

Supplementary Information

Double Competitive Immunodetection of Small Analyte: Realization for Highly Sensitive Lateral Flow Immunoassay of Chlo-Ramphenicol

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$$\begin{aligned}
 \frac{d([P] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction1}).k2" \cdot [AP] - "(\text{reaction1}).k1" \cdot [A] \cdot [P]) \\
 \frac{d([A] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction1}).k2" \cdot [AP] - "(\text{reaction1}).k1" \cdot [P] \cdot [A]) \\
 \frac{d([AP] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction1}).k1" \cdot [A] \cdot [P] - "(\text{reaction1}).k2" \cdot [AP]) \\
 \frac{d([R] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction2}).k2" \cdot [PR] - "(\text{reaction2}).k1" \cdot [R] \cdot [P]) \cdot h \\
 \frac{d([PR] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction2}).k1" \cdot [R] \cdot [P] - "(\text{reaction2}).k2" \cdot [PR]) \cdot h \\
 h &= \begin{cases} \text{Time} < T, & 0 \\ \text{else,} & 1 \end{cases}
 \end{aligned}$$

A

$$\begin{aligned}
 \frac{d([A] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_1}).k2" \cdot [AP] - "(\text{reaction_1}).k1" \cdot [A] \cdot [P]) \\
 \frac{d([AP] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_1}).k1" \cdot [A] \cdot [P] - "(\text{reaction_1}).k2" \cdot [AP]) \\
 \frac{d([P] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_1}).k2" \cdot [AP] - "(\text{reaction_1}).k1" \cdot [A] \cdot [P] + "(\text{reaction_2}).k2" \cdot [PC] - "(\text{reaction_2}).k1" \cdot [P] \cdot [C] + "(\text{reaction_3}).k2" \cdot [PCP] - "(\text{reaction_3}).k1" \cdot [P] \cdot [PC]) \\
 \frac{d([C] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_2}).k2" \cdot [PC] - "(\text{reaction_2}).k1" \cdot [P] \cdot [C]) \\
 \frac{d([PC] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_2}).k1" \cdot [P] \cdot [C] - "(\text{reaction_2}).k2" \cdot [PC] - "(\text{reaction_3}).k1" \cdot [P] \cdot [PC] + "(\text{reaction_3}).k2" \cdot [PCP]) \\
 \frac{d([PCP] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_3}).k1" \cdot [P] \cdot [PC] - "(\text{reaction_3}).k2" \cdot [PCP]) \\
 \frac{d([AR] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_4}).k1" \cdot [A] \cdot [R] - "(\text{reaction_4}).k2" \cdot [AR]) \cdot h \\
 \frac{d([R] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_4}).k2" \cdot [AR] - "(\text{reaction_4}).k1" \cdot [A] \cdot [R] + "(\text{reaction_5}).k2" \cdot [CR] - "(\text{reaction_5}).k1" \cdot [C] \cdot [R] + "(\text{reaction_6}).k2" \cdot [PCR] - "(\text{reaction_6}).k1" \cdot [PC] \cdot [R]) \cdot h \\
 \frac{d([CR] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_5}).k1" \cdot [C] \cdot [R] - "(\text{reaction_5}).k2" \cdot [CR] + "(\text{reaction_7}).k2" \cdot [PCR] - "(\text{reaction_7}).k1" \cdot [P] \cdot [CR]) \cdot h \\
 \frac{d([PCR] \cdot V_{\text{compartment}})}{dt} &= V_{\text{compartment}} \cdot ("(\text{reaction_6}).k1" \cdot [PC] \cdot [R] - "(\text{reaction_6}).k2" \cdot [PCR] + "(\text{reaction_7}).k1" \cdot [P] \cdot [CR] - "(\text{reaction_7}).k2" \cdot [PCR]) \cdot h \\
 h &= \begin{cases} \text{Time} < T, & 0 \\ \text{else,} & 1 \end{cases}
 \end{aligned}$$

B

Figure S1. Differential equations systems for describing the kinetics of affine interactions for standard competitive LFIA (A) and proposed alternative scheme of competitive immunochromatography (B).

Species

#	Name	Compartment	Type	Unit	Initial Concentration [Unit]	Concentration [Unit]	Rate [Unit/s]	Reactions	Initial Expression [Unit]
1	B	compartment	ode	mmol/ml	1e-08	nan	nan	3	
2	BA	compartment	ode	mmol/ml	0	nan	nan	4	
3	A	compartment	ode	mmol/ml	1e-07	nan	nan	4	
4	ABA	compartment	ode	mmol/ml	0	nan	nan	1	
5	BP	compartment	ode	mmol/ml	0	nan	nan	4	
6	P	compartment	ode	mmol/ml	1e-08	nan	nan	4	
7	PBP	compartment	ode	mmol/ml	0	nan	nan	1	
8	ABP	compartment	ode	mmol/ml	0	nan	nan	2	
9	BR	compartment	ode	mmol/ml	0	nan	nan	3	
10	R	compartment	ode	mmol/ml	1e-08	nan	nan	3	
11	ABR	compartment	ode	mmol/ml	0	nan	nan	2	
12	PBR	compartment	ode	mmol/ml	0	nan	nan	2	

Reactions

#	Name	Reaction	Rate Law	Flux [mmol/s]	Noise Expression
1	reaction1	B + A = BA	Mass action (reversible)	nan	
2	reaction2	BA + A = ABA	Mass action (reversible)	nan	
3	reaction3	B + P = BP	Mass action (reversible)	nan	
4	reaction4	BP + P = PBP	Mass action (reversible)	nan	
5	reaction5	BA + P = ABP	Mass action (reversible)	nan	
6	reaction6	BP + A = ABP	Mass action (reversible)	nan	
7	reaction7	B + R = BR	Mass action (reversible)	nan	
8	reaction8	BR + A = ABR	Mass action (reversible)	nan	
9	reaction9	BR + P = PBR	Mass action (reversible)	nan	
10	reaction10	BA + R = ABR	Mass action (reversible)	nan	
11	reaction11	BP + R = PBR	Mass action (reversible)	nan	
	New Reaction				

Expression [Unit] or [Unit/s]
$(\text{reaction1}).k2*[BA] - (\text{reaction1}).k1*[A]*[B] + ((\text{reaction3}).k2*[BP] - (\text{reaction3}).k1*[B]*[P]) * \text{Values}[h1]$
$(\text{reaction1}).k1*[B]*[A] + (\text{reaction2}).k2*[ABA] - (\text{reaction1}).k2*[BA] - (\text{reaction2}).k1*[BA]*[A] + ((\text{reaction5}).k2*[ABP] - (\text{reaction5}).k1*[BA]*[P]) * \text{Values}[h1]$
$(\text{reaction1}).k2*[BA] + (\text{reaction2}).k2*[ABA] - (\text{reaction1}).k1*[B]*[A] - (\text{reaction2}).k1*[A]*[BA] + ((\text{reaction6}).k2*[ABP] - (\text{reaction6}).k1*[A]*[BP]) * \text{Values}[h1]$
$(\text{reaction2}).k1*[A]*[BA] - (\text{reaction2}).k2*[ABA]$
$((\text{reaction3}).k1*[B]*[P] + (\text{reaction4}).k2*[PBP] + (\text{reaction5}).k2*[ABP] - (\text{reaction3}).k2*[BP] - (\text{reaction4}).k1*[BP]*[P] - (\text{reaction6}).k1*[A]*[BP]) * \text{Values}[h1]$
$((\text{reaction3}).k2*[BP] + (\text{reaction4}).k2*[PBP] + (\text{reaction5}).k2*[ABP] - (\text{reaction3}).k1*[B]*[P] - (\text{reaction4}).k1*[BP]*[P] - (\text{reaction5}).k1*[BA]*[P]) * \text{Values}[h1]$
$((\text{reaction4}).k1*[P]*[BP] - (\text{reaction4}).k2*[PBP]) * \text{Values}[h1]$
$((\text{reaction5}).k1*[P]*[BA] + (\text{reaction6}).k1*[A]*[BP] - (\text{reaction5}).k2*[ABP] - (\text{reaction6}).k2*[ABP]) * \text{Values}[h1]$
$((\text{reaction7}).k1*[B]*[R] + (\text{reaction8}).k2*[ABR] + (\text{reaction9}).k2*[PBR] - (\text{reaction7}).k2*[BR] - (\text{reaction8}).k1*[BR]*[A] - (\text{reaction9}).k1*[P]*[BR]) * \text{Values}[h2]$
$((\text{reaction7}).k2*[BR] + (\text{reaction10}).k2*[ABR] + (\text{reaction11}).k2*[PBR] - (\text{reaction7}).k1*[B]*[R] - (\text{reaction10}).k1*[R]*[BA] - (\text{reaction11}).k1*[R]*[BP]) * \text{Values}[h2]$
$((\text{reaction8}).k1*[BR]*[A] + (\text{reaction10}).k1*[R]*[BA] - (\text{reaction8}).k2*[ABR] - (\text{reaction10}).k2*[ABR]) * \text{Values}[h2]$
$((\text{reaction9}).k1*[BR]*[P] + (\text{reaction11}).k1*[BP]*[R] - (\text{reaction9}).k2*[PBR] - (\text{reaction11}).k2*[PBR]) * \text{Values}[h2]$

Figure S2. Parameters of the proposed model of double competitive LFIA.