Electronic Supplementary Information

A two-dimensional mathematical model for analyzing effects of capture probe properties on the performance of lateral flow assays

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The production rate equations

In the above concentration-governing equations, the concentration production rates $F_{PA}$, $F_{RA}$, $F_{RPA_1}$, and $F_{RPA_2}$ correspond to complexes PA, RA, and RPA in Eqs. (1-4). Based on the chemical reaction kinetics, the reagent production rates $F_{PA}$, $F_{RA}$, $F_{RPA_1}$, and $F_{RPA_2}$ correspond to Eqs. (1-4), respectively and are given as follows:

\[
F_{PA} = k_a \cdot C_A \cdot C_p - k_d \cdot C_{PA}
\]

\[
F_{RA} = k_a \cdot (C_{R_p} - C_{RA} - C_{RPA}) \cdot C_A - k_d \cdot C_{RA}
\]

\[
F_{RPA_1} = k_a \cdot (C_{R_p} - C_{RA} - C_{RPA}) \cdot C_{PA} - k_d \cdot C_{RPA}
\]

\[
F_{RPA_2} = k_a \cdot C_{RA} \cdot C_p - k_d \cdot C_{RPA}
\]

According to the nucleic acid hybridization kinetics model\[^{[33]}\], $k_a$ and $k_d$ are evaluated to be the same in the Eqs. (1) to (4). $k_a$ and $k_d$ are calculated to be $3.0 \times 10^{-5}$ (nM$^{-1}$·s$^{-1}$) and $1.0 \times 10^{-7}$ (1/s), respectively. Furthermore, the $F_{RPA}$, which are produced by the above two approaches shown in Eqs. (16-17) are expressed as follows:

\[
F_{RPA} = F_{RPA_1} + F_{RPA_2}
\]

Discretization and solution procedure

The two dimensional calculation domain is discretized by using the inner node method with uniform grid spacing (Fig. S1). The concentration-governing equations (9)–(18) and the initial and boundary conditions Eqs. (19)–(23) are discretized by finite control volume method. The implicit scheme is applied for unsteady terms. Therefore, the discretized...
The discretized production rates of the total complex $A$ produced by the above two Eqs. (2c–d) are given as:

\[
\frac{C_{A,i,j}^{k+1} - C_{A,i,j}^k}{\Delta t} = D_A \frac{C_{A,i,j}^{k+1} - 2C_{A,i,j}^k + C_{A,i,j}^{k-1}}{\Delta x^2} + D_A \frac{C_{A,i,j+1}^{k+1} - 2C_{A,i,j}^k + C_{A,i,j-1}^{k+1}}{\Delta y^2} - \frac{U}{2\Delta x} C_{A,i,j}^{k+1} - C_{A,i,j}^k - F_{PA,i,j}^{k+1}
\] (S1)

The discretized concentration-governing equation of report particle $P$ is given as:

\[
\frac{C_{P,i,j}^{k+1} - C_{P,i,j}^k}{\Delta t} = D_p \frac{C_{P,i,j}^{k+1} - 2C_{P,i,j}^k + C_{P,i,j}^{k-1}}{\Delta x^2} + D_p \frac{C_{P,i,j+1}^{k+1} - 2C_{P,i,j}^k + C_{P,i,j-1}^{k+1}}{\Delta y^2} - \frac{U}{2\Delta x} C_{P,i,j}^{k+1} - C_{P,i,j}^k - F_{PA,i,j}^{k+1}
\] (S2)

The discretized concentration-governing equations of complex $PA$ are written as:

\[
\frac{C_{PA,i,j}^{k+1} - C_{PA,i,j}^k}{\Delta t} = D_p \frac{C_{PA,i,j}^{k+1} - 2C_{PA,i,j}^k + C_{PA,i,j}^{k-1}}{\Delta x^2} + D_p \frac{C_{PA,i,j+1}^{k+1} - 2C_{PA,i,j}^k + C_{PA,i,j-1}^{k+1}}{\Delta y^2} - \frac{U}{2\Delta x} C_{PA,i,j}^{k+1} - C_{PA,i,j}^k + F_{PA,i,j}^{k+1}
\] (S3)

Correspondingly, the discretized balance equation of production rate of $RA$ is given as:

\[
\frac{C_{RA,i,j}^{k+1} - C_{RA,i,j}^k}{\Delta t} = F_{RA,i,j}^{k+1} - F_{RPA,i,j}^{k+1}
\] (S4)

The discretized balance equations of production rates of $RPA$ are expressed as:

\[
\frac{C_{RPA,i,j}^{k+1} - C_{RPA,i,j}^k}{\Delta t} = F_{RPA,i,j}^{k+1}
\] (S5)

The discretized production rates of complex $PA$ are given as:

\[
F_{PA,i,j}^k = k_a \cdot C_{A,i,j}^k \cdot C_{P,i,j}^k - k_d \cdot C_{PA,i,j}^k
\] (S6)

The discretized production rate of complex $RA$ is written as:

\[
F_{RA,i,j}^k = k_a \cdot \left( C_{R}^k - C_{RA,i,j}^k - C_{RPA,i,j}^k \right) C_{A,i,j}^k - k_d \cdot C_{RA,i,j}^k
\] (S7)

The discretized production rates of complex $RPA$ produced by the three methods shown in Eqs. (2c–d) are given as:

\[
F_{RPA,i,j}^k = k_a \cdot \left( C_{R}^k - C_{RA,i,j}^k - C_{RPA,i,j}^k \right) C_{PA,i,j}^k - k_d \cdot C_{RPA,i,j}^k
\] (S8)

\[
F_{RPA,i,j}^k = k_a \cdot C_{RA,i,j}^k \cdot C_{P,i,j}^k - k_d \cdot C_{RPA,i,j}^k
\] (S9)

The discretized production rates of the total complex $RPA$ produced by the above two methods are given as:
As shown in the above discretized equations, they are solved simultaneously with the SIMPLE algorithm [1].

\[ F_{\text{RPA}_{i,j}}^k = F_{\text{RPA}_{i,j}}^{k-1} + F_{\text{RPA}_{i,j}}^{k-1} \]  \hspace{1cm} (S10)

Initial conditions:

\[ C_{\text{Al}_{i,j}}^{0} = 0 \text{, } C_{\text{Pl}_{i,j}}^{0} = 0 \text{, } C_{\text{RA}_{i,j}}^{0} = 0 \text{, } C_{\text{PA}_{i,j}}^{0} = 0 \text{ and } C_{\text{RPA}_{i,j}}^{0} = 0 \text{ \hspace{1cm} } t = 0 \]  \hspace{1cm} (S11)

Left boundary conditions:

\[ C_{\text{Al}_{i,j}} = C_{\text{A}} \text{, } C_{\text{Pl}_{i,j}} = C_{\text{P}} \text{, } C_{\text{PA}_{i,j}} = 0 \text{ nM} \text{ \hspace{1cm} } x = 0 \]  \hspace{1cm} (S12)

Right boundary conditions:

\[ \frac{C_{\text{Al}_{i,2}}^{k} - C_{\text{Al}_{i,2}}^{k}}{\Delta x} = 0 \text{, } \frac{C_{\text{Pl}_{i,2}}^{k} - C_{\text{Pl}_{i,2}}^{k}}{\Delta x} = 0 \text{, } \frac{C_{\text{PA}_{i,2}}^{k} - C_{\text{PA}_{i,2}}^{k}}{\Delta x} = 0 \text{ \hspace{1cm} } x = L \]  \hspace{1cm} (S13)

Up boundary conditions:

\[ \frac{C_{\text{Al}_{i,1}}^{k} - C_{\text{Al}_{i,2}}^{k}}{\Delta y} = 0 \text{, } \frac{C_{\text{Pl}_{i,1}}^{k} - C_{\text{Pl}_{i,2}}^{k}}{\Delta y} = 0 \text{, } \frac{C_{\text{PA}_{i,1}}^{k} - C_{\text{PA}_{i,2}}^{k}}{\Delta y} = 0 \text{ \hspace{1cm} } y = H \]  \hspace{1cm} (S14)

Down boundary conditions:

\[ \frac{C_{\text{Al}_{i,1}}^{k} - C_{\text{Al}_{i,2}}^{k}}{\Delta y} = 0 \text{, } \frac{C_{\text{Pl}_{i,1}}^{k} - C_{\text{Pl}_{i,2}}^{k}}{\Delta y} = 0 \text{, } \frac{C_{\text{PA}_{i,1}}^{k} - C_{\text{PA}_{i,2}}^{k}}{\Delta y} = 0 \text{ \hspace{1cm} } y = 0 \]  \hspace{1cm} (S15)

The reaction rates of the complex RA, RPA, and RPA₂ on the fiber surface at test line are given as:

\[-D_{A} \frac{C_{\text{Al}_{i,j}}^{k} - C_{\text{Al}_{i,j-1}}^{k}}{\Delta y} = F_{\text{RA}_{i,j}} \text{, } -D_{P} \frac{C_{\text{Pl}_{i,j}}^{k} - C_{\text{Pl}_{i,j-1}}^{k}}{\Delta y} = F_{\text{RPA}_{2,i,j}} \text{, } \]
\[-D_{P} \frac{C_{\text{PA}_{i,j}}^{k} - C_{\text{PA}_{i,j-1}}^{k}}{\Delta y} = F_{\text{RPA}_{1,i,j}} \text{ \hspace{1cm} } x_{1} < x \leq x_{2} \]  \hspace{1cm} (S15)

The above discretized equations are solved simultaneously with the SIMPLE algorithm [1]. As shown in Figure S2, the solution procedure includes the inner iteration for solving the governing and boundary equations at each time level and the outer iteration for the evolving time steps. The inner iteration is terminated until the convergence criterion of Eq. (16) is satisfied. The convergence values then evolve to another time step for the outer iteration.

\[ \max |\Delta C_{\text{RPA}_{i,j}}^{k(a)}| = \left| \frac{C_{\text{RPA}_{i,j}}^{k(a)} - C_{\text{RPA}_{i,j}}^{k(a-1)}}{C_{\text{RPA}_{i,j}}^{k(a)}} \right| < 10^{-3} \]  \hspace{1cm} (S16)
Numerical investigation on distribution formats of capture probe

To investigate the influence of capture probe distribution, we performed simulations by using different slope distributions of capture probes (e.g., \( k = \omega(Y) = 1.03 \times 10^{-7}, 7.33 \times 10^{-8}, 2.93 \times 10^{-8}, \) 0 mol/m² in Eq. (25)) to detect a series of inlet target HIV concentrations (e.g., \( C_{\text{A,in}} = 1, 5, 10, 50, \) and 100 nM) (Fig. S3a). With the same slope \( k \), the signal on the top surface \( S_{\text{top}} \) is directly proportional to the target HIV concentration, which presents an agreement with the observation when the target HIV concentration remains a relative low value (Fig. S3b). With a fixed \( C_{\text{A,in}} \), a larger \( k \) will produce a higher \( S_{\text{top}} \), because more capture probe immobilized near the top surface of test line can provide stronger capture capacity to form RPA. To give an intuitive explanation, we presented the distributions of amount of RPA in different layers for various slope distributions of capture probes (Fig. S3c). Obviously, the amount of RPA in the top layer (13th layer) is increased with an increasing slope \( k \). This result indicates that \( S_{\text{top}} \) depends on the amount of capture probe on the top layer. In addition, \( S \) at 13th layer is smaller than \( S \) at 12th layer. This is mainly because that a relative low velocity appears at the top boundary, resulting a small amount of reagents (A, PA) passing through the region above 13th layer and a reduced production of RPA.

Reference

Figure S1. Spatial discretization diagram
Figure S2. Solution procedure
Figure S3. Numerical investigation on the effects of capture probe’s distribution. (a) The capture probe distributions with various slope in thickness direction of T-line; (b) Comparison of the top signal intensity between different slopes of capture probe distributions; (c) The signal intensity in different layers under various slopes of capture probe distributions.