Real-time Recognition of *Mycobacterium tuberculosis* and Lipoarabinomannan using the Quartz Crystal Microbalance

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Equations used to calculate equilibrium association and rate constants

θ	degree of surface coverage	Δm	change in mass
α_0	correlation factor	$\Delta m_{\max}$	maximum change in mass
μ	rate at which atoms strike the surface per unit area	k_a	associate rate constant
v_1	rate of evaporation from a completely covered surface	k_d	disassociation rate constant
K_a	equilibrium association constant	t	time
C	liquid concentration	τ	tau

(S-1) $\theta = \alpha_0\mu/(v_1 + \alpha_0\mu)$

(S-2) $\theta = C/(K_a^{-1} + C)$

(S-3) $\theta = \Delta m/\Delta m_{max}$

(S-4) $\Delta m = \Delta m_{\max} K_a C/(1 + K_a C)$

(S-5) $C/\Delta m = C/\Delta m_{\max} + 1/\Delta m_{\max} K_a$

(S-6) $\Delta m = -\Delta m/K_a C + \Delta m_{\max}$

(S-7) $1/\Delta m = 1/K_a \Delta m_{max} C + 1/\Delta m_{max}$

(S-8) $v_a = k_a C(1 - \theta)$

(S-9) $v_d = k_d \theta$

(S-10) $k_a C(1 - \theta) = k_d \theta$

(S-11) $\theta(t) = k_a C/(k_a C + k_d)\{1 - \exp[-(k_a C + k_d)t]\}$

(S-12) $\theta(t) = \theta[1 - \exp(-t/\tau)]$

(S-13) $\ln(1 - \Delta m/\Delta m_{\max}) = -t/\tau$

(S-14) $-1/\tau = k_a C + k_d$

The equation used to define Langmuir adsorption isotherms in gaseous environments (Eq. S-1), has also been used to describe isotherms occurring in liquid (Eq. S-2).[1] In liquid environments the degree of surface coverage can be represented by the change in mass at a given time over the maximum change in mass for the given concentration (Eq. S-3). Incorporating this equation into the mathematical equation for Langmuir adsorption isotherms (Eq. S-4), and rearranging allows for graphical determination of binding constants by varying the concentration of protein in solution (Eqs. S-5 through S-7).[2-4] These equations allow direct calculation of equilibrium constants which would not be as definitive if $\Delta m_{\max}$ were instead approached asymptomatically as is traditionally done.

At equilibrium, the velocities of adsorption and desorption at the surface of the crystal can be described in terms of respective rate constants and degree of surface coverage (Eqs. S-8 and S-9).[5, 6] The

association and dissociation velocities can be assumed to be equal at equilibrium, and can be algebraically rearranged to form the general Langmuir isotherm (Eq. S-10).

Monolayer formation with time is equal to the velocity of association minus the velocity of dissociation. Integrating this differential equation using a Laplace transform (boundary condition: $\theta = 0\ at\ t = 0$) results in Eq. S-11.[7] Substituting the Langmuir isotherm using the principle of microscopic reversibility and allowing $k_a C + k_d$ to be represented by τ^{-1} presents a more simplified equation (Eq. S-12).[7, 8] Using the assumption that the maximum degree of surface coverage is one and $\theta(t) = \Delta m/\Delta m_{max}$, τ can be calculated at various concentrations (Eq. S-13) and then graphed as C versus τ^{-1} to extrapolate k_a and k_d which can be used for comparison with isotherm methods (Eq. S-14).[3, 9]

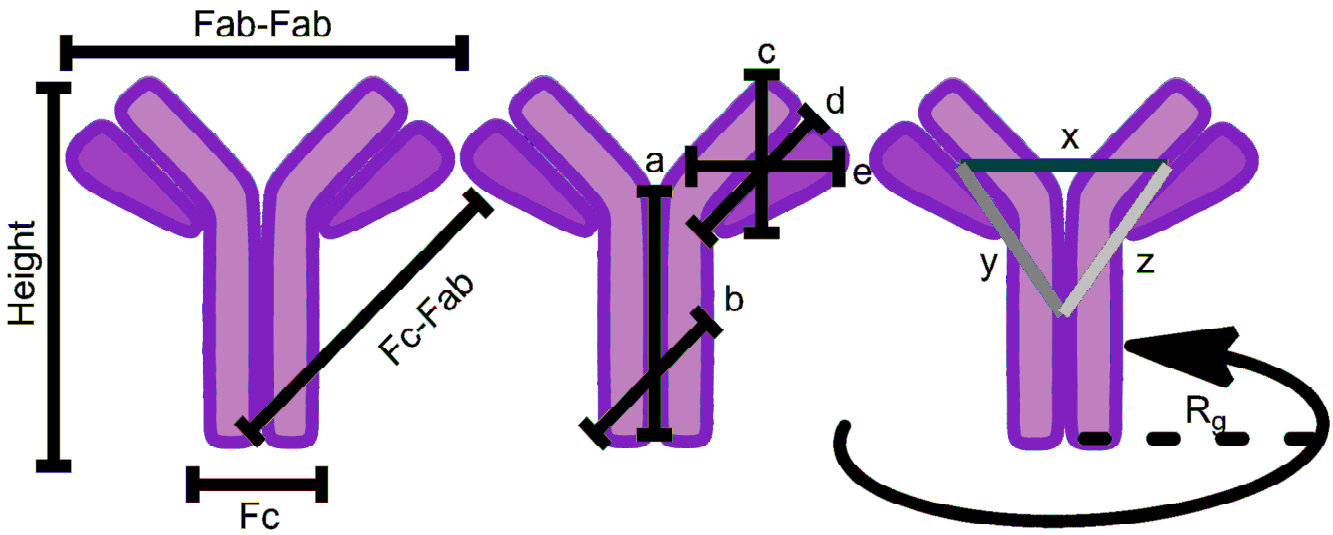

Figure S-1. Literature reports the dimensions of IgG antibodies as follows: Fab-Fab (14.2 nm)[10], height (11.5, 14.0 nm)[11], Fc-Fab (13.6 nm)[12], Fc (0.91[13], 3.8[10] nm), Rg (6,[14] 5.5[15], 5.6[16] nm), a (8.5 nm)[10], b (4.5 nm)[10], c*d*e (7x5x4 nm)[10], x-y-x (8.3-7.3-7.9 nm)[17]. These were used to develop spherical (using Rg) and elliptical (using d and b for the width, Fab-Fab for the length) representations that could be used to examine possible orientations of the IgG molecules immobilized on a QCM crystal through binding of its Fc region to Protein A, thus predicting maximum surface coverage.

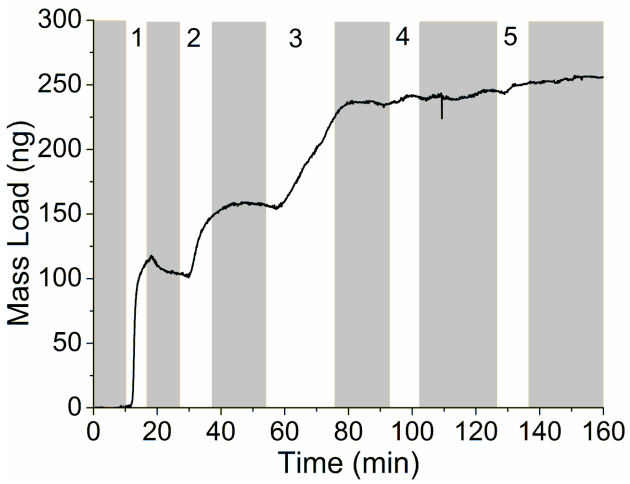

Figure S-2. Reusability of the α-LAM immunosensor is demonstrated with sequential binding of Mtb and then LAM. The total mass detected in each step is as follows: (1) 101 ng protein A, θ =17% (2) 53 ng BSA, θ =106% (3) 83 ng α-LAM, θ =18% (4) 4 ng Mtb, θ =0.3% (5) 6 ng LAM, θ =13%.

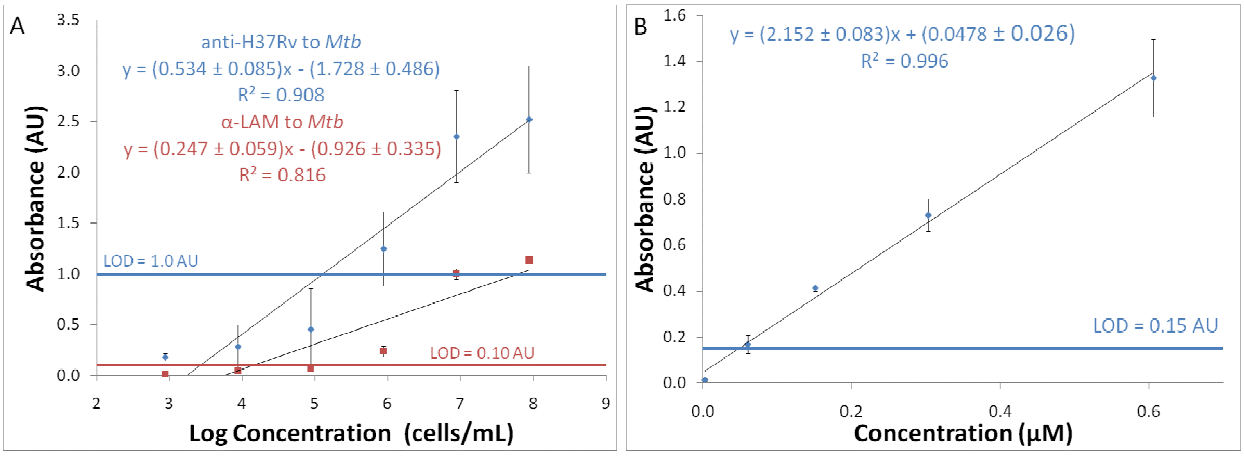

Figure S-3. Detection of *Mtb* using anti-H37Rv and α-LAM (a) and of LAM using α-LAM (b) in an ELISA.

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