Electronic Supplementary Material (ESI) for Lab on a Chip.

Lab on a Chip
Supplementary Information

Development of Quantitative Radioactive Methodologies on Paper to Determine Important Lateral-Flow Immunoassay Parameters

Garrett L. Mosley,[1] Phuong Nguyen,[1] Benjamin M. Wu,[1,2], and Daniel T. Kamei[1,]*

[1]Department of Bioengineering, University of California, Los Angeles, CA 90095
[2]Division of Advanced Prosthodontics & Weintraub Center for Reconstructive Biotechnology, School of Dentistry, University of California, Los Angeles, CA 90095

*Corresponding Author: Daniel T. Kamei, kamei@seas.ucla.edu

S.1 Full derivation of the species balance equation described in the Materials and Methods section entitled "Derivation of species balance equation used to calculate $k_{r,b}$ and $k_{f,b}$"

Let us first consider binding of free target molecules in the bulk suspension to individual antibody binding sites on probes in the bulk suspension, where the probe has n number of antibodies on its surface. We treat each antibody binding site as being distinguishable so that there are separate probe concentrations for each binding permutation represented by $P_{b[symbols]}$ where the symbols within the brackets denote the unique sites that are occupied. For example, $P_{b[\alpha,\beta]}$ represents the concentration of probe with binding sites α and β occupied by target molecules. **Figure S1** illustrates an example scenario where the probe has 4 available binding sites. Note that we model the two binding sites of each antibody by treating each binding site as an independent interaction and assuming no cooperativity in binding (a standard assumption with antibody binding).

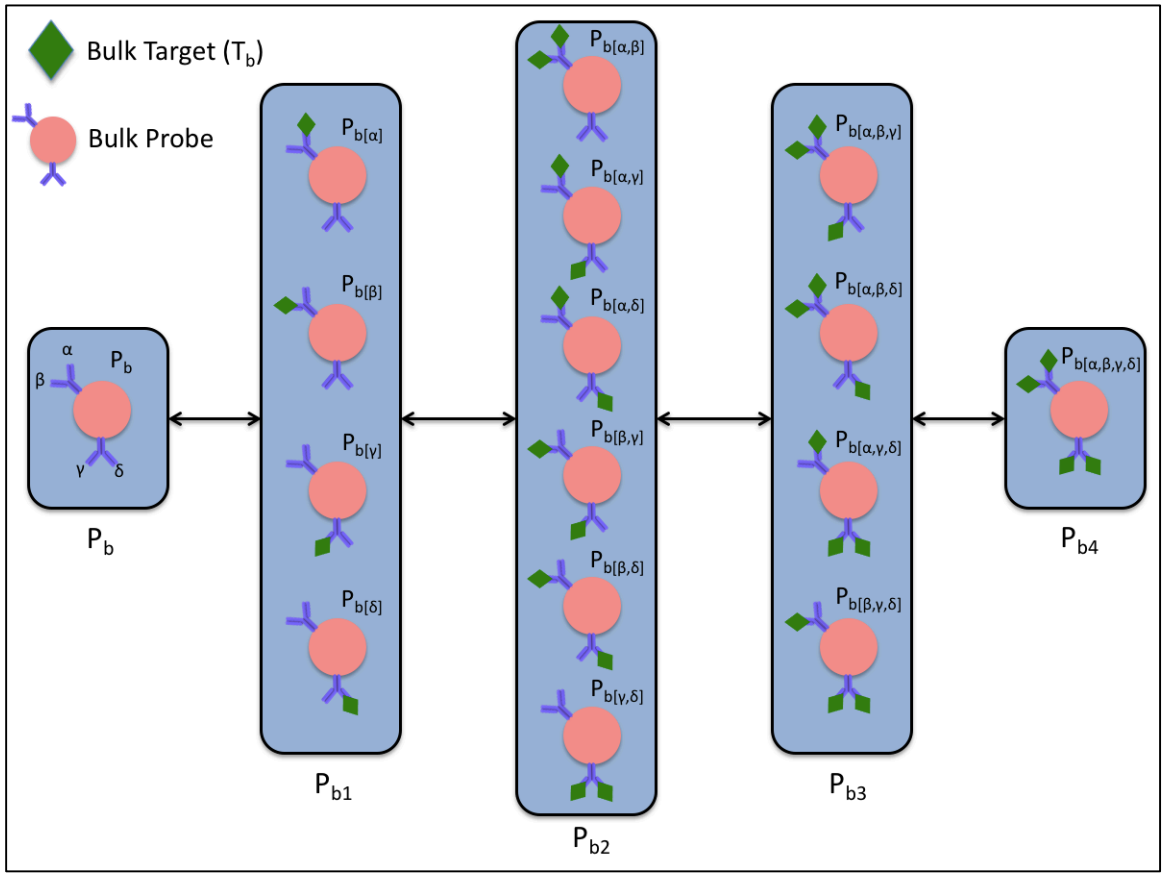

Figure S1. Schematic of bulk probe binding to bulk target. The schematic depicts the binding between a bulk probe (P_b) with 4 antibody binding sites (i.e., n=2 antibodies bound) to bulk target (T_b). Each antibody binding site is distinguishable by location, thus demonstrating the 16 possible binding permutations ($P_{b[symbols]}$) for this example. Each binding interaction is governed by the rate constants $k_{r,b}$ and $k_{f,b}$. The summation of the probe species with a given number of bound targets is represented as P_{bj}, where j is the number of bound targets. Note that this schematic depicts probes with 4 antibody binding sites for the purpose of clarity; however, the probes used during the experimental procedures had 8 antibody binding sites.

Each transition from one unique bound state to another can be described by expressions of rates of formation (**Eq. (1)**) and disappearance (**Eq. (2)**):

$$k_{f,b} P_{b[symbols]} T_b \tag{1}$$

$$k_{r,b}P_{b[symbols]} \tag{2}$$

where $P_{b[symbols]}$ is the molar concentration of the unique probe species that is undergoing the transition, T_b is the molar concentration of target in the bulk suspension, and $k_{f,b}$ and $k_{r,b}$ are the forward and reverse binding rate constants, respectively.

We assume each antibody site has equal binding affinity for the target. This allows us to treat each unique probe species with a given amount of bound target as having equal concentrations, which will be denoted as $P_{b[j]}$ where j is the total number of target molecules bound to the antibody sites, and j ranges from 0 to 2n. For example, $P_{b[\alpha,\beta,\gamma,]}$, $P_{b[\alpha,\beta,\delta]}$, $P_{b[\alpha,\gamma,\delta]}$, and $P_{b[\beta,\gamma,\delta]}$ have equal concentration values, and each of these concentrations is represented as $P_{b[3]}$. The rate of change of any given unique probe species $P_{b[j]}$ is a summation of the rates of formation and disappearance for its transitions between the other unique probe species, which can be mathematically represented as follows:

$$\frac{dP_{b[j]}}{dt} = jk_{f,b}P_{b[j-1]}T_b - jk_{r,b}P_{b[j]} - (2n-j)k_{f,b}P_{b[j]}T_b + (2n-j)k_{r,b}P_{b[j+1]} \tag{3}$$

where j is the number of bound antibody sites, (2n-j) is the number of available antibody sites after j sites are bound, n is the total number of antibodies per probe, $P_{b[j]}$, $P_{b[j-1]}$, and $P_{b[j+1]}$ are the molar concentrations of unique probe species with j, j-1, and j+1 number of targets bound, respectively, and T_b is the molar concentration of target in the bulk solution.

Considering that the concentration of each unique probe species $P_{b[j]}$ is equal to each other for a given number of bound targets j (e.g., $P_{b[\alpha,\beta,\gamma,]} = P_{b[\alpha,\beta,\delta]} = P_{b[\alpha,\gamma,\delta]} = P_{b[\beta,\gamma,\delta]} = P_{b[3]}$), the summation of all the $P_{b[j]}$ values for a given j can be mathematically described by multiplying by the number of binding permutations:

$$P_{bj} = \frac{(2n)!}{(2n-j)!\,j!} \cdot P_{b[j]} \tag{4}$$

where P_{bj} is the sum of all $P_{b[j]}$ species concentrations for a given j value, i.e., the cumulative concentration for each j value. The total number of permutations is equal to the total number of bound states with the same energy, i.e., the degeneracy, and it is equal to the binomial coefficient of 2n choose j. Using **Eq. (4)**, several equations can be developed for the unique probe species $P_{b[j]}$, $P_{b[j-1]}$, and $P_{b[j+1]}$ to describe them in terms of their corresponding cumulative concentrations P_{bj}, P_{bj-1}, and P_{bj+1}, respectively:

$$P_{b[j]} = \frac{P_{bj}}{\left(\frac{(2n)!}{(2n-j)!\,j!}\right)} \tag{5}$$

$$P_{b[j-1]} = \frac{P_{bj-1}}{\left(\frac{(2n)!}{(2n-(j-1))!(j-1)!}\right)} \tag{6}$$

$$P_{b[j+1]} = \frac{P_{bj+1}}{\left(\frac{(2n)!}{(2n-(j+1))!(j+1)!}\right)} \tag{7}$$

Equation (3) can now be written in terms of the cumulative concentrations by substituting in **Eqs. (5)**, **(6)**, and **(7)** to yield:

$$\frac{dP_{b[j]}}{dt} = jk_{f,b}T_b \frac{P_{bj-1}}{\frac{(2n)!}{(2n-(j-1))!(j-1)!}} - jk_{r,b}\frac{P_{bj}}{\frac{(2n)!}{(2n-j)!\,j!}}$$

$$-(2n-j)k_{f,b}T_b \frac{P_{bj}}{\frac{(2n)!}{(2n-j)!\,j!}} + (2n-j)k_{r,b}\frac{P_{bj+1}}{\frac{(2n)!}{(2n-(j+1))!(j+1)!}} \tag{8}$$

We can now derive the rate of change for the cumulative concentration of probe for a given number of bound targets j by taking the derivative of **Eq. (4)** with respect to time and substituting in **Eq. (8)**. After simplifying, the equation is given by:

$$\frac{dP_{bj}}{dt} = \left(2n-\left(j-1\right)\right)k_{f,b}T_bP_{bj-1} - jk_{r,b}P_{bj} - \left(2n-j\right)k_{f,b}T_bP_{bj} + \left(j+1\right)k_{r,b}P_{bj+1} \tag{9}$$

which holds true when (1≤j≤2n-1). The j=0 and j=2n expressions cannot be represented by the above equation as they represent completely unbound probe and completely bound probe, respectively. Specifically, the rates of change for bound target values of j=0 and j=2n can be written as:

$$\frac{dP_b}{dt} = -2nk_{f,b}T_bP_b + k_{r,b}P_{b1} \tag{10}$$

$$\frac{dP_{b2n}}{dt} = k_{f,b}T_bP_{b2n-1} - 2nk_{r,b}P_{b2n} \tag{11}$$

To experimentally determine the rate constants, radiolabeled Tf is used to quantify the change in molar concentration of total target bound to the antibody binding sites at varying points in time. This measurement can be described mathematically as a summation of the rates of change of the cumulative probe species concentrations from **Eqs. (9)**, **(10)**, and **(11)**, multiplied by their respective number of targets bound j:

$$\frac{d\left(\sum_{j=0}^{2n} jP_{bj}\right)}{dt} = 2n\left(k_{f,b}T_bP_{b2n-1} - 2nk_{r,b}P_{b2n}\right) + 0\left(-2nk_{f,b}T_bP_b + k_{r,b}P_{b1}\right) +$$

$$\sum_{j=1}^{2n-1}\left(j\left(\left(2n-\left(j-1\right)\right)k_{f,b}T_bP_{bj-1} - jk_{r,b}P_{bj} - \left(2n-j\right)k_{f,b}T_bP_{bj} + \left(j+1\right)k_{r,b}P_{bj+1}\right)\right) \tag{12}$$

Equation (12) can be simplified to:

$$\frac{d\left(\sum_{j=0}^{2n} jP_{bj}\right)}{dt} = k_{f,b}T_b\left(\sum_{j=0}^{2n} 2nP_{bj} - \sum_{j=0}^{2n} jP_{bj}\right) - k_{r,b}\left(\sum_{j=0}^{2n} jP_{bj}\right) \quad (13)$$

The first summation on the right-hand side of **Eq. (13)** represents the total molar concentration of antibody binding sites (available and bound) within the solution, which we will denote as A_0. The remaining summations in **Eq. (13)** represent the total molar concentration of bound antibody binding sites within the suspension, which we will denote as B. Making these notation substitutions, **Eq. (13)** is given by:

$$\frac{dB}{dt} = k_{f,b}T_b\left(A_0 - B\right) - k_{r,b}B \quad (14)$$

Equation (14) can be used to calculate the binding rate constants for our specific experimental designs.

S.2 Validating constant bulk probe assumption ($P_b = P_{b,0}$) over varying volumetric flow rates used in the calculation of $k_{f,s}$

S.2.1 Materials and Methods

DGNPs and LFA membranes were setup as described in the Materials and Methods section of the main manuscript. To vary the volumetric flow rate during DGNP binding, solutions of gold buffer, which did not contain DGNPs, were initially flowed along the test strip for varying durations of time (0, 1, and 3 minutes) before the LFA test strips were switched to a gold buffer suspension containing DGNPs. DGNP binding was quantified using methods described in the manuscript.

The average volumetric flow rate (μL/min) for each condition was determined by dividing the volume of the DGNP solution by the length of time that it took the DGNP solution to flow up the LFA test strip.

S.2.2 Results

We see from the data (**Table S1**) that over the range of volumetric flow rates tested, the total percentage of DGNPs bound to the test line remains low, which indicates that the constant bulk probe concentration assumption is valid over the range of volumetric flow rates tested.

Table S1. Validating constant probe assumption. Quantification of the percentage of DGNPs that bound to the test line over varying average volumetric flow rates.

Average Volumetric Flow Rate (μL/min)	Bound DGNPs (fmol)	Bound DGNPs (%)
4.4	685	5.0%
3.2	605	4.4%
2.9	587	4.3%