Supporting Information for "A Porous SiO_2 Interferometric Biosensor for Quantitative Determination of Protein Interactions: Binding of Protein A to Immunoglobulins Derived from Different Species" by Michael P. Schwartz, Sara D. Alvarez, and Michael J. Sailor

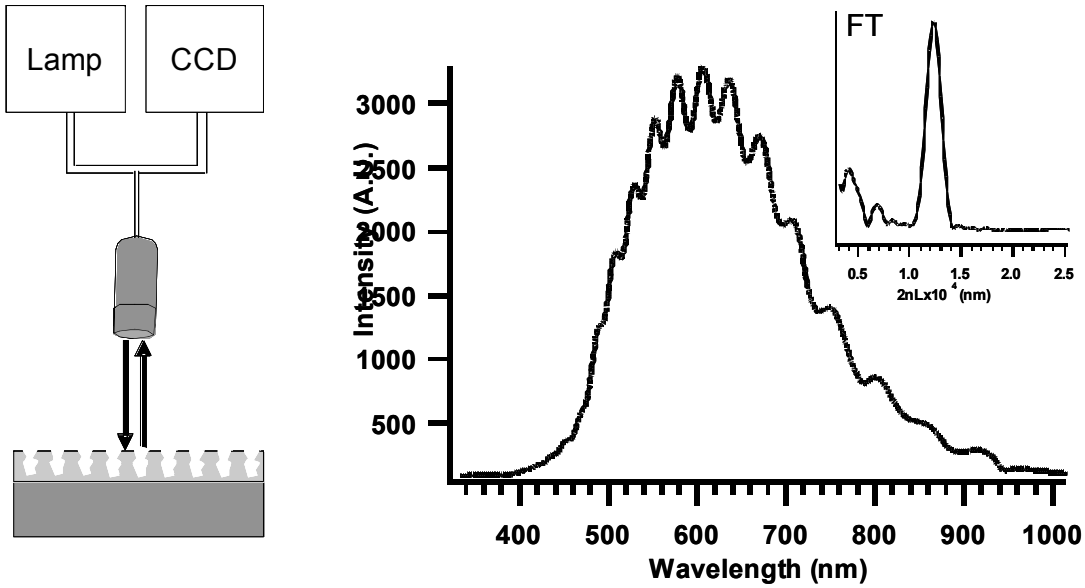

Figure S1. Experimental design for biosensing experiments using a porous SiO_2 interferometer. Light is fed through one end of a bifurcated fiber optic cable, focused through a lens onto the sensor film at normal incidence, collected through the same optics, and input to a CCD spectrometer. Light reflected from the film displays Fabry-Perot interference fringes, leading to the spectrum shown. The position of the peak in the Fourier transform (inset) yields the value 2nL, twice the optical thickness of the film.

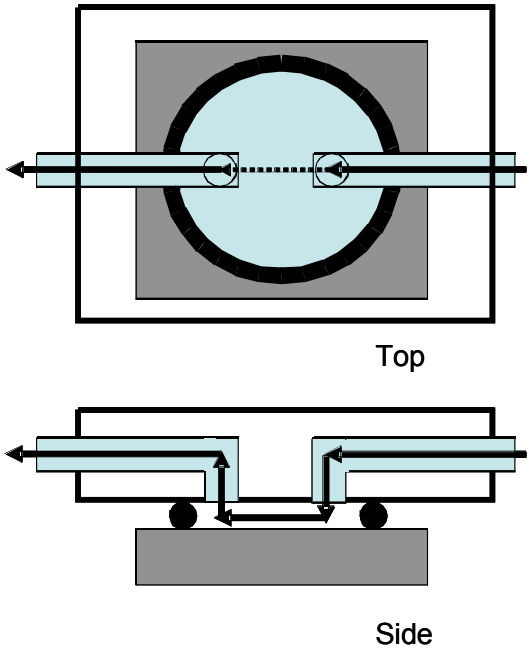

Figure S2. Schematic representation of the flow cell design used in this study. A porous silicon chip (gray square) is sealed under a plexiglass block using an o-ring. Inlet and outlet ports drilled into the plexiglass block are located above the porous silicon surface. The light from the tungsten lamp is focused on a spot between the entrance and the exit port through the plexiglass block. This configuration is not optimized to minimize mixing and mass transfer effects, which can lead to errors in determining kinetic rate constants.

Determination of Binding Constants from Equilibrium Binding Data.

The reaction of surface adsorbed protein A with IgG can be defined by the expression

$$A_{ads} + [IgG] \underset{kd}{\overset{ka}{\rightleftharpoons}} AG \quad (1)$$

where A_{ads} represents the available adsorbed protein A binding sites, [IgG] represents the concentration of free IgG in solution, and AG represents the surface-bound protein A/IgG complex. The equilibrium dissociation constant is defined as:

$$K_d = \frac{A_{ads}[IgG]}{AG} \quad (2)$$

which can be rearranged to be expressed in terms of product formed:

$$AG = \frac{A_{ads}[IgG]}{K_d} \quad (3)$$

A_{ads}, an unknown quantity, can also be expressed in terms of product formed, and substituted into Eq. 3:

$$AG = \frac{(AG_{max} - AG)[IgG]}{K_d} = \frac{AG_{max}[IgG] - AG[IgG]}{K_d} \quad (4)$$

where AG_{max} is the maximum number of available binding sites (a constant equal to the initial number of protein A sites that can form complex). Since the change in optical thickness of the porous silicon layer is directly proportional to the total amount of adsorbed species, AG is proportional to ΔOT_f, and AG_{max} is proportional to ΔOT_{max}. ΔOT_f is the value of ΔOT measured at steady-state for a given [IgG]. The proportionality constant is the same for both ΔOT_{max} and ΔOT_f, and therefore ΔOT_f and ΔOT_{max} can be substituted for AG and AG_{max}, respectively. Substitution into Eq. 4 and rearrangement yields:

$$\Delta OT_f * K_d = (\Delta OT_{max}[IgG] - \Delta OT_f [IgG]) \quad (5)$$

Solving Eq. 5 for ΔOT_f results in the following expression:

$$\Delta OT_f = \frac{\Delta OT_{max}[IgG]}{K_d + [IgG]} \quad (6)$$

K_d can be obtained by plotting [IgG] vs. ΔOT_f and numerically fitting the data to Eq. 6. Due to significant non-ideal behavior observed at high surface coverage (See Results and Discussion), ΔOT_{max} is also determined from the numerical fit.

Time-Dependent Kinetic Analysis.

Kinetic data could be obtained from flow cell experiments using a nonlinear least squares fitting method.[9,30,31] The use of nonlinear least squares methods for optical biosensing was demonstrated for SPR in 1993,[30] and the same principles from that work can be applied to the porous silicon interferometers. As discussed in the Experimental section, optical thickness is directly related to refractive index inside the porous silicon matrix, so the spectrum provides a measure of the amount of protein binding that occurs in the film.

The quantitative determination of equilibrium and kinetic binding constants is based on solving the time-dependent form of Eq. 1,

$$A_{ads} + [IgG] \underset{kd}{\overset{ka}{\rightleftharpoons}} AG$$

where k_a is the adsorption rate constant, k_d is the dissociation rate constant, A_{ads} is available surface adsorbed protein A binding sites, [IgG] is the concentration of IgG in solution, and AG is the surface bound protein A/IgG complex. The rate of AG formation can be expressed in terms of formation and dissociation of products:

$$\frac{d\,AG}{dt} = k_a(A_{ads})[IgG] - k_d(AG) \quad (7)$$

In order to quantitatively determine binding constants using Eq. 7, the unknowns AG and A_{ads} must be

expressed in terms of the measurable quantity, optical thickness. Substituting available protein A binding sites A_{ads} with AG_{max} - AG, the equation becomes

$$\frac{d\,\mathrm{AG}}{dt} = k_a[\mathrm{IgG}](\mathrm{AG}_{max} - \mathrm{AG}) - k_d(\mathrm{AG}) \tag{8}$$

where AG_{max} is the maximum available protein A/IgG binding sites, which is a constant equal to the initial number protein A binding sites. AG is directly proportional to the change in optical thickness (ΔOT) and AG_{max} is proportional to the maximum change in optical thickness (ΔOT_{max}) by the same proportionality constant, and can therefore be substituted into Eq. 8:

$$\frac{d(\Delta\,\mathrm{OT})}{dt} = k_a[\mathrm{IgG}](\Delta\,\mathrm{OT}_{max} - \Delta\,\mathrm{OT}) - k_d\,\Delta\,\mathrm{OT} \tag{9}$$

Eq. 9 can be rearranged to give

$$\frac{d(\Delta\,\mathrm{OT})}{dt} = k_a[\mathrm{IgG}]\Delta\,\mathrm{OT}_{max} - (k_a[\mathrm{IgG}] + k_d)\Delta\,\mathrm{OT} \tag{10}$$

Integration of Eq. 10 and simplification results in the equation

$$\Delta\,\mathrm{OT} = \frac{k_a[\mathrm{IgG}]\Delta\,\mathrm{OT}\,max}{k_a[\mathrm{IgG}] + k_d}[1 - e^{-(ka[IgG]+kd)\,t}] \tag{11}$$

which can be simplified by combining constants into a single term, C

$$\Delta OT = C[1 - e^{-(ka[IgG]+kd)t}] \quad (12)$$

Numerical fitting of Eq. 12 yields the fit parameters C and (k_a[IgG]+k_d).

In order to determine $K_d = k_d/k_a$, the quantity k_d must also be determined. The dissociation rate constant, k_d, can be determined using the expression

$$\frac{d(\Delta OT)}{dt} = -k_d \Delta OT \quad (13)$$

which can be integrated to yield

$$\Delta OT = \Delta OT_0 * e^{-k_d t} \quad (14)$$

where ΔOT_0 is the sensor response at the time of initiation of buffer flow. For Eq. 14 to be valid, the solution must be continually refreshed so that reassociation cannot occur. The dissociation rate constant, k_d, can be directly determined by numerically fitting the dissociation curve to eq. 14. Then, using the value obtained for k_d, the association rate constant, k_a, can be determined by fitting Eq. 12 to the association data and solving for the exponential term using the known concentration and k_d value.