{on}} c_w(R_T - B),
\]

where \( k_{\text{on}} \) is the on rate constant, \( R_T \) is the receptor concentration, \( B \) is the bound antigen concentration, and \( c_w(x) \) is the suspended concentration of antigen along the wall. The rate of dissociation is \( k_{\text{off}} B \), where \( k_{\text{off}} \) is the off rate constant. The time rate of change of antigen bound to the immobilized antibodies, \( \frac{\partial B}{\partial t} \), is equal to the rate of association minus the rate of dissociation

\[
\frac{\partial B}{\partial t} = k_{\text{on}} c_w(R_T - B) - k_{\text{off}} B \tag{6}
\]

The rate of antigen binding to immobilized antibodies must be balanced by the diffusive flux of antigen at the binding surface, \( y = 0 \), such that

\[
\frac{\partial c_w}{\partial t} = D \frac{\partial^2 c_w}{\partial y^2} \bigg|_{y=0} \tag{7}
\]

Eqns (5), (6) and (7) are solved with an immobilized antibody concentration \( R_T = 3.3 \times 10^{-11} \text{ M m} \) (i.e.

\[
2 \times 10^{16} \text{ molecules m}^{-3}
\]

and an initial suspended antigen concentration of \( 10^{-10} \text{ M} \). Under the standard, passive format where there is no micro-stirring (Fig. 5a), binding locally depletes the suspended antigen concentration. Because diffusion is the only transport mechanism, the depleted region surrounding the sensor surface grows with time and reduces the rate of binding. When the functionalized surface is located near the center of an electrode pair (Fig. 5b), and the electrodes are energized at \( V = 6 \text{ V rms} \) and \( f = 200 \text{ kHz} \), electrothermally-driven circulation redistributes the depleted concentration throughout the domain, and the sensor effectively sees higher suspended analyte concentration, resulting in a higher binding rate. Fig. 6 shows that an applied voltage of \( V = 6 \text{ V rms} \) produces a 7-fold increase in bound antigen compared to the 0 V rms (passive) case. For an applied voltage of \( V = 12 \text{ V rms} \), the increase jumps to 14-fold. The predicted maximum velocity and temperature rise are listed in Table 1. These velocities (\( u_{\max} \approx 0.4–6 \text{ mm s}^{-1} \)) vary with voltage to the 4th power, which is characteristic of electrothermal flow. These velocities are characteristic of other types of AC electrokinetic flows. For example, Bazant and Squires predict a 0.7 mm s\(^{-1}\) velocity generated by charge-induced AC electroosmosis. To be conservative, we will focus on the 6 V rms case, for which the numerical simulations suggest a factor of 7 increase in binding rate. Higher binding rates may be achieved with higher voltages. The following parameters were used in the numerical

Table 1 Predicted maximum velocity and temperature rise for electrothermally generated flow. A doubling of applied voltage results in a factor of four increase in temperature rise, and a factor of 16 increase in velocity. \( a_w = 0.66 \text{ S m}^{-1} \); \( f = 300 \text{ kHz} \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied potential/V rms</td>
<td>6</td>
</tr>
<tr>
<td>Model result: ( u_{\max}/\text{mm s}^{-1} )</td>
<td>0.38</td>
</tr>
<tr>
<td>Model result: ( \Delta T_{\max}/\text{K} )</td>
<td>4.1</td>
</tr>
</tbody>
</table>
The Damköhler number is the ratio of reaction velocity to diffusion velocity. If the reaction rate is fast compared with the transport of analyte to the reaction surface (i.e. through diffusion), the binding rate is diffusion-limited. On the other hand, if the diffusion is fast and the reaction rate slow, the binding rate is reaction-rate limited. The Damköhler number is the ratio of reaction velocity \( k_{on}R_T \) to diffusion velocity \( D/h^{20} \)

\[
Da = \frac{k_{on}R_T h}{D}
\]

For large \( Da \), slow diffusion limits the binding rate, and any increase in transport of analyte to the binding surface—through channel flow, or through electrothermally-driven micro-stirring—will increase binding rate. On the other hand, for small \( Da \), a slow reaction limits the binding rate, and increases in the transport rate through electrothermal flow will not improve the binding rate. A series of numerical simulations were conducted for various \( Da \). The binding enhancement factor, \( Be = B/B_0 \), is defined as the ratio of bound antigen resulting from electrothermal micro-stirring to the bound antigen without electrothermal stirring \( (B_0) \). For each parameter set, the binding enhancement factor, \( Be = B/B_0 \) at \( t = 100 \) s was calculated and is shown in Fig. 7. \( Da \) is varied by varying either \( k_{on} \) (diamonds) or \( D \) (squares). The data collapse onto one curve thereby confirming that \( Be \) depends on \( Da \), and not independently on \( k_{on} \) or \( D \). From this plot we can predict, for a given assay, how much binding improvement electrothermally-driven micro-stirring can provide. The dependence of \( Be \) on \( Da \) is not sensitive to initial concentration. The same simulations were run for \( Da \) of 10 \times \) higher and 10 \times \) lower initial concentrations \( (c_0 = 1 \text{ nM and } c_0 = 0.01 \text{ nM}) \). The resulting binding enhancement factor was within 5\% of the plotted values \( (c_0 = 0.1 \text{ nM}) \) for all values of \( Da \).

Table 2 shows typical Damköhler numbers for bio-molecule sensing systems. \( Da \) is calculated both for a DNA hybridization system and for an antigen antibody system for each of three microchannel heights. These \( Da \) numbers show that while significant binding enhancement is obtained for antigen–antibody assays of height greater than about 400 \( \mu \text{m} \), little binding enhancement is obtained for DNA hybridization sensors. The smaller molecules and lower \( k_{on} \) suggests that the system is reaction-rate limited. Within antigen–antibody assays, the systems which are the best candidates for electrothermal micro-stirring enhancement are those with the highest \( Da \), that is, those with large sample volume, large \( k_{on} \), large \( R_T \), that is, tightest receptor surface packing, or small diffusivity. Diffusivity varies inversely with molecule size, and is therefore reduced with the attachment of a label or a linker, such as for a sandwich immunoassay. The example antigen, Troponin I, is a mid-size protein (24 kDa). The method of micro-stirring is not limited to microfluidic channels, but can be optimized for

![Fig. 7](image-url) Simulation results showing that the binding enhancement factor, \( Be = B/B_0 \), increases with increasing Damköhler number. Each point represents two time-dependent simulations: one with and one without electrothermal micro-stirring; reported is the ratio of bound antigen \( B \) at 100 s for these two simulations. These simulations were run with initial concentration \( c_0 = 0.1 \text{ nM} \). Results (not shown) for \( c_0 = 1 \text{ nM} \) and \( c_0 = 0.01 \text{ nM} \) agree within 5\% of these values.
Table 2  Damköhler number, \( Da \), for antigen–antibody and for DNA hybridization systems of three different characteristic sizes. When the \( Da \) values calculated in this table are mapped onto Fig. 7, it is evident that DNA systems are not diffusion limited, and no significant binding enhancement is expected through electrothermal micro-stirring. The antigen–antibody system, however, is diffusion-limited, for sufficiently large length scales, and significant enhancement is possible. Assay binding rate constants were obtained from ref. 23 and 24.

<table>
<thead>
<tr>
<th>Analyte + immobilized receptor</th>
<th>( Da )</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 base ss-DNA + compl. ssDNA</td>
<td>0.006 0.06 0.6</td>
</tr>
<tr>
<td>Antigen + antibody (Troponin I)</td>
<td>300 3000</td>
</tr>
<tr>
<td>( Da = \frac{k_{\text{act}} R_T h D}{D} )</td>
<td></td>
</tr>
<tr>
<td>ssDNA: ( k_{\text{act}} = 1.4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} ); ( R_T = 3.3 \times 10^{-12} \text{ M m} ); ( D = 3 \times 10^{-10} \text{ m}^2 \text{ s}^{-1} )</td>
<td></td>
</tr>
<tr>
<td>Troponin I: ( k_{\text{act}} = 10^6 \text{ M}^{-1} \text{ s}^{-1} ); ( R_T = 1.7 \times 10^{-11} \text{ M m} ); ( D = 2.3 \times 10^{-11} \text{ m}^2 \text{ s}^{-1} )</td>
<td></td>
</tr>
</tbody>
</table>

ELISA tests in microtitre plates, or microarray assays where the sample floods a slide spotted with different antibodies.

### 4.3 Flow-through simulations

We now investigate binding enhancement from micro-stirring in microchannels with pressure-driven flow. In these sensors, the pressure-driven flow continually exposes the sensor to fresh analyte, and so we do not expect that the binding enhancement through micro-stirring will be as significant as for non-pressure driven flow. Also, the microchannel is smaller in these flow-through simulations than the simulations discussed in sections 4.1 and 4.2, to better represent microscale flow-through channels (e.g. MEMS based) which often have smaller fluid volume than static immunoassays (e.g. microarray). The channels are 100 \( \mu \text{m} \) in height, so the Damköhler number is reduced by a factor of 5, as compared with the same assay in the larger microcavity simulations (sections 4.1 and 4.2). For these simulations, a parabolic velocity inlet boundary condition is prescribed. Electrothermal forces distort the parabolic flow around the electrode gap to produce a circulating flow as shown in Fig. 8. For this case (average inlet velocity \( u = 67 \text{ \mu m s}^{-1} \); \( V = 6 \text{ V}_{\text{rms}} \)) the electrothermally generated velocity (\( \sim 300 \text{ \mu m s}^{-1} \)) is large compared with the average base flow (67 \( \text{ \mu m s}^{-1} \)). In the convection–diffusion model, zero initial concentration is prescribed throughout the microchannel. A concentration of \( c_0 = 0.1 \text{ nM} \) is introduced at the inlet (left hand side) of the channel for \( t > 0 \). For the passive case, where there is no applied voltage, the base flow is parabolic, and analyte is transported downstream most rapidly at the channel center. This is shown in Fig. 9a, where the highest concentrations of analyte extend through the center of the channel. When an electric potential is applied, the electrothermally-induced motion redirects the concentration profile (Fig. 9b). The optimum position of the functionalized surface is near the leading edge of the downstream electrode.

In this case, where convective transport from pressure-driven flow through the microchannel is important, the Peclet number must be considered as well as the Damköhler number. The Peclet number is defined as \( Pe = \frac{uhD}{D} \), which is the ratio of convective to diffusive transport. Fig. 10 shows the
binding enhancement factor \( Be \) for \( t = 100 \text{ s} \), for various Peclet numbers, and as a function of \( Da \). As in the non-flow-through format (Fig. 7), the binding enhancement factor \( Be \) has a strong dependence on \( Da \). In this flow-through case, however, there is an additional strong dependence on \( Pe \). Fig. 10 indicates that the binding enhancement factor \( Be \) is lower for larger \( Pe \), that is, for faster flows. While electrothermal micro-stirring may not be able to increase reaction rates in channels with high flow rates, it may provide more efficient capture of antigen, thereby allowing reduced flow rates. In cases where a high flow rate has been required to achieve sufficiently high binding rates in a transport-limited regime, the addition of electrothermal stirring to the channel allows the flow rate to be reduced while maintaining the same high binding rate. This effectively reduces required sample volume.

### 4.4 Hydrodynamic interactions near the wall

Although this paper focuses on electrothermally-generated micro-stirring, circulating fluid motion can also be generated through AC electroosmosis, for certain conditions in low electrical conductivity buffers. An important consideration for this application to heterogeneous assays is the near-wall velocity. It is conceivable that sufficiently high near-wall flow could be produced such that the drag on the bound antigen would overcome the bond energy and pull the antigen out of the bond. Because electroosmotic flow is driven by ions in the electrical double-layer near the electrodes, the flow velocity is highest near the wall. The electrothermal driving force, in contrast, is highest where the electrical conductivity gradient dotted into the electric field is highest. For the current geometry, this is adjacent to the electrode edges near the gap, diminishing rapidly away from the edge (Fig. 4). As a result, the electrothermally-generated flow produces less shear-induced dissociation than electroosmotic flow, and therefore is a better choice for immunoassay enhancement. In order to verify that the electrothermal flow in this model does significantly affect dissociation, we follow Leckband and Israelachvili’s analysis of bonds subject to external forces. \(^{21}\)

The intrinsic lifetime of a bond \( \tau_0 \) is related to its activation barrier \( E_0 \) by

\[
\tau_0 = \frac{1}{v_0} \exp(\frac{E_0}{kT})
\]

where \( v_0 \) is the vibration frequency of the bond. When an external force, \( F \), is applied, the bond lifetime is reduced according to the Bell equation

\[
\tau = \frac{1}{v_0} \exp(\frac{(E_0 -Fd)/kT}{})
\]

where \( d \) is the bond length. Therefore, by comparing \( E_0 \) and \( F_d \), where \( F_d \) is the force on the antigen from hydro-dynamic drag, we can determine whether drag has a significant effect on bond lifetime. The free energy of a typical antigen–antibody bond is \(^{22}\)

\[
E_0 = -kT \ln(K_A) \approx 8 \times 10^{-20} \text{J}
\]

where the binding affinity \( K_A = k_{on}/k_{off} = 10^9 \text{M}^{-1} \). We will consider an electrothermal velocity of 10 mm s\(^{-1}\) at 10 \( \mu \text{m} \) from the wall (which is almost double the highest velocity presented in this paper, 6 mm s\(^{-1}\) for \( V = 12 \text{ Vrms} \)). Assuming a linear velocity profile near the wall, we estimate the velocity scale at \( y = 20 \text{ nm} \) from the wall to be a maximum of \( \approx 20 \mu \text{m s}^{-1} \). According to the Stokes’ solution for creeping flow over a sphere, \(^{20}\) the Stokes’ drag force is \( F_D = 4 \times 10^{-15} \text{ N} \) for a protein with diameter 20 nm. Over a bond length of \( d = 1 \text{ nm} \), this yields a dislocation energy \( F_{bd} \approx 4 \times 10^{-24} \text{ J} \), insignificant compared to the \( 8 \times 10^{-20} \text{ J} \) bond energy for typical immunoassays. Even for a bond length of \( d = 10 \text{ nm} \), the bond energy would still be more than 3 orders of magnitude higher than \( F_{bd} \). Therefore, we predict that the fluid motion resulting from electrothermal flow will not create significant dissociation of the bound analyte.

### 5 Conclusions

Electrothermally-generated fluid motion was investigated experimentally and numerically. Experimental measurements of AC electrokinetically-driven velocity fields suggest that electrothermal forces are more important than AC electroosmosis for generation of fluid motion in high conductivity \((\sigma = 0.66 \text{ S m}^{-1})\) buffers, which are applicable for many bioassays. Numerical simulations were conducted to predict electrothermally-generated fluid motion. The model predicts, for 5 V\(_{\text{rms}}\) applied, a temperature rise on the order of 2.9 K, which results in velocities on the order of 100 \( \mu \text{m} \) s\(^{-1}\). The numerical model flow pattern closely matches experimental measurements with an applied voltage of \( V = 7 \text{ Vrms} \). The total power consumption of the electrothermal flow was 4.3 mW for a 200 \( \mu \text{m} \) deep cavity.

This model was used, together with a first order reaction model, to predict binding enhancement in heterogeneous reactions, for example, immunoassays. The model suggests that electrothermally-generated micro-stirring can increase the binding rate for non-flow-through assays, such as a microarray or microtiter plate, by a factor of seven, for 6 V\(_{\text{rms}}\) applied potential. A series of simulations with different parameters show that the binding enhancement depends strongly on the Damköhler number. The binding rate is increased by a factor of 7 with electrothermal micro-stirring, for \( Da = 10^4 \). However, the binding rate is only increased by a factor of 3 for \( Da \approx 100 \), and little increase in binding rate is observed for \( Da \approx 1 \).

Electrothermally-generated micro-stirring can also improve binding rates for flow-through assays, for example in BioMEMS or lab-on-a-chip devices. In these cases, the binding enhancement factor depends on the Peclet number as well as the Damköhler number. Higher Peclet numbers, which correspond to higher flow rates, yield lower binding enhancement through electrothermal micro-stirring. For example, with a Damköhler number of \( 3 \times 10^3 \), and a Peclet number of 100, a factor of nearly 8 improvement is possible. However, with a Peclet number of 1000, the expected improvement is reduced to a factor of about 3. The numerical simulations reported here indicate that electrothermal micro-stirring can be a useful technique for increasing binding rates in heterogeneous assays, particularly for diffusion-limited reactions.
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