Note: Four-port microfluidic flow-cell with instant sample switching

Christopher A. MacGriff,¹ Shaopeng Wang,² and Nongjian Tao^{1,2}

¹*School of Electrical, Computer, and Energy Engineering, Arizona State University, Tempe, Arizona 85287, USA*

²*Center for Bioelectronics and Biosensors, Biodesign Institute, Arizona State University, Tempe, Arizona 85287, USA*

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A simple device for high-speed microfluidic delivery of liquid samples to a surface plasmon resonance sensor surface is presented. The delivery platform is comprised of a four-port microfluidic cell, two ports serve as inlets for buffer and sample solutions, respectively, and a high-speed selector valve to control the alternate opening and closing of the two outlet ports. The time scale of buffer/sample switching (or sample injection rise and fall time) is on the order of milliseconds, thereby minimizing the opportunity for sample plug dispersion. The high rates of mass transport to and from the central microfluidic sensing region allow for SPR-based kinetic analysis of binding events with dissociation rate constants (k_d) up to 130 s^{-1} . The required sample volume is only $1\text{ }\mu\text{L}$, allowing for minimal sample consumption during high-speed kinetic binding measurement. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4826359>]

In its most popular application, surface plasmon resonance (SPR) technology provides real-time measurement of the extent of solute adsorption to a sensor surface; the rates of association k_a ($\text{Mol}^{-1}\text{ s}^{-1}$) and dissociation k_d (s^{-1}) between molecular binding partners can be extracted from an SPR signal. In general, the observed kinetic measurement data are a function of analyte mass transport and analyte/receptor binding affinity. The binding reaction is mass transport limited (MTL) if hydrodynamic forces retard the reaction rates of association and/or dissociation.

A common example of MTL occurs when the diffusion of the analyte near the sensor surface is comparable or slower than the binding of the analyte to the surface.¹ The delivery speed of analyte to the sensing region, before it diffuses to the sensor surface, is another potentially limiting factor. Although it is typically assumed to have a negligible effect in most popular kinetic binding models, this speed becomes important when the binding reactions have fast kinetics.² Small molecule binding reactions are less likely to be limited by slow diffusion rates than their large molecule analogues, as an analyte's diffusion coefficient D scales inversely with its molecular weight MW as $D \sim (MW)^{-\frac{1}{3}}$. However, this increased diffusion rate during sample convection to the sensing region may limit the availability of a quickly associating analyte or prolong removal during the dissociation phase. The large dispersion rate and fast binding kinetics characteristic of small molecules make their measurement particularly susceptible to this type of MTL.

The no-slip boundary condition in viscous fluid flow restricts the velocity of a fluid immediately adjacent to a solid boundary, which can manifest as a parabolic fluid velocity profile in microfluidic tubing. As analytes randomly diffuse during transport they experience disparate convective forces according to their position in the tubing. Consequently, it takes a finite amount of time for the fluid in the sensing region to transition between 100% buffer and 100% sample. This “transition” time (or injection rise and fall time) can limit

the chemical reaction on the sensor surface by reducing the availability of analyte during the association phase (reducing measured k_a), or insufficiently removing unbound analytes during dissociation (reducing measured k_d). Formally, this type of MTL invalidates the “continuously replenished analyte bulk concentration” assumption of popular kinetic analysis models.³ A minimal transition time between buffer and sample solution on the sensor surface is necessary to measure the fast kinetic rates of bi-molecular interactions.

The presented four-port microfluidic device addresses the specific mass-transport limitation of sample dispersion during convective transport in flow cell-based SPR detection platforms. The device is particularly useful for small molecule kinetic measurements: rapidly switching between buffer and sample solutions on the order of milliseconds. This enables the detection of binding events that occur two orders of magnitude faster than the current limits of popular commercial SPR systems (Table I) and expands the dynamic range of kinetic rate constant determination to values of $k_d < 130\text{ s}^{-1}$.⁴ Another advantage of this device is the minimal requirement of sample volume. Binding kinetics can be measured with as

TABLE I. Summary of the kinetic measurement capabilities of several major commercial SPR platforms.

Company	Instrument	Measurement limits		
		k_a ($\text{M}^{-1}\text{ s}^{-1}$)	k_d (s^{-1})	Sample Volume (μL)
Biorad	ProteOn XPR 36	3×10^6	0.6	95
GE Healthcare	Biacore T200	5×10^7	1	22
	Biacore X100	1×10^7	0.1	20
	Biacore 3000	1×10^7	0.1	>21
	Biacore 4000	5×10^6	1	30
SensiQ Technologies	(all products)	1×10^8	0.1	5
Plexera	PlexArray	1×10^6	0.01	50
IBIS	IBIS-MX96	5×10^6	0.05	n/a

FIG. 1. A schematic of the microfluidic delivery platform. (a) A syringe pump pushes buffer solution (blue) loaded in two identical syringes through the two inlet ports at a constant flow rate. One of the syringes is connected to an injection valve, allowing for the introduction of sample to the microfluidic device. The two outlet ports are connected to a high-speed stream selector valve. The selector valve allows for a single outlet port to flow at a time (open: solid line; closed: dotted line). By selecting which port is open, the direction of flow through the device and the composition of the solution (sample or buffer) in the central sensing area are controlled. (b) The relationship between the selector valve and the fluidic flow through the microfluidic device is illustrated. Buffer and sample (red) solutions flow through the device simultaneously, while only one solution flows over the central sensing area. The mixture (orange) of the solutions exits the device through the outlet port selected by the selector valve. The alternating opening and closing of the two outlets ports reverses the direction of flow and determines which solution flows over the central sensing area.

little as $1 \mu\text{L}$ of analyte solution, which enables measurement for precious samples.

The four-port flow cell has two inlets and two outlets and is described in detail in Figure 1. Buffer flows into one inlet, and the sample flows into the other. The two outlets are connected to a high-speed switching valve (Low Pressure Stream Selector Valve 1/16" and microelectric actuator, VICI Valco Instruments). Only one outlet is open at any given time, controlling the direction of flow through the microfluidic device and the composition of the solution localized to the sensor surface. Depending on which outlet is opened, the buffer or sample flows exclusively over the sensor surface, transitioning between the two rapidly. The small distance the sample travels to the sensing region minimizes the opportunity for sample dispersion (i.e., increased transition time). Note that the sample plug experiences dispersive effects due to diffusion of its constituent molecules as it travels from the injection valve to the microfluidic flow cell. However, the sample is not introduced to the sensing region of the flow cell until the switching valve selects the appropriate outlet port. In this way, the diluted samples in the front and tail ends of the sample plug are never introduced to the sensing region, cir-

cumventing the dispersive effects of this initial travel time. The transition time will scale with the physical dimensions of the device and is inversely proportional to the fluid flow rate. At a volumetric flow rate of $5 \mu\text{L}/\text{min}$, the devices pictured in Figures 2(b) and 2(c) transitioned between buffer and sample in 26 ms and 7.6 ms, respectively. Multiple samples can be injected in series, with a finite time interval to ensure the samples are well separated by the buffer.

A two-dimensional computer simulation of the four-port microfluidic device was conducted using COMSOL's Convection and Diffusion in combination with the incompressible Navier-Stokes fluid dynamics application modes, and followed the assembly of the Fluid Valve (COMSOL Model ID: 315).⁵ The mass transport of $50 \mu\text{M}$ of a 100 Da molecule ($D = 4 \times 10^{-10} \text{ m}^2/\text{s}$) with a constant influx rate of $5 \mu\text{L}/\text{min}$ was modeled using the physical dimensions of the device in Figure 2(b). The simulated fluids were assumed to be Newtonian, and the default no-slip boundary conditions were employed. The alternate closing of the outlet ports was modeled as local changes in fluid viscosity ($\mu \rightarrow \infty$). A summary of the simulated mass transport of the sample through the device is summarized in Figure 3. Empirical measurement of the de-

FIG. 2. (a) Image of the four-port microfluidic device taken with an optical microscope using transmitted light. PDMS is cured on an SU-8 mold ($60 \mu\text{m}$ height) fabricated using common soft lithography techniques. The device is press-clamped onto an Au sensor chip or permanently bound to a glass microscope slide with Au sensor regions using oxygen plasma. (b) Target receptors (e.g., IgG) are immobilized on an Au sensor surface prior to binding PDMS device. (c) A small Au sensor chip is fabricated on a glass microscope slide, allowing for a permanent bond between the PDMS device and the surrounding glass surface using oxygen plasma. Target receptors are bound to the surface using flow-based immobilization methods.

FIG. 3. (a) COMSOL simulation of sample flow through four port flow cell. Buffer flow from the top left inlet and the sample solution flows from the top right inlet. The bottom two outlets are connected to a switching valve. The bottom left outlet is opened in the first three pictures, then is switched closed as the bottom right outlet is opened. The flow through the center sensing region reverses direction as the sample solution flows out the open outlet. (b) The sample concentration in the center sensor region of the flow cell is plotted in red as a function of time. The black scatter plot is the experimental data showing the SPR sensor signal. The experimental data match the COMSOL simulation almost exactly. (c) Experimental data showing an IgG/algG binding for three discrete printed spots. The microfluidic device is pressed onto an Au sensor chip pre-printed with IgG.

vice's performance (170 mM ethanol in water, MW 46 Da, $D = 1.2 \times 10^{-10} \text{ m}^2/\text{s}$) and the computed sample concentration profile at a central point (i.e., sensing region) in the flow cell device is plotted as a function of time in Figure 3(b).

The device functionality was tested using a model IgG anti-IgG binding event on a homemade SPR platform. A non-contact piezoelectric inkjet printer (Engineering Arts LLC) was used to immobilize a uniform pattern of human IgG spots (via EDC/NHS coupling) with $200 \mu\text{m}$ spacing as previously described.⁶ Prior to the print, the Au sensor chip was functionalized with 5 mM of 50:1 PEG-OH:PEG-COOH in a 4:1% v/v ethanol to water solution.

$1 \times \text{PBS}$ was used as a running buffer and filled the microfluidic device and tubing prior to the sample injection. A $1 \mu\text{L}$ sample solution of $20 \mu\text{g}/\text{mL}$ goat anti-human IgG was injected into the injection valve and allowed to run through the microfluidic device until the front edge of the dispersed sample had passed through the microfluidic device, the sample was then introduced to the sensing region of the device for 20 s then removed. Local areas on the sensor surface without immobilized IgG were used as reference regions. The reference-subtracted SPR sensorgrams for the binding event are shown in Figure 3(c). This experiment was repeated three times with three different microfluidic flow cells and Au sensor chips. The measured binding affinities ($K_D = 22 \text{ nM}$, 12 nM , and 40 nM) are consistent with values reported in the literature, while the measured association rate constants ($k_a = 1.069 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, $1.460 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, and $6.785 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) and dissociation rate constants ($k_d = 0.023 \text{ s}^{-1}$, 0.017 s^{-1} , and 0.027 s^{-1}) are faster than expected.⁷ This is due to the short recording time and single injection concentration. The high frame rate of the CCD cam-

era (Pike, Allied Vision Technologies) rapidly fills the computer's memory buffer, setting an upper bound on the total recording time. Neither the association nor dissociation process was measured for a sufficiently long time to make an accurate measurement of the slow kinetics of this proof-of-principle binding event. These experimental artifacts are easily overcome and are irrelevant for the measurement of fast kinetic rate constants.

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⁴Measurement limits of kinetic rate constant of commercial SPR products listed in Table I were found in the product spec sheets in the corresponding manufacturer websites (Proteon: <http://www.bio-rad.com/en-us/product/proteon-xpr36-protein-interaction-array-system>; Biacore: https://www.biacore.com/lifesciences/products/systems_overview/index.html; SensiQ: <http://www.sensiqtech.com/products/>; Plexera: <http://www.plexera.com/Products/index.php>; IBIS: http://www.ibis-spr.nl/p_23.php) or by phone or email solicitation in June 2013.

⁵See <http://www.comsol.com/model/gate-valve-in-pipe-branch-12707> for COMSOL Model ID: 12707, Gate Valve in a Pipe Branch, which demonstrates the same principle of modeling gate behavior as a localized change in viscosity.

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