Electronic Supplementary Information (ESI) file

Continuous Variables Logic via Coupled Automata Using a DNAzyme Cascade with Feedback

S. Lilienthal[a], M. Klein[a], R. Orbach[a], I. Willner[a], F. Remacle[a,b], and R. D. Levine*[,a,c]

[a] Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
[b] Department of Chemistry, University of Liege, B6c, 4000 Liege, Belgium
[c] Department of Molecular and Medical Pharmacology, David Geffen School of Medicine and Department of Chemistry and Biochemistry University of California, Los Angeles, CA 90095

Table of Contents

Section I: The three layered DNAzymes cascade

Shown below as Equation (S1) is the kinetic scheme corresponding to Figure1 of the main text

$$
\left.\begin{aligned}
\frac{d[\mathrm{D_3}](t)}{dt} &= k_+[\mathrm{D_3H_2}](t) - k_-[\mathrm{D_3}](t)[\mathrm{H_2}](t) \\
\frac{d[\mathrm{D_3H_2}](t)}{dt} &= k_-[\mathrm{D_3}](t)[\mathrm{H_2}](t) - k_+[\mathrm{D_3H_2}](t)
\end{aligned}\right\} \text{3rd layer}
$$

$$
\left.\begin{aligned}
\frac{d[\mathrm{D_2}](t)}{dt} &= k_+[\mathrm{D_3H_2}](t) - k_-[\mathrm{D_2}](t)[\mathrm{H_1}](t) + k_+[\mathrm{D_2H_1}](t) \\
\frac{d[\mathrm{D_2H_1}](t)}{dt} &= k_-[\mathrm{D_2}](t)[\mathrm{H_1}](t) - k_+[\mathrm{D_2H_1}](t)
\end{aligned}\right\} \text{2nd layer} \qquad \text{(S1)}
$$

$$
\left.\begin{aligned}
\frac{d[\mathrm{D_1}](t)}{dt} &= k_+[\mathrm{D_2H_1}](t) - k_-[\mathrm{D_1}](t)[\mathrm{FQ}](t) + k_+[\mathrm{D_1FQ}](t) \\
\frac{d[\mathrm{D_1FQ}](t)}{dt} &= k_-[\mathrm{D_1}](t)[\mathrm{FQ}](t) - k_+[\mathrm{D_1FQ}](t)
\end{aligned}\right\} \text{1st layer}
$$

$$
\frac{d[\mathrm{F}](t)}{dt} = k_+[\mathrm{D_1FQ}](t) \quad \text{fluorescence}
$$

Section II: Solving analytically for one layer

We here solve the coupled kinetic equations for the first layer

$$\frac{d[\mathrm{D_1}](t)}{dt} = -k_-[\mathrm{D_1}](t)[\mathrm{FQ}](t) + k_+[\mathrm{D_1FQ}](t)$$

$$\frac{d[\mathrm{FQ}](t)}{dt} = -k_-[\mathrm{D_1}](t)[\mathrm{FQ}](t)$$

$$\frac{d[\mathrm{D_1FQ}](t)}{dt} = k_-[\mathrm{D_1}](t)[\mathrm{FQ}](t) - k_+[\mathrm{D_1FQ}](t) = -\frac{d[\mathrm{D_1}](t)}{dt}$$

$$\frac{d[\mathrm{F}](t)}{dt} = k_+[\mathrm{D_1FQ}](t)$$

We simplify the task by taking advantage of the conservation of the DNAzyme, meaning that $[\mathrm{D_1FQ}](t) + [\mathrm{D_1}](t) = const = [\mathrm{D_1}](0)$ and assuming that no initial $\mathrm{D_1FQ}$ is poresent.

Then the equation for the fluorescence becomes

$$\frac{d[\mathrm{F}](t)}{dt} = k_+[\mathrm{D_1FQ}](t) = k_+\left([\mathrm{D_1}](0) - [\mathrm{D_1}](t)\right)$$

Using the conservation $[\mathrm{D_1FQ}](t) = [\mathrm{D_1}](0) - [\mathrm{D_1}](t)$

$$\begin{aligned}\frac{d[\mathrm{D_1}](t)}{dt} &= -\left(k_-[\mathrm{FQ}](t)\right)[\mathrm{D_1}](t) + k_+[\mathrm{D_1FQ}](t) \\ &= -\left(k_-[\mathrm{FQ}](t)\right)[D_1](t) + k_+\left([\mathrm{D_1}](0) - [\mathrm{D_1}](t)\right) \\ &= -\left(k_-[\mathrm{FQ}](t) + k_+\right)\left([\mathrm{D_1}](t) - [\mathrm{D_1}](0)\right) - \left(k_-[\mathrm{FQ}](t)\right)[\mathrm{D_1}](0)\end{aligned}$$

$$\frac{d\left([\mathrm{D_1}](t) - [\mathrm{D_1}](0)\right)}{dt} = -\left(k_-[\mathrm{FQ}](t) + k_+\right)\left([\mathrm{D_1}](t) - [\mathrm{D_1}](0)\right) - \left(k_-[\mathrm{FQ}](t)\right)[\mathrm{D_1}](0)$$

If FQ is in great excess so that its concentration is effectively constant in time

$$\frac{d\left([\mathrm{D_1}](t) - [\mathrm{D_1}](0)\right)}{dt} = -\left(k_-[\mathrm{FQ}] + k_+\right)\left([\mathrm{D_1}](t) - [\mathrm{D_1}](0)\right) - \left(k_-[\mathrm{FQ}]\right)[\mathrm{D_1}](0)$$

We next define an auxiliary variable $y \equiv \left([\mathrm{D_1}](t) - [\mathrm{D_1}](0)\right)$, $y(t=0) = 0$ and use a well known mathematical trick for solving a linear non homogeneous differential equation $dy/dt = -ky + c$ with a constant c that is not time dependent. We define a new variable $\exp(kt)y(t)$ and rewrite the differential equation as

$$d\exp(kt)y/dt = k\exp(kt)y + \exp(kt)dy/dt$$
$$= k\exp(kt)y - k\exp(kt)y + \exp(kt)c$$
$$= \exp(kt)c$$

Integrating both sides from 0 to t

$$\exp(kt)y - y(0) = k^{-1}c\left(\exp(kt) - 1\right)$$
$$y(t) = k^{-1}c\left(1 - \exp(-kt)\right) + y(0)\exp(-kt)$$

$\frac{dy}{dt} = -\kappa y - \left(k_{-}[\mathrm{FQ}]\right)[\mathrm{D}_1](0)$ and using the preliminary results above

$$y = \left([\mathrm{D}_1](t) - [\mathrm{D}_1](0)\right) = \kappa^{-1}\left(k_{-}[\mathrm{FQ}]\right)[\mathrm{D}_1](0)\left(1 - \exp(-\kappa t)\right)$$

With the solution for $\left([\mathrm{D}_1](t) - [\mathrm{D}_1](0)\right)$ we continue to solve for $[F](t)$

$$\frac{d[F](t)}{dt} = k_{+}\left([D_1](0) - [D_1](t)\right) = -k_{+}y$$
$$[F](t) - [F](0) = -k_{+}\int_0^t y(t')dt'$$

Integrating y(t)

$$\int_0^t \left(1 - \exp(-\kappa t')\right)dt' = t + \kappa^{-1}\left(\exp(-\kappa t) - 1\right)$$

Final result:

$$[F](t) = [F](0) + k_{+}\kappa^{-1}\left(k_{-}[FQ]\right)[D_1](0)\left\{-\kappa^{-1}\left(1 - \exp(-\kappa t)\right)\right\}$$
$$= [F](0) + [D_1](0)x\kappa\left(t - \kappa^{-1}\left(1 - \exp(-\kappa t)\right)\right)$$
$$= [F](0) + [D_1](0)x\kappa t - [D_1](0)x\left(1 - \exp(-\kappa t)\right)$$

In the experiments reported here there is no initial fluorophore $[F](0) = 0$.

We then have that the fluorescence has a zero intercept at zero time and it is a sum of a transient term that decays exponentially with the rate κ, and a term $[D_1](0)x\kappa t$ that increases linearly with time. This is the term that gives rise to the observed linear rise of the signal with time. A key point is that the coefficient is $[D_1](0)$, the initial concentration of D_1. Thereby we have the linear term for equation (2) of the main text. It shows how the initial concentration determines the slope of the linear term.

An analytical solution for the model of two or three layers is also possible and the final results are quoted in Section III of this ESI

Section III: The results of solving analytically for the kinetics of two layers

When we include the second layer in the model the result is

$$\begin{aligned}\text{Fluorescence} &= [\mathrm{F}](t=0)+3[\mathrm{D}_2](t=0)x^2(1 - \exp(-\kappa t))-[\mathrm{D}_1](t=0)x(1 - \exp(-\kappa t))\\ &\quad([\mathrm{D}_1](t=0)-(2+\exp(-\kappa t))[\mathrm{D}_2](t=0))x\kappa t+\\ &\quad\frac{1}{2}[\mathrm{D}_2](t=0)x^2\kappa^2t^2\\ &\xrightarrow{\kappa t>1}[\mathrm{F}](t=0)+3[\mathrm{D}_2](t=0)x^2-[\mathrm{D}_1](t=0)x+\\ &\quad\left([\mathrm{D}_1](t=0)-2[\mathrm{D}_2](t=0)x\right)x\kappa t+\\ &\quad\frac{1}{2}[\mathrm{D}_2](t=0)x^2\kappa^2t^2\end{aligned} \tag{S2}$$

In the limit when x <<1 and for times longer than the induction period, $\kappa t > 1$, we have for the second layer the result shown in the main text,

$$\begin{aligned}\text{Fluorescence} &\xrightarrow{x<<1}[\mathrm{F}](t=0)+[\mathrm{D}_1](t=0)x\kappa t+\frac{1}{2}[\mathrm{D}_2](t=0)x^2\kappa^2t^2\\ &=\text{Fluorescence 1st layer}+\frac{1}{2}[\mathrm{D}_2](t=0)x^2\kappa^2t^2\end{aligned} \tag{S3}$$

Table S1: DNA sequences for the complete three layer system, Figure 1 of the main text.

Hairpin 1	GATATCAGCGATCCGGAACGGCACCCATGT TACTCTrAGGTTACTACGCTGATA
Hairpin 2	TAGTAACAGCGATCCGGAACGGCACCCATGT AGAGTArAGGACACAGGCTGTTACTA
Dz 1	GATATCAGCGATCCGGAACGGCACCCATGT TACTCT
Dz 2	TAGTAACAGCGATCCGGAACGGCACCCATGT AGAGTA
Dz 3	CTGTGTCAGCGATCCGGAACGGCACCCATGTTACTCT
FQ	/56-FAM/AGAGTATrAGGATATC/3IABkFQ/

Figure S1

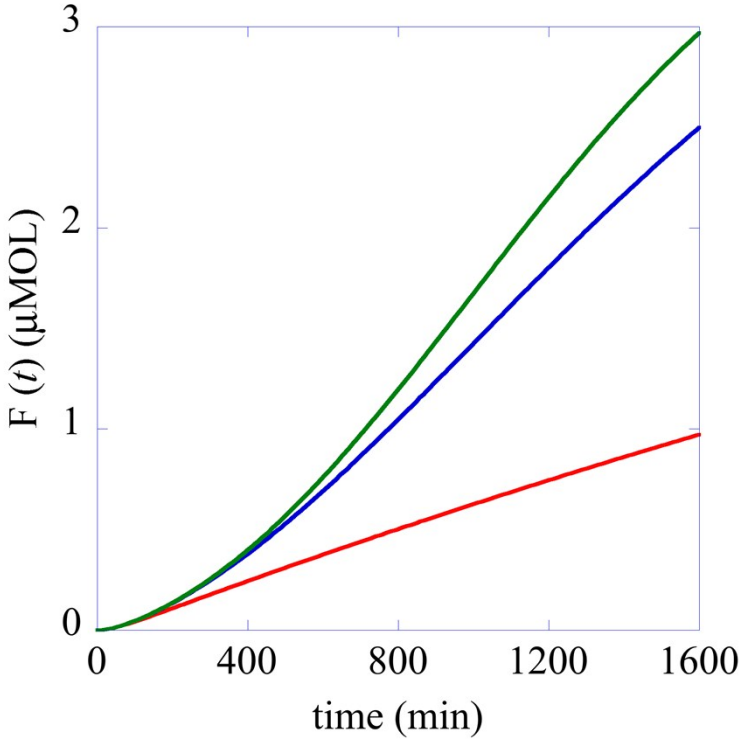


Figure S1. Results of a mumerical integration for the concentration of the fluorophore, [F(t)] in □MOL for the one layer system (red), two layer system (blue) and three layer system (green) obtained from the kinetic model (Eq. S1) using the values of the rate constants fitted to the experimental data (Table 1) and the experimental initial concentrations in substrate. The initial concentrations in all DNAzymes are non zero emphasize the capabilities of the experimental system to operate as an integrator outside of the limiting conditions of the analytical model. One layer initial concentrations (in μMOL): [D_1(t=0)]=0.33, [FQ(t = 0)] = 4. Two layer system: [D_1(t=0)]=0.33, [FQ(t = 0)] = 4, [D_2(t=0)] =0.33, [H_1(t = 0)] = 4. Three layer system: [D_1(t=0)]=0.33, [FQ(t = 0)] = 4, [D_2(t=0)] =0.33, [H_1(t = 0)] = 4, [D_3(t=0)] =0.66, [H_2(t = 0)] = 4.

Figure S2. Stepwise integration a-la Euler of three concatenated AND gates. A(0) is the input and the concentrations are shown in time steps of a small increment δt

$$\frac{d[C]}{dt} = [A]$$
$$\frac{d[E]}{dt} = [C]$$
$$\frac{d[P]}{dt} = [E]$$

	A	C	E	P
0	A(0)	0	0	0
δt	A(0)	A(0)	0	0
2δt	A(0)	2A(0)	A(0)	0
3δt	A(0)	3A(0)	3A(0)	A0
4δt	A(0)	4A(0)	6A(0)	4A(0)
5δt	A(0)	5A(0)	10A(0)	10A(0)
6δt	A(0)	6A(0)	15A(0)	20A(0)
7δt	A(0)	7A(0)	21A(0)	35A(0)

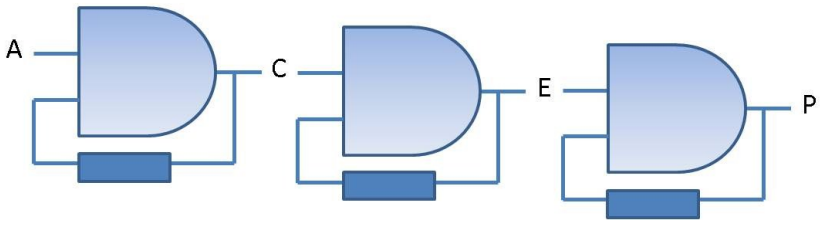


Figure S3. An integrator in the notation of electronic circuits

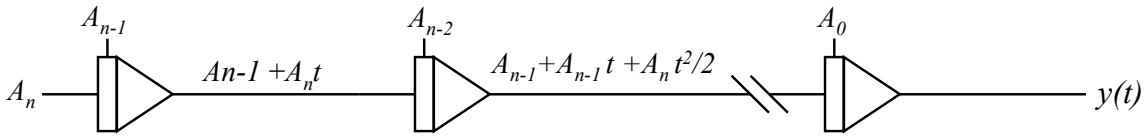
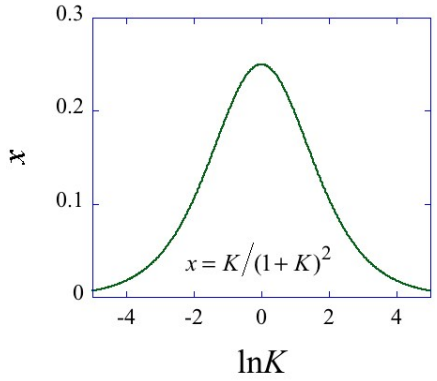


Figure S4. The reduced variable x as a function of the dimensionless constant $K = k_{-}\left[\text{substrate}\right]/k_{+}$. For best results one needs to operate at a value of x that is well below 1.



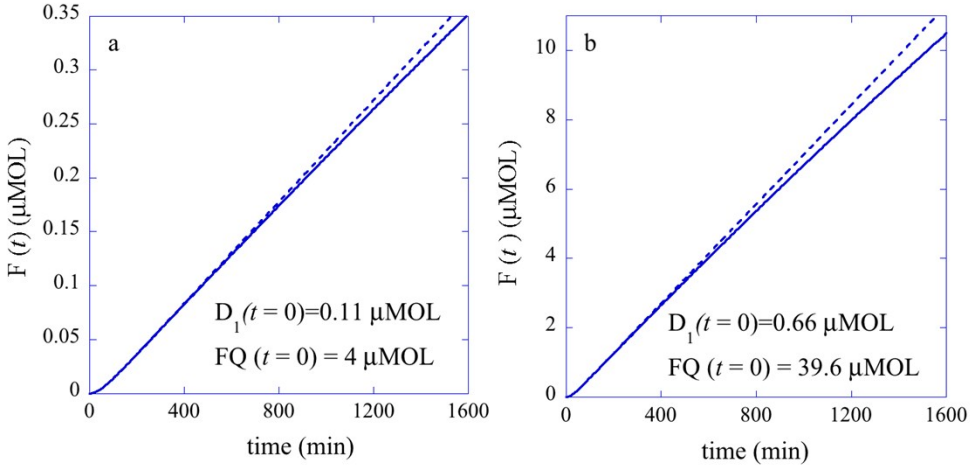


Figure S5. Comparison between the numerical solution of the kinetic scheme (full lines) and the solution of the analytical model, dashed line, for a one layer system. The values of the rate constants are the values obtained from the fit of the one layer experimental fluorescence reported in Table I. In panel a) the initial concentrations in DNAzyme is divided by 6 and that of the substrate is left unchanged compared to the experimental values : $[D_1(t=0)]=0.11$, $[FQ(t = 0)] = 4$. In Panel b), the initial concentration in DNAzyme is left unchanged and that of the substrate is multiplied by 6. $[D_1(t=0)]=0.66$, $[FQ(t = 0)] = 39.6$. Concentrations are given in µMOL. Both cases correspond to an excess of substrate but two different values of the κ (Eq. (3) of the main text) and x parameters (Eq. (4) of the main text) which allow to asses the validity of the analytical solution. In panel a), the values of x and κ are the same as those of the fit to the experimental result since the two parameters do not depend on the initial concentration of DNAzymes : x=0.1 and κ = 0.0224. In panel b) κ = 0.04376, x = 0.25. The numerical solution in panel b) deviates more from the analytical one because the values of the κ and x parameters are further away from their optimal values. See also Equation (5) of the main text and section II above.

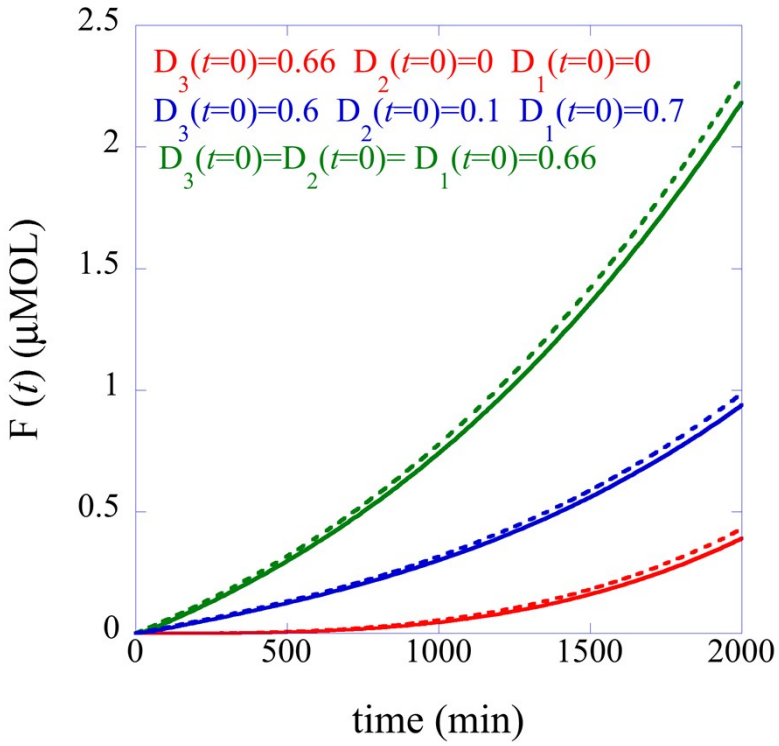


Figure S6. Numerical solutions (solid line) and analytical solutions (dashes) for a 3 layer system that obeys of the conditions of validity of the analytical model. The numerical integration of the non-linear rate equations was done using a Runge-Kutta fifth order scheme. Each layer has the same set of rate constants, k_{-} = 6 MOL^{-1} min^{-1} and k_{+}=0.05 min^{-1}, the concentration of substrate for each layer is 133.2 μMOL, which leads to x = 0.015 and κ□□□□□□□□. x <<1, which leads to a good agreement between the analytical model (Eqs. (7)-(9)) and the results of the numerical integration of the kinetic scheme. The different computations correspond to different sets of initial concentrations of DNAzymes, as indicated in the figure. In this regime of parameters, the initial concentrations in DNAzymes fully control the values of the coefficients of the polynomial as in equation (2) of the main text.

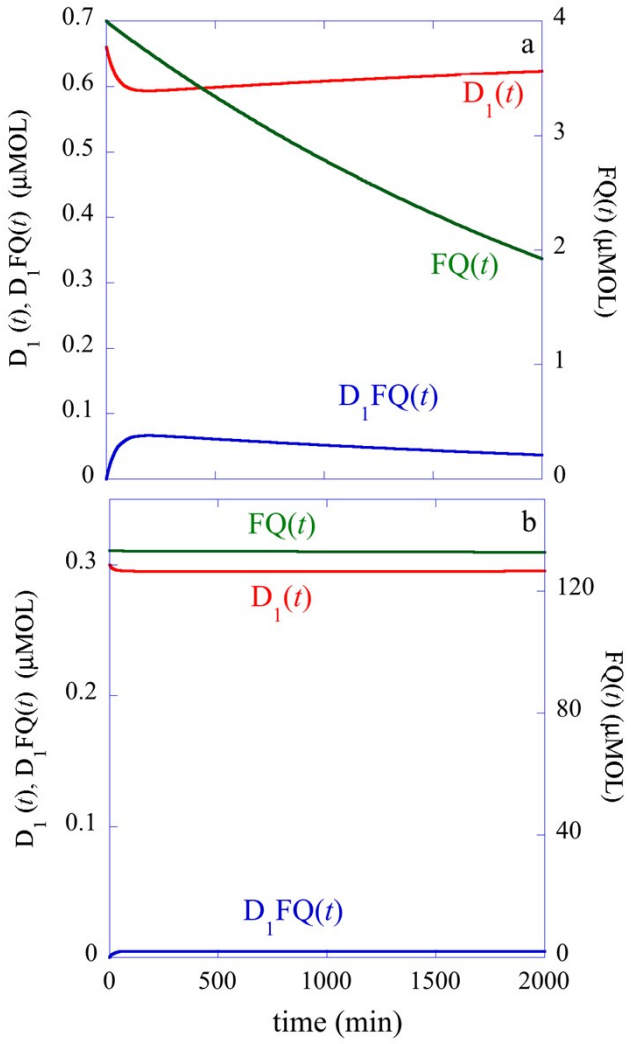


Figure S7. The 'steady state' where the concentration of the DNAzyme and the adduct settle to a constant under two different set of conditions for one layer. (a) Using the rate constants fitted to the experimental data of one layer and with the experimental initial concentration. The substrate FQ, right ordinate, is not in great excess compared to $[D_1(t=0)]$. It is therefore depleted by the reaction and while the feedback strives to keep $[D_1(t)]$ constant in time, it succeeds but only approximately. (b) Same conditions as in Figure S6. $[FQ(t=0)]$ is high enough that the DNAzyme reaches a steady state and the output of the integrator is strictly linear in time.

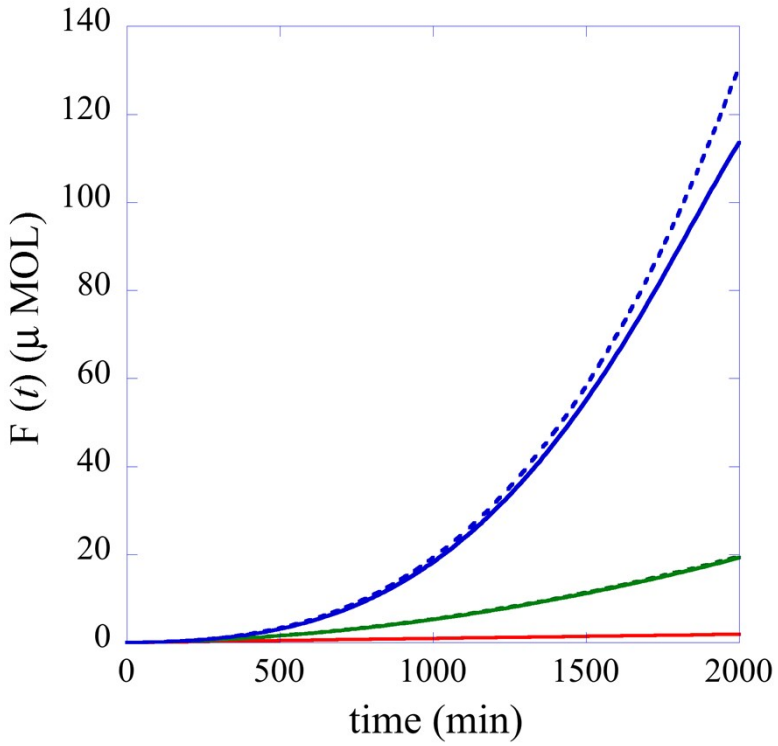


Figure S8. The model is accurate when K >1. Flourescence computed by numerical integration, solid lines, and by the model, dashed lines, for K = 15.96. $k_{\_}$ =1200 min^{-1} MOL^{-1} , k_{+} =10^{-2} min^{-1}. κ = 0.169 and x =$5.5 \cdot 10^{-2}$. The initial concentration of substrate for each layer is 133.2 μMOL and the initial concentration of the DNAzyme for each layer is 0.1 μMOL.