# Linear-quadratic rational polynomial families versus Hill in an analysis of human thymidine kinase 1 data

Tomas Radivoyevitch*

Department of Epidemiology and Biostatistics

Case Western Reserve University, Cleveland, Ohio 44106 USA

*corresponding author

Email addresses:
TR: txr24@case.edu

# Abstract

### Background

The Hill reaction rate model is useful because it is flexible enough to represent many reactions and because it characterizes the extent to which biochemical reactions exhibit cooperativity. Its main disadvantage is that it cannot discriminate between cooperativity mediated by changes in enzyme activity versus substrate binding affinity.

### Results

The linear-quadratic rational polynomial (LQRP) reaction rate models explored here assume: (1) that binding of enzyme monomers to form dimers is well approximated as being infinitely tight at the enzyme concentrations of the data, (2) that the behavior of any higher order enzyme structures (if they exist) is dominated by the behavior of dimers within such structures, and (3) that the enzyme concentrations are so low relative to the lowest substrate concentration that free substrate concentrations approximately equal total substrate concentrations. In addition to these assumptions, hypotheses of dissociation constants $K$ being equal to each other or infinitely large, and hypotheses of enzyme activities $k$ being equal to each other or zero, yields a family of $k$-$K$ LQRP models. These $k$-$K$ LQRPs map, typically many-to-one, into data fitting equivalent canonical LQRPs. The canonical LQRPs were fitted to human thymidine kinase 1 data. Predictions of the top models (ranked by the Akaike Information Criterion) suggest subsequent experiments to distinguish TK1 cooperativity mechanisms mediated primarily by changes in $k$, $K$ or both $k$ and $K$.

### Conclusions

Compared to single model fits of the empirical Hill model, fits of families of rational polynomials, such as those presented here, are preferable because they yield predictions that can guide subsequent experiments to elucidate mechanisms.

## Background

The empirical Hill model of reaction fluxes $v$ as a function of substrate concentrations $S$ is

$$v = \frac{V_{\max}\left(\frac{S}{S_{50}}\right)^n}{1+\left(\frac{S}{S_{50}}\right)^n} \quad (1)$$

where $V_{\max}$ is the maximum flux, $S_{50}$ is the substrate concentration at which the flux is half maximum, and $n$ is greater than 1 for positive cooperativity and less than 1 for negative cooperativity. This model is useful because it is flexible enough to represent many reactions, and because it characterizes the extent to which biochemical reactions exhibit cooperativity. Its disadvantage is that it cannot discriminate between cooperativity mediated by changes in enzyme activity versus cooperativity mediated by changes in substrate binding affinity.

The linear-quadratic rational polynomial (LQRP) models explored here assume: (1) that binding of enzyme monomers to form enzyme dimers is approximately infinitely tight across the enzyme concentrations of interest, (2) that the behavior of any higher order enzyme structures (if they exist) is dominated by the behavior of dimers within such structures, and (3) that enzyme concentrations are so low relative to the lowest substrate concentration that free substrate concentrations approximately equal total substrate concentrations. Added to these assumptions are hypotheses of various dissociation constants $K$ being equal to each other or being infinitely large, and hypotheses of enzyme complex activities $k$ being equal to each other or being well approximated as zero. These hypotheses generate a family of $k$-$K$ LQRP models/hypotheses (see Table 1) which map, typically many-to-one, into data fitting equivalent canonical $V$-α LQRPs (e.g. Eqs. 3-9 below). This paper explores the fitting of $V$-α LQRP models first to simulated Hill data with Hill coefficients of .4, .7, 1.3, and 1.6, and then to published human thymidine kinase 1 data [1-5].

# Results

### The LQRP model family

LQRP hypotheses/models were created as parameter constraints applied to the model

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}} [E_0] \quad (2)$$

where $k_i$ and $K_i$ are the activity and dissociation constants of the *i*th potentially active enzyme complex in Figure 1 and $[E_0]$ is the total concentration of enzyme *dimers*. Because Eq. (2) has six parameters where at most four can be identified (see Eq. 3 below), it follows that at least two parameters must be eliminated from Eq. (2) by parameter constraints before fitting the resulting model to a dataset. This leads to many models. Indeed, 37 *k*-*K* LQRPs (Table 1) arise from Eq. (2) without the use of $k_i = 0$ constraints (these will be dealt with later). These *k*-*K* LQRPs can be grouped into 7 data fitting equivalence classes that can be represented by the following 7 canonical *V*-α LQRPs:

4-parameter model

$$v = \frac{V_1 S + V_\infty \alpha_2 S^2}{1 + 2\alpha_1 S + \alpha_2 S^2} \quad (3)$$

3-parameter models

$$v = \frac{V_\infty \left(\alpha_1 S + \alpha_2 S^2\right)}{1 + 2\alpha_1 S + \alpha_2 S^2} \quad (4)$$

$$v = \frac{V_1 S + V_\infty \alpha_1^2 S^2}{\left(1 + \alpha_1 S\right)^2} \quad (5)$$

$$v = \frac{V_1 S + V_\infty \alpha_1^2 S^2}{1 + \alpha_1 S + \alpha_1^2 S^2} \quad (6)$$

2-parameter models

$$v = \frac{V_\infty \left(.5\alpha_1 S + \alpha_1^2 S^2\right)}{1 + \alpha_1 S + \alpha_1^2 S^2} \quad (7)$$

$$v = \frac{V_\infty \alpha_1 S}{1 + \alpha_1 S} \quad (8)$$

$$v = \frac{V_\infty \alpha_1 S^2}{1 + \alpha_1 S^2} \qquad (9)$$

where $V_\infty$ is the flux in the limit of large $S$. Examples of derivations of these equations follow:

$K_1 = K_2 \neq K_3$ and $k_1 = k_2 \neq k_3$ (row 1 in Table 1) yields Eq. (3)

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}} [E_0] = \frac{2k_1 \frac{S}{K_1} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + 2\frac{S}{K_1} + \frac{S^2}{K_1 K_3}} [E_0] \qquad (10)$$

if $V_1 = 2k_1[\mathrm{E}_0]/K_1$, $V_\infty = 2k_3[\mathrm{E}_0]$, $\alpha_1 = 1/K_1$, and $\alpha_2 = 1/(K_1 K_3)$. Rows in Table 1 that map to Eq. (3) represent mechanisms in which cooperativity is mediated by changes in both $k$ and $K$, save two (rows 11 and 12) that assume equalities across the $k$ if the condition $2k_3 = k_1 + k_2$ is classified as such (and it is here). One of these two rows is the case of a rigid asymmetric dimer defined by $K_1 \neq K_2 = K_3$ and $2k_3 = k_1 + k_2$ (row 12). This case reduces to a model of a mixture of two independent monomer enzymes that use the same substrate as follows

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}} [E_0] = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + (k_1 + k_2) \frac{S^2}{K_1 K_2}}{\left(1 + \frac{S}{K_1}\right)\left(1 + \frac{S}{K_2}\right)} [E_0]$$

$$= \frac{k_1 \frac{S}{K_1}\left(1 + \frac{S}{K_2}\right) + k_2 \frac{S}{K_2}\left(1 + \frac{S}{K_1}\right)}{\left(1 + \frac{S}{K_1}\right)\left(1 + \frac{S}{K_2}\right)} [E_0] = \left( \frac{k_1 \frac{S}{K_1}}{\left(1 + \frac{S}{K_1}\right)} + \frac{k_2 \frac{S}{K_2}}{\left(1 + \frac{S}{K_2}\right)} \right] [E_0] = \frac{V_{m1} \frac{S}{K_1}}{\left(1 + \frac{S}{K_1}\right)} + \frac{V_{m2} \frac{S}{K_2}}{\left(1 + \frac{S}{K_2}\right)} \qquad (11).$$

Since substrate will always bind the site with the tighter of $K_1$ and $K_2$ first, positive cooperativity cannot be represented by this model, though negative cooperativity can. Rewriting Eq. (11) as

$$v = \frac{\left(\frac{k_1}{K_1} + \frac{k_2}{K_2}\right) S + (k_1 + k_2) \frac{S^2}{K_1 K_2}}{1 + \left(\frac{1}{K_1} + \frac{1}{K_2}\right) S + \frac{S^2}{K_1 K_2}} [E_0] = \frac{V_1 S + V_\infty \alpha_2 S^2}{1 + 2\alpha_1 S + \alpha_2 S^2} \qquad (11b)$$

shows that, although $\alpha_1$ and $\alpha_2$ are free to be any real coefficients in Eq. (3), they are restricted in (11b) to yield real roots. Further, $\alpha_1$ cannot increase or decrease without increasing or decreasing $\alpha_2$, and *vice-versa*, and if $k_1 + k_2$ increases or decreases to increase or decrease $V_\infty$, so too must $V_1 = \frac{k_1}{K_1} + \frac{k_2}{K_2}$ also increase or decrease, and *vice-versa*. Thus, when forced into the structure of Eq. (3), we see that Eq. (11) is a subset of Eq. (3), though both are four parameter models. Since Eq. (3) can fit any dataset as well as Eq. (11), but it can also fit positive cooperativity data which Eq. (11) cannot, it follows that Eq. (3) will always tie (if $n < 1$) or do better than (if $n > 1$) Eq. (11), i.e. Eq. (11) does not create an additional *V-α* model since *v-S* data cannot discriminate it from Eq. (3) *k-K* models/hypotheses.

$K_1 = K_2 \neq K_3$ and $k_1 + k_2 = 2k_3$ (row 10) yields Eq. (4)

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}}[E_0] = \frac{2k_3\left(\frac{S}{K_1} + \frac{S^2}{K_1 K_3}\right)}{1 + 2\frac{S}{K_1} + \frac{S^2}{K_1 K_3}}[E_0] = \frac{V_\infty\left(\alpha_1 S + \alpha_2 S^2\right)}{1 + 2\alpha_1 S + \alpha_2 S^2} \quad (12).$$

Further, all of the other Eq. (4) rows in Table 1 are also consistent with cooperativity being mediated by inequalities in *K*'s rather than *k*'s.

$K_1 = K_2 = K_3$ in rows 17 to 20 yield Eq. (5)

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}}[E_0] = \frac{\left(k_1 + k_2\right)\frac{2S}{K_1} + 2k_3 \frac{S^2}{K_1^2}}{1 + 2\frac{S}{K_1} + \frac{S^2}{K_1^2}}[E_0] = \frac{V_1 S + V_\infty \alpha_1^2 S^2}{1 + 2\alpha_1 S + \alpha_1^2 S^2} = \frac{V_1 S + V_\infty \alpha_1^2 S^2}{\left(1 + \alpha_1 S\right)^2} \quad (13).$$

These are *k* cooperativity mechanisms.

$K_1 = K_3$ and $K_2 = \infty$ and $k_1 \neq k_3$ (row 28) maps to Eq. (6)

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}}[E_0] = \frac{k_1 \frac{S}{K_1} + 2k_3 \frac{S^2}{K_1^2}}{1 + \frac{S}{K_1} + \frac{S^2}{K_1^2}}[E_0] = \frac{V_1 S + V_\infty \alpha_1^2 S^2}{1 + \alpha_1 S + \alpha_1^2 S^2} \quad (14)$$

and is an example of a mixed *k*-*K* cooperativity mechanism.

$K_1 = K_3$ and $K_2 = \infty$ and $k_1 = k_3$ (row 29), a *K* cooperativity mechanism, maps to Eq. (7) as

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}}[E_0] = \frac{2k_3\left(.5\frac{S}{K_1} + \frac{S^2}{K_1^2}\right)}{1 + \frac{S}{K_1} + \frac{S^2}{K_1^2}}[E_0] = \frac{V_\infty\left(.5\alpha_1 S + \alpha_1^2 S^2\right)}{1 + \alpha_1 S + \alpha_1^2 S^2} \quad (15)\,.$$

$k_1 = k_2 = k_3$ and $K_1 = K_2 = K_3$ (row 21) yields Eq. (8)

$$v = \frac{k_3\left(2\frac{S}{K_1} + 2\frac{S^2}{K_1^2}\right)}{1 + 2\frac{S}{K_1} + \frac{S^2}{K_1^2}}[E_0] = \frac{k_3 2\frac{S}{K_1}\left(1 + \frac{S}{K_1}\right)}{\left(1 + \frac{S}{K_1}\right)\left(1 + \frac{S}{K_1}\right)}[E_0] = \frac{k_3 2\frac{S}{K_1}}{\left(1 + \frac{S}{K_1}\right)}[E_0] = \frac{V_\infty \alpha_1 S}{1 + \alpha_1 S} \quad (16)$$

or the Hill model in Eq. (1) with $n = 1$, i.e. the Michaelis-Menten model. The Eq. (8) rows of 30, 31 and 32 in Table 1 prove that inequalities in *K*'s and *k*'s do not imply cooperativity.

$K_1 = \infty$, $K_2 = \infty$ and $K_1 K_3$ finite (row 34) yields Eq. (9)

$$v = \frac{k_1 \frac{S}{K_1} + k_2 \frac{S}{K_2} + 2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S}{K_1} + \frac{S}{K_2} + \frac{S^2}{K_1 K_3}}[E_0] = \frac{2k_3 \frac{S^2}{K_1 K_3}}{1 + \frac{S^2}{K_1 K_3}}[E_0] = \frac{V_\infty \alpha_2 S^2}{1 + \alpha_2 S^2} \quad (17)$$

or the Hill model in Eq. (1) with $n = 2$, $S_{50} = 1/\sqrt{\alpha_2} = \sqrt{K_1 K_3}$ , and $V_{max} = V_\infty = 2k_3\,[E_0]$. This model hypothesizes that although $K_1$ and $K_2$ are so large that single substrate enzyme dimers cannot be detected above noise, $K_3$ is tight enough that $K_1 K_3$ products must be treated as

being finite, i.e. it hypothesizes that the presence of substrate saturated dimers can be detected. This equation represents a *K* cooperativity mechanism because the values of $k_1$ and $k_2$ can be anything and can thus be thought of as being equal to $k_3$.

Other *V*-$\alpha$ LQRPs arise when $k_i = 0$ hypotheses are applied to Table 1 rows as follows:

*Eq. (3) derivates*

$k_3 = 0$ applied to any of the Eq. (3) rows of Table 1 yields

$$v = \frac{V_1 S}{1 + 2\alpha_1 S + \alpha_2 S^2} \qquad (18)$$

which, along with Eqs. (24) and (27), is distinguished by its drops back to zero at large *S*.

$k_3 \neq 0$, $k_1 = 0$ and $k_2 = 0$ applied to the Eq. (3) rows in Table 1 in which neither $k_1$ nor $k_2$ are constrained to equal $k_3$ yields

$$v = \frac{V_\infty \alpha_2 S^2}{1 + 2\alpha_1 S + \alpha_2 S^2} \qquad (19), \text{ and}$$

$k_3 \neq 0$ and $k_1 = 0$ or $k_2 = 0$ in Eq. (3) rows where one of $k_1$ and $k_2$ is constrained to equal $k_3$ yields, via rows 2 and 3,

$$v = \frac{V_\infty (.5\alpha_1 S + \alpha_2 S^2)}{1 + 2\alpha_1 S + \alpha_2 S^2} \qquad (20),$$

via row 5,

$$v = \frac{V_\infty (.5\sqrt{\alpha_2} S + \alpha_2 S^2)}{1 + 2\alpha_1 S + \alpha_2 S^2} \qquad (21),$$

via row 6,

$$v = \frac{V_\infty (.5\alpha_1 S + \alpha_2 S^2)}{1 + (\alpha_1 + \sqrt{\alpha_2}) S + \alpha_2 S^2} \qquad (22),$$

and via rows 8 and 9,

$$v = \frac{V_\infty(.5\alpha_1 S + \alpha_1\alpha_2 S^2)}{1 + (\alpha_1 + \alpha_2)S + \alpha_1\alpha_2 S^2} \qquad (23)\,.$$

*Eq. (4) derivates*

In each of the Eq. (4) models in Table 1, $k_1$ and $k_2$ are constrained to either equal $k_3$ or sum to $2k_3$, and since $k_3 \neq 0$, else the rate law trivially vanishes, no new LQRP's are formed.

*Eq. (5) derivates*

If $k_3 = 0$ these rows yield

$$v = \frac{V_1 S}{(1 + \alpha_1 S)^2} \qquad (24)\,,$$

else, row 18 yields

$$v = \frac{V_\infty \alpha_1^2 S^2}{(1 + \alpha_1 S)^2} \qquad (25)\,,$$

rows 19 and 20 yield

$$v = \frac{.5V_\infty \alpha_1 S + V_\infty \alpha_1^2 S^2}{(1 + \alpha_1 S)^2} \qquad (26)\,,$$

and row 17 reproduces Eq. (5).

*Eq. (6) derivates*

With $k_3 = 0$ row 28 creates

$$v = \frac{V_1 S}{1 + \alpha_1 S + \alpha_1^2 S^2} \qquad (27)$$

and with $k_1 = 0$ it creates

$$v = \frac{V_\infty \alpha_1^2 S^2}{1 + \alpha_1 S + \alpha_1^2 S^2} \qquad (28)\,.$$

*Eq. (7), Eq. (8) and Eq. (9) derivates*

When $k$ =0 hypotheses are applied to these Table 1 rows the models either collapse to $v = 0$ or remain unchanged by either a factor of 1 or 2 being absorbed into $V_\infty$. Thus, no additional *V*-$\alpha$ LQRP models are formed.

**LQRP versus Hill model with n = .4, .7, 1.3 and 1.6**

Hill models with $V_{max} = 1$, $S_{50} = 1$ μM, and $n$ = (.4, .7, 1.3, and 1.6) were simulated with 1% added noise at 100 logarithmically spaced $S$ values between .1 and 10 μM. The resulting "data" was then fitted to Eqs (3-9) and Eqs (18-28) to yield the plots in Figure 2 and the parameter estimates in Table 2 (reproducible by the R [6] script in Additional File 1). Using the Akaike Information Criterion (AIC) [7] and a $\Delta AIC_c$ (see Methods) cut-off of 15 for model rejection [8] (models not rejected have asterisks in the legend in Fig. 2), Eqs. (3) and (4) fit the hill data well at all four $n$ values, and Hill coefficients closer to 1 (.7 and 1.3) were fitted well by more LQRPs than Hill coefficients further from 1 (.4 and 1.6).

**LQRP fits to the Birringer et al human thymidine kinase 1 data**

The complete set of *V*-α LQRP models was fitted to recent human thymidine kinase 1 data of Birringer et al. [1] (Table 8 and Additional File 2). The fits are shown in Fig. 3 (which can be regenerated by Additional File 3). With models ranked by $AIC_c$, Table 3 shows that the Hill model was far from being selected as the best model. The top model, Eq. (25), is consistent with $k_1 = k_2 = 0$ and $K_1 = K_2 = K_3$ (Table 1, row 18), i.e. an extreme case of positive cooperativity mediated by changes in $k$ (since $k_3 > 0$) rather than changes in $K$. This *k*-*K* LQRP is

$$v = \frac{2k_3 \frac{S^2}{K_1^2}}{1 + 2\frac{S}{K_1} + \frac{S^2}{K_1^2}} 30 \times 153 \times 10^{-6} \qquad (29)$$

where $[E_0]$=153 pM [1] (306 pM was the monomer concentration) and the reaction vessel volume was 30 µL [1]. Upon fitting Eq. (29) to this data the parameter estimates, upon conversion to seconds, were $k_3$ = 0.2746 (0.2545333, 0.2962) $sec^{-1}$ and $K_1$ = 0.266 (0.207, 0.337) µM (95% confidence intervals are shown in parentheses, see Additional File 4). Meanwhile, the Hill parameter estimates of the original data analysis were $k_{cat}$=.28/sec, $n$=1.3, and $S_{50}$ =.51 µM [1]. These activities are low relative to maximum velocities on the order of 10 µmole/min/mg found by Munch-Peterson's group [2, 3, 5] and others [4]. Since 10 µmoles per minute is 167 nmoles/sec, and since 1 mg of protein is 1mg/($25x10^6$ mg/mole) = 40 nmoles, 10 µmoles/min/mg is roughly 4 turnovers per second, i.e. the activity in Birringer et al. is roughly 16-fold lower than expected based on earlier studies.

The second best fitting model (Eq. 28) proposes $k_1$ is zero and that the value of $k_2$ does not matter since $K_2 = \infty$. Thus, this model is consistent with cooperativity being mediated by both activity changes and affinity changes. The third best model (Eq. 7) proposes that $k_1$ is the same as $k_3$ and that $k_2$ does not matter since $K_2 = \infty$, i.e. it proposes affinity changes as the mechanism. Finally, the fourth best model is Eq. (26) which derives from rows 19 and 20 in Table 1 which claim that $k_1$ or $k_2$ is zero while $k_3$ is not, and that the $K$ are all the same, i.e. it too supports changes in $k$ as the mechanism of cooperativity. Thus, the top 4 models place 2.5 votes in favor of activity changes and 1.5 votes in favor of binding affinity changes. The 5th best model, the Michaelis-Menten model (Eq. 8), is disfavored by $\Delta AIC_c > 3.5$. Of the $\Delta AIC_c$ gap of ~4 that follows this two parameter model, ~2 is due to the small sample correction term (see Methods) and 2 is due to an additional parameter (the 6th to 10th models

are all three parameter models). This suggests that if three parameter models are to compete, additional data is needed to increase the sum of squared errors component of the $AIC_c$ and thus decrease the relative importance of parameter numbers.

### LQRP fits to other human thymidine kinase 1 data

Tables 4 to 7 show the results of fitting the LQRPs to other data [2-5]. Strikingly, the top 4 models of the dataset of Frederiksen et al.[2] (Table 4) are the same as those for Birringer et al. [1]. One of these top 4 models (Eq. 7) is also the best model of the Berenstein et al. [3] data (Table 5). The data of Li et al. [4] is different in that the Michaelis-Menten model wins (the Hill coefficient was 1.01, see Table 6), and the data of Munch-Petersen et al. [5] is different in that the 4 parameter model of Eq. (3) (see Table 7) is its clear winner.

### Joint LQRP fits to human thymidine kinase 1 data

Datasets with Hill coefficients of 1.2 to 1.3 [1-3, 5] (i.e. excluding the non-cooperative data of Li et al.) were amplitude normalized by dividing their fluxes $v$ by $V_{max}$ of their Hill model fits (see Additional File 5). These datasets were then fitted jointly to Eqs (3)-(9) and Eqs (18)-(28). Eq. (7) won this competition, and oddly, the Hill model came in second place with a $\Delta AIC_c$ of ~2 (not shown). If the Hill model approximates an average of more than one LQRP, this result could indicate that the datasets are too different to merge. The 1993 dataset [5] was thus removed. The result was approximately the same, i.e. Eq. (7) won and the Hill model came in second. The 2000 dataset [3] was then also removed, leaving just the merged normalized data of Birringer et al. [1] and Frederiksen et al. [2]. The Hill model then fell out of the competition and Eq. (25) came in first place and Eq. (7) in second (with a $\Delta AIC_c$ of ~2.5). These top two models differ in their predictions the most at thymidine concentrations greater than 100 μM and between .2 and .5 μM (see Fig. 4 and Additional File 6). Subsequent experiments should thus be performed at these concentrations.

## Discussion

Since there are 7 Eq. (8) rows in Table 1, the Michaelis-Menten model does not suggest a mechanism. Similarly, Eq. (3) appears in 12 rows in Table 1, so its victory in fitting the data of Munch-Petersen et al. [5] also leaves the underlying *k-K* model/mechanism undefined. In contrast, Eq. (7) appears in Table 1 only once, and Eq. (25) is strictly a derivative of row 18, so when one of these *V-α* LQRP models is the best, a specific *k-K* hypothesis is supported.

It should be clear that LQRPs cannot represent datasets with Hill coefficients greater than 2 and that, to handle datasets with Hill coefficients up to 4, Fig. 1 needs to be replaced by a tetramer model. The number of models/hypotheses then becomes large. A need for automated *k-K* model enumeration approaches is therefore anticipated.

## Conclusions

Compared to single model fits of the empirical Hill model, fits of families of rational polynomials such as those presented here are preferable because they yield predictions that can guide subsequent experiments to elucidate mechanisms.

# Methods

### Data

All of the datasets were digitized using plotDigitizer [9]. The ATP concentrations in all of the datasets was 2.5 mM or higher (see Table 8) and at such concentrations TK1 is a tetramer [5]. It is thus assumed that such tetramers are loose dimers of tight dimers such that interactions within dimers dominate interactions between dimers. This notion has some support [10]. The highest enzyme concentration in any of the datasets was that of Birringer et al at 306 pM, and the lowest substrate concentration appears to have been 50 nM. Given the large gap between these concentrations, it is safe to assume that total substrate concentrations approximately equal free substrate concentrations; if this were not the case, total concentration constraint polynomials in the free concentrations [11] could instead have been used to model the data.

### Model selection

The Akaike Information Criterion (AIC) is $2*P$ – 2*log-likelihood [7] which, in the case of normally distributed errors, is $AIC = 2*P + N*\log(2\pi) + N*\log(SSE/N) + N$ where $P$ is the number of estimated parameters (including the variance), $N$ is the number of data points and SSE is the sum of squared errors. The small sample size corrected $AIC_c = AIC + 2*P(P+1)/(N-P-1)$ was used for model comparisons, as recommended in [8], as this correction is significant at the small sample size of the data in Fig. 3 (in contrast, this correction is negligible at the $N = 100$ sample size of the simulated data of Fig. 2). $\Delta AIC_c$ is the difference between the $AIC_c$ of a particular model and the best/lowest $AIC_c$ of all models tested.

### Parameter Estimates

The 95% confidence intervals of the parameter estimates of Eq. (29) were obtained using `nls` and `confint` of the statistical programming environment R [6], see Additional File 4.

## Competing interests

TR has no competing interests.

## Authors' contributions

TR performed the work and wrote the paper.

## Acknowledgements

This work was supported by the National Cancer Institute under grant K25 CA104791.

## References

1. Birringer MS, Perozzo R, Kut E, Stillhart C, Surber W, Scapozza L, Folkers G: **High-level expression and purification of human thymidine kinase 1: quaternary structure, stability, and kinetics**. *Protein Expr Purif* 2006, **47**(2):506-515.
2. Frederiksen H, Berenstein D, Munch-Petersen B: **Effect of valine 106 on structure-function relation of cytosolic human thymidine kinase. Kinetic properties and oligomerization pattern of nine substitution mutants of V106**. *Eur J Biochem* 2004, **271**(11):2248-2256.
3. Berenstein D, Christensen JF, Kristensen T, Hofbauer R, Munch-Petersen B: **Valine, not methionine, is amino acid 106 in human cytosolic thymidine kinase (TK1). Impact on oligomerization, stability, and kinetic properties**. *J Biol Chem* 2000, **275**(41):32187-32192.
4. Li CL, Lu CY, Ke PY, Chang ZF: **Perturbation of ATP-induced tetramerization of human cytosolic thymidine kinase by substitution of serine-13 with aspartic acid at the mitotic phosphorylation site**. *Biochem Biophys Res Commun* 2004, **313**(3):587-593.
5. Munch-Petersen B, Tyrsted G, Cloos L: **Reversible ATP-dependent transition between two forms of human cytosolic thymidine kinase with different enzymatic properties**. *J Biol Chem* 1993, **268**(21):15621-15625.
6. Ihaka R, Gentleman R: **R:a language for data analysis and graphics**. *Journal of Computational and graphical statistics* 1996, **5**:299-314.
7. Akaike H: **A new look at the statistical model identification**. *IEEE Transactions on Automatic Control* 1974, **19**:716-723.
8. Burnham KP, Anderson DR: **Model Selection and Multimodel Inference: A Practical-Theoretic Approach**: Springer-Verlag; 2002.
9. **Plot Digitizer** [http://plotdigitizer.sourceforge.net/]
10. Segura-Pena D, Lichter J, Trani M, Konrad M, Lavie A, Lutz S: **Quaternary structure change as a mechanism for the regulation of thymidine kinase 1-like enzymes**. *Structure* 2007, **15**(12):1555-1566.
11. Radivoyevitch T: **Equilibrium model selection: dTTP induced R1 dimerization**. *BMC Syst Biol* 2008, **2**(1):15.

# Figures

### Figure 1 – Possible states of an infinitely tightly bound asymmetric enzyme dimer

### Figure 2 – Simulated data fits

Comparison of LQRP fits to hill model "data" simulated at different *n* values. The rows are models with 4, 3, 3, 3, 2 and 2 parameters, respectively. Models with $\Delta AIC_c < 15$ are indicated by an asterisk. Parameters are given in Table 2. For details see Additional File 1.

### Figure 3 – V-α LQRP fits to the hTK1 data of Berringer et al [1]

Units are pmoles/min and μM. For details see Additional Files 2 and 3.

### Figure 4 – Top 2 models of data of Birringer et al [1] and Frederiksen et al [2] pooled

Substrate concentrations where model predictions differ most are excellent conditions for subsequent model discrimination experiments. See Additional File 6.

# Tables

### Table 1 – LQRP k-K Models

These models were derived from Eq. (2) using constraints to eliminate at least 2 parameters

| row | Eq | $k_1$ | $k_2$ | $k_3$ | $K_1$ | $K_2$ | $K_3$ | Numerator | Denominator |
|---|---|---|---|---|---|---|---|---|---|
| 1[s] | 3 | $k_1$ | $k_1$ | $k_3$ | $K_1$ | $K_1$ | $K_3$ | $2k_1\frac{S}{K_1}+2k_3\frac{S^2}{K_1K_3}$ | $1+2\frac{S}{K_1}+\frac{S^2}{K_1K_3}$ |
| 2 | 3 | $k_3$ | $k_2$ | $k_3$ | $K_1$ | $K_1$ | $K_3$ | $(k_3+k_2)\frac{S}{K_1}+2k_3\frac{S^2}{K_1K_3}$ | $1+2\frac{S}{K_1}+\frac{S^2}{K_1K_3}$ |
| 3 | 3 | $k_1$ | $k_3$ | $k_3$ | $K_1$ | $K_1$ | $K_3$ | $(k_1+k_3)\frac{S}{K_1}+2k_3\frac{S^2}{K_1K_3}$ | $1+2\frac{S}{K_1}+\frac{S^2}{K_1K_3}$ |
| 4 | 3 | $k_1$ | $k_1$ | $k_3$ | $K_1$ | $K_2$ | $K_1$ | $k_1\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+2k_3\frac{S^2}{K_1^2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1^2}$ |
| 5 | 3 | $k_3$ | $k_2$ | $k_3$ | $K_1$ | $K_2$ | $K_1$ | $\left(k_3\frac{1}{K_1}+k_2\frac{1}{K_2}\right)S+2k_3\frac{S^2}{K_1^2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1^2}$ |
| 6 | 3 | $k_1$ | $k_3$ | $k_3$ | $K_1$ | $K_2$ | $K_1$ | $\left(k_1\frac{1}{K_1}+k_3\frac{1}{K_2}\right)S+2k_3\frac{S^2}{K_1^2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1^2}$ |
| 7 | 3 | $k_1$ | $k_1$ | $k_3$ | $K_1$ | $K_2$ | $K_2$ | $k_1\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{2k_3S^2}{K_1K_2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_2}$ |
| 8 | 3 | $k_3$ | $k_2$ | $k_3$ | $K_1$ | $K_2$ | $K_2$ | $\left(\frac{k_3}{K_1}+\frac{k_2}{K_2}\right)S+\frac{2k_3S^2}{K_1K_2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_2}$ |
| 9 | 3 | $k_1$ | $k_3$ | $k_3$ | $K_1$ | $K_2$ | $K_2$ | $\left(\frac{k_1}{K_1}+\frac{k_3}{K_2}\right)S+\frac{2k_3S^2}{K_1K_2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_2}$ |
| .10 | 4 | $k_1+k_2=2k_3$ | | | $K_1$ | $K_1$ | $K_3$ | $2k_3\left(\frac{S}{K_1}+\frac{S^2}{K_1K_3}\right)$ | $1+2\frac{S}{K_1}+\frac{S^2}{K_1K_3}$ |
| 11 | 11b | $k_1+k_2=2k_3$ | | | $K_1$ | $K_2$ | $K_1$ | $\left(\frac{k_1}{K_1}+\frac{k_2}{K_2}\right)S+(k_1+k_2)\frac{S^2}{K_1^2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1^2}$ |
| 12 | 11b | $k_1+k_2=2k_3$ | | | $K_1$ | $K_2$ | $K_2$ | $\left(\frac{k_1}{K_1}+\frac{k_2}{K_2}\right)S+(k_1+k_2)\frac{S^2}{K_1K_2}$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_2}$ |
| 13 | 4 | $k_3$ | $k_3$ | $k_3$ | $K_1$ | $K_2$ | $K_3$ | $2k_3\left(\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_3}\right)$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_3}$ |
| 14[s] | 4 | $k_3$ | $k_3$ | $k_3$ | $K_1$ | $K_1$ | $K_3$ | $2k_3\left(\frac{S}{K_1}+\frac{S^2}{K_1K_3}\right)$ | $1+2\frac{S}{K_1}+\frac{S^2}{K_1K_3}$ |
| 15 | 4 | $k_3$ | $k_3$ | $k_3$ | $K_1$ | $K_2$ | $K_1$ | $2k_3\left(.5\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1^2}\right)$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1^2}$ |
| 16 | 4 | $k_3$ | $k_3$ | $k_3$ | $K_1$ | $K_2$ | $K_2$ | $2k_3\left(.5\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_2}\right)$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S+\frac{S^2}{K_1K_2}$ |

| row | Eq | $k_1$ | $k_2$ | $k_3$ | $K_1$ | $K_2$ | $K_3$ | Numerator | Denominator |
|---|---|---|---|---|---|---|---|---|---|
| 17 | 5 | $k_1$ | $k_2$ | $k_3$ | $K_1$ | $K_1$ | $K_1$ | $\frac{k_1+k_2}{K_1}S+\frac{2k_3}{K_1^2}S^2$ | $\left(1+\frac{S}{K_1}\right)^2$ |
| 18[s] | 5 | $k_1$ | $k_1$ | $k_3$ | $K_1$ | $K_1$ | $K_1$ | $\frac{2k_1}{K_1}S+\frac{2k_3}{K_1^2}S^2$ | $\left(1+\frac{S}{K_1}\right)^2$ |
| 19 | 5 | $k_3$ | $k_2$ | $k_3$ | $K_1$ | $K_1$ | $K_1$ | $\frac{k_3+k_2}{K_1}S+\frac{2k_3}{K_1^2}S^2$ | $\left(1+\frac{S}{K_1}\right)^2$ |
| 20 | 5 | $k_1$ | $k_3$ | $k_3$ | $K_1$ | $K_1$ | $K_1$ | $\frac{k_1+k_3}{K_1}S+\frac{2k_3}{K_1^2}S^2$ | $\left(1+\frac{S}{K_1}\right)^2$ |
| 21 | 8 | $k_3$ | $k_3$ | $k_3$ | $K_1$ | $K_1$ | $K_1$ | $2k_3\frac{S}{K_1}$ | $1+\frac{S}{K_1}$ |
| 22 | 3 | X | $k_2$ | $k_3$ | ∞ | $K_2$ | $K_3$ | $\frac{k_2}{K_2}S+\frac{2k_3}{K_1K_3}S^2$ | $1+\frac{1}{K_2}S+\frac{S^2}{K_1K_3}$ |
| 23 | 4 | X | $k_3$ | $k_3$ | ∞ | $K_2$ | $K_3$ | $2k_3\left(\frac{.5}{K_2}S+\frac{1}{K_1K_3}S^2\right)$ | $1+\frac{1}{K_2}S+\frac{S^2}{K_1K_3}$ |
| 24 | 3 | X | $k_2$ | $k_3$ | ∞ | $K_2$ | $K_2$ | $\frac{k_2}{K_2}S+\frac{2k_3}{K_1K_2}S^2$ | $1+\frac{1}{K_2}S+\frac{S^2}{K_1K_2}$ |
| 25 | 4 | X | $k_3$ | $k_3$ | ∞ | $K_2$ | $K_2$ | $2k_3\left(\frac{.5}{K_2}S+\frac{1}{K_1K_2}S^2\right)$ | $1+\frac{1}{K_2}S+\frac{S^2}{K_1K_2}$ |
| 26 | 3 | $k_1$ | X | $k_3$ | $K_1$ | ∞ | $K_3$ | $\frac{k_1}{K_1}S+\frac{2k_3}{K_1K_3}S^2$ | $1+\frac{1}{K_1}S+\frac{S^2}{K_1K_3}$ |
| 27 | 4 | $k_3$ | X | $k_3$ | $K_1$ | ∞ | $K_3$ | $2k_3\left(\frac{.5}{K_1}S+\frac{1}{K_1K_3}S^2\right)$ | $1+\frac{1}{K_1}S+\frac{S^2}{K_1K_3}$ |
| 28 | 6 | $k_1$ | X | $k_3$ | $K_1$ | ∞ | $K_1$ | $\frac{k_1}{K_1}S+\frac{2k_3}{K_1^2}S^2$ | $1+\frac{1}{K_1}S+\frac{S^2}{K_1^2}$ |
| 29 | 7 | $k_3$ | X | $k_3$ | $K_1$ | ∞ | $K_1$ | $2k_3\left(\frac{.5}{K_1}S+\frac{1}{K_1^2}S^2\right)$ | $1+\frac{1}{K_1}S+\frac{S^2}{K_1^2}$ |
| 30 | 8 | $k_1$ | $k_2$ | X | $K_1$ | $K_2$ | ∞ | $\left(\frac{k_1}{K_1}+\frac{k_2}{K_2}\right)S$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S$ |
| 31 | 8 | $k_1$ | $k_1$ | X | $K_1$ | $K_2$ | ∞ | $k_1\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S$ | $1+\left(\frac{1}{K_1}+\frac{1}{K_2}\right)S$ |
| 32 | 8 | $k_1$ | $k_2$ | X | $K_1$ | $K_1$ | ∞ | $\left(\frac{k_1+k_2}{K_1}\right)S$ | $1+\frac{2}{K_1}S$ |
| 33[s] | 8 | $k_1$ | $k_1$ | X | $K_1$ | $K_1$ | ∞ | $k_1\frac{2}{K_1}S$ | $1+\frac{2}{K_1}S$ |
| 34[s] | 9 | X | X | $k_3$ | ∞ | ∞ | $K_3$ | $2k_3\frac{S^2}{K_1K_3}$ | $1+\frac{S^2}{K_1K_3}$ |
| 35 | 8 | X | $k_2$ | X | ∞ | $K_2$ | ∞ | $k_2\frac{1}{K_2}S$ | $1+\frac{1}{K_2}S$ |

| row | Eq | $k_1$ | $k_2$ | $k_3$ | $K_1$ | $K_2$ | $K_3$ | Numerator | Denominator |
|---|---|---|---|---|---|---|---|---|---|
| 36 | 8 | $k_1$ | X | X | $K_1$ | ∞ | ∞ | $k_1 \frac{1}{K_1} S$ | $1 + \frac{1}{K_1} S$ |
| 37[n] | | X | X | X | ∞ | ∞ | ∞ | 0 | 1 |

X = any finite value is acceptable since the parameter is not in the model

[S] = models that assume a symmetric dimer, i.e. $K_1 = K_2$ and $k_1 = k_2$.

[n] = null model not mapped to an equation

**Table 2 - Fits to simulated Hill data**

| EQ | n | SSE | AICc | $\alpha_1$ | $V_\infty$ | $V_1$ | $\alpha_2$ | <n> |
|---|---|---|---|---|---|---|---|---|
| hill | 0.4 | 0.02295 | -233.55 | 1.2336 | 0.9189 | | | 0.4469 |
| hill | 0.7 | 0.02283 | -233.81 | 1.1033 | 0.9685 | | | 0.736 |
| hill | 1.3 | 0.02269 | -234.11 | 1.0557 | 0.9908 | | | 1.3382 |
| hill | 1.6 | 0.02267 | -234.15 | 1.0482 | 0.9946 | | | 1.6436 |
| eq3 | 0.4 | 0.02295 | -231.07 | 14.255 | 0.753 | 9.999 | 19.809 | |
| eq3 | 0.7 | 0.02274 | -231.52 | 5.516 | 0.9 | 2.886 | 8.111 | |
| eq3 | 1.3 | 0.02258 | -231.88 | 0.551 | 1.006 | 0.453 | 1.238 | |
| eq3 | 1.6 | 0.02252 | -232.02 | 0.197 | 0.999 | 0.205 | 1.025 | |
| eq4 | 0.4 | 0.0329 | -215.53 | 5.087 | 1.02 | | 0.877 | |
| eq4 | 0.7 | 0.02431 | -230.66 | 2.216 | 0.931 | | 1.673 | |
| eq4 | 1.3 | 0.02253 | -234.46 | 0.479 | 0.997 | | 1.055 | |
| eq4 | 1.6 | 0.02252 | -234.49 | 0.201 | 1 | | 1.039 | |
| eq5 | 0.4 | 0.08354 | -168.94 | 4.777 | 0.662 | 3.161 | | |
| eq5 | 0.7 | 0.04897 | -195.64 | 1.749 | 0.836 | 1.462 | | |
| eq5 | 1.3 | 0.02709 | -225.24 | 2.261 | 1.054 | 0.041 | | |
| eq5 | 1.6 | 0.06048 | -185.09 | 1.978 | 1.133 | 0 | | |
| eq6 | 0.4 | 0.14564 | -141.15 | 6.615 | 0.631 | 1.168 | | |
| eq6 | 0.7 | 0.12868 | -147.34 | 1.819 | 0.771 | 0.886 | | |
| eq6 | 1.3 | 0.02259 | -234.32 | 1.118 | 1.007 | 0.454 | | |
| eq6 | 1.6 | 0.03091 | -218.66 | 1.512 | 1.071 | 0 | | |
| eq18 | 0.4 | 0.08355 | -168.94 | 2.387 | | 3.161 | 0 | |
| eq18 | 0.7 | 0.04897 | -195.64 | 0.874 | | 1.462 | 0 | |
| eq18 | 1.3 | 0.04032 | -205.36 | 0.263 | | 0.754 | 0.02 | |
| eq18 | 1.6 | 0.08575 | -167.63 | 0.158 | | 0.639 | 0.028 | |
| eq19[a] | 0.4 | 0.08354 | -168.94 | 17751663 | 0.662 | | 1.7E+08 | |
| eq19[a] | 0.7 | 0.04897 | -195.64 | 16743770 | 0.836 | | 58566375 | |
| eq19 | 1.3 | 0.0268 | -225.79 | 1.943 | 1.044 | | 4.736 | |
| eq19 | 1.6 | 0.02577 | -227.75 | 0.394 | 1.032 | | 1.787 | |
| eq20[a] | 0.4 | 0.03585 | -211.24 | 17463314 | 0.706 | | 67178398 | |
| eq20 | 0.7 | 0.0228 | -233.87 | 7.35 | 0.895 | | 11.949 | |
| eq20 | 1.3 | 0.02306 | -233.31 | 0.737 | 1.019 | | 1.75 | |
| eq20 | 1.6 | 0.02304 | -233.34 | 0.269 | 1.016 | | 1.287 | |
| eq21[a] | 0.4 | 0.07465 | -174.57 | 49.069 | 0.668 | | 384.913 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| eq21 | 0.7 | 0.03451 | -213.15 | 21.285 | 0.853 | | 58.981 | |
| eq21 | 1.3 | 0.02256 | -234.39 | 0.455 | 0.994 | | 0.978 | |
| eq21 | 1.6 | 0.02643 | -226.48 | 0.118 | 0.952 | | 0.563 | |
| eq22 | 0.4 | 0.0231 | -233.21 | 35.802 | 0.746 | | 34.074 | |
| eq22 | 0.7 | 0.0229 | -233.65 | 5.481 | 0.908 | | 4.705 | |
| eq22 | 1.3 | 0.02411 | -231.07 | 0.55 | 1.033 | | 2.541 | |
| eq22 | 1.6 | 0.03091 | -218.66 | 0 | 1.071 | | 2.286 | |
| eq23 | 0.4 | 0.023 | -233.42 | 23.289 | 0.757 | | 0.645 | |
| eq23 | 0.7 | 0.02365 | -232.03 | 4.72 | 0.921 | | 0.51 | |
| eq23 | 1.3 | 0.04354 | -201.53 | 1.084 | 1.093 | | 2.98 | |
| eq23 | 1.6 | 0.12038 | -150.67 | 0.923 | 1.177 | | 2.671 | |
| eq7 | 0.4 | 0.14831 | -142.61 | 5.232 | 0.63 | | | |
| eq7 | 0.7 | 0.13026 | -149.1 | 2.093 | 0.774 | | | |
| eq7 | 1.3 | 0.02284 | -236.16 | 1.011 | 1.005 | | | |
| eq7 | 1.6 | 0.057 | -190.42 | 0.889 | 1.073 | | | |
| eq8 | 0.4 | 0.08354 | -171.3 | 4.776 | 0.662 | | | |
| eq8 | 0.7 | 0.04897 | -198.01 | 1.749 | 0.836 | | | |
| eq8 | 1.3 | 0.06972 | -180.35 | 0.771 | 1.126 | | | |
| eq8 | 1.6 | 0.17539 | -134.22 | 0.659 | 1.215 | | | |
| eq9 | 0.4 | 0.28491 | -109.96 | 35.052 | 0.588 | | | |
| eq9 | 0.7 | 0.35878 | -98.44 | 6.448 | 0.702 | | | |
| eq9 | 1.3 | 0.1178 | -154.12 | 1.55 | 0.895 | | | |
| eq9 | 1.6 | 0.0472 | -199.85 | 1.215 | 0.952 | | | |
| eq24 | 0.4 | 0.72007 | -63.61 | 0.376 | | 1.072 | | |
| eq24 | 0.7 | 0.33923 | -101.24 | 0.25 | | 0.795 | | |
| eq24 | 1.3 | 0.05783 | -189.7 | 0.177 | | 0.66 | | |
| eq24 | 1.6 | 0.08593 | -169.89 | 0.166 | | 0.647 | | |
| eq25 | 0.4 | 0.11167 | -156.8 | 12.457 | 0.646 | | | |
| eq25 | 0.7 | 0.10466 | -160.04 | 5.026 | 0.796 | | | |
| eq25 | 1.3 | 0.02714 | -227.52 | 2.301 | 1.053 | | | |
| eq25 | 1.6 | 0.06048 | -187.46 | 1.978 | 1.133 | | | |
| eq26 | 0.4 | 0.09286 | -166.02 | 7.906 | 0.656 | | | |
| eq26 | 0.7 | 0.06188 | -186.32 | 2.969 | 0.823 | | | |
| eq26 | 1.3 | 0.05104 | -195.94 | 1.337 | 1.1 | | | |
| eq26 | 1.6 | 0.13818 | -146.14 | 1.151 | 1.183 | | | |
| eq27 | 0.4 | 0.99154 | -47.61 | 0.379 | | 0.839 | | |
| eq27 | 0.7 | 0.51661 | -80.21 | 0.265 | | 0.651 | | |
| eq27 | 1.3 | 0.11481 | -155.41 | 0.2 | | 0.57 | | |
| eq27 | 1.6 | 0.10245 | -161.11 | 0.191 | | 0.566 | | |
| eq28 | 0.4 | 0.1487 | -142.48 | 8.846 | 0.629 | | | |
| eq28 | 0.7 | 0.16767 | -136.47 | 3.717 | 0.765 | | | |
| eq28 | 1.3 | 0.03519 | -214.54 | 1.742 | 0.999 | | | |
| eq28 | 1.6 | 0.03091 | -221.02 | 1.512 | 1.071 | | | |

[a]Fits to these models did not converge.

**Table 3 - LQRP fits to Birringer et al's 2006 data**

| EQ | SSE | AICc | $\alpha_1$ | $V_\infty$ | $V_1$ | $\alpha_2$ | n |
|---|---|---|---|---|---|---|---|
| eq25 | 0.00026 | -57.67 | 3.765 | 0.151 | | | |
| eq28 | 0.00028 | -56.93 | 2.756 | 0.145 | | | |
| eq7 | 0.00029 | -56.86 | 1.658 | 0.145 | | | |
| eq26 | 0.00034 | -55.35 | 2.316 | 0.156 | | | |
| eq8 | 0.00039 | -54.1 | 1.37 | 0.158 | | | |
| eq22 | 0.00026 | -50.48 | 0.833 | 0.148 | | 6.732 | |
| eq20 | 0.00027 | -50.23 | 1.293 | 0.148 | | 4.635 | |
| eq9 | 0.00061 | -49.99 | 3.645 | 0.132 | | | |
| eq1 | 0.00028 | -49.88 | 1.941 | 0.144 | | | 1.297 |
| eq6 | 0.00028 | -49.71 | 2.747 | 0.145 | 0 | | |
| eq4 | 0.00029 | -49.6 | 0.815 | 0.146 | | 2.704 | |
| eq24 | 0.00065 | -49.5 | 0.272 | | 0.149 | | |
| eq21 | 3.00E-04 | -49.33 | 1.459 | 0.149 | | 3.996 | |
| eq19 | 0.00032 | -48.79 | 0.946 | 0.144 | | 6.156 | |
| eq23 | 0.00033 | -48.49 | 2.145 | 0.155 | | 3.006 | |
| eq18 | 0.00037 | -47.41 | 0.576 | | 0.2 | 0.022 | |
| eq5 | 0.00037 | -47.25 | 0.96 | 0.157 | 0.207 | | |
| eq27 | 0.00105 | -45.19 | 0.303 | | 0.127 | | |
| eq3 | 0.00026 | -38.57 | 2.5287 | 0.1496 | 0.0205 | 10.1832 | |

**Table 4 - LQRP fits to Fredericksen et al's 2004 data**

| EQ | SSE | AICc | $\alpha_1$ | $V_\infty$ | $V_1$ | $\alpha_2$ | n |
|---|---|---|---|---|---|---|---|
| eq25 | 0.65514 | 11.12 | 3.904 | 9.408 | | | |
| eq7 | 0.79543 | 13.06 | 1.666 | 9.051 | | | |
| eq28 | 0.92358 | 14.56 | 2.895 | 8.988 | | | |
| eq26 | 1.05517 | 15.89 | 2.325 | 9.75 | | | |
| eq5 | 0.65514 | 17.12 | 3.904 | 9.408 | 0 | | |
| eq19 | 0.65509 | 17.12 | 3.967 | 9.414 | | 15.415 | |
| eq22 | 0.68674 | 17.59 | 1.067 | 9.305 | | 6.973 | |
| eq20 | 0.7127 | 17.97 | 1.424 | 9.276 | | 4.955 | |
| eq1 | 0.71901 | 18.05 | 1.891 | 9.096 | | | 1.263 |
| eq8 | 1.35414 | 18.38 | 1.366 | 9.925 | | | |
| eq21 | 0.75662 | 18.56 | 1.083 | 9.247 | | 3.199 | |
| eq4 | 0.77582 | 18.81 | 0.92 | 9.167 | | 2.643 | |
| eq6 | 0.77785 | 18.84 | 2.002 | 9.068 | 5.973 | | |
| eq18 | 0.9075 | 20.38 | 0.501 | | 11.706 | 0.028 | |
| eq23 | 0.92916 | 20.62 | 1.852 | 9.71 | | 5.483 | |
| eq24 | 2.21962 | 23.33 | 0.238 | | 8.575 | | |
| eq3 | 0.65156 | 26.07 | 6.0618 | 9.4465 | -4.7865 | 23.7351 | |
| eq9 | 2.95794 | 26.2 | 4.071 | 8.092 | | | |
| eq27 | 4.128 | 29.53 | 0.256 | | 7.132 | | |

**Table 5 - LQRP fits to Berenstein et al's 2000 data**

| EQ | SSE | AICc | $\alpha_1$ | $V_\infty$ | $V_1$ | $\alpha_2$ | n |
|---|---|---|---|---|---|---|---|
| eq7 | 3.07179 | 26.96 | 1.708 | 16.921 | | | |
| eq6 | 2.83282 | 29.57 | 1.255 | 16.798 | 16.794 | | |
| eq4 | 3.05477 | 30.7 | 0.899 | 16.946 | | 2.919 | |
| eq21 | 3.0714 | 30.78 | 0.843 | 16.915 | | 2.89 | |
| eq1 | 3.25408 | 31.65 | 2.014 | 16.881 | | | 1.275 |
| eq20 | 3.53592 | 32.89 | 1.347 | 17.017 | | 5.359 | |
| eq25 | 4.63669 | 33.14 | 4.285 | 17.133 | | | |
| eq3 | 2.77059 | 33.9 | 0.6384 | 16.8003 | 18.6378 | 1.2412 | |
| eq22 | 3.89233 | 34.33 | 1.165 | 17.084 | | 8.022 | |
| eq28 | 5.05865 | 34.45 | 2.888 | 16.922 | | | |
| eq26 | 5.55593 | 35.85 | 2.776 | 17.265 | | | |
| eq5 | 4.50722 | 36.53 | 3.914 | 17.167 | 7.39 | | |
| eq19 | 4.57175 | 36.75 | 3.117 | 17.081 | | 14.274 | |
| eq23 | 5.23599 | 38.78 | 2.352 | 17.25 | | 4.741 | |
| eq18 | 5.35286 | 39.11 | 0.775 | | 27.835 | 0.001 | |
| eq8 | 6.92409 | 39.15 | 1.705 | 17.327 | | | |
| eq9 | 13.69352 | 49.38 | 2.847 | 16.298 | | | |
| eq24 | 288.1286 | 95.08 | 0.086 | | 7.656 | | |
| eq27 | 388.9926 | 99.58 | 0.09 | | 6.266 | | |

**Table 6 - LQRP fits to Li et al's 2004 data**

| EQ | SSE | AICc | $\alpha_1$ | $V_\infty$ | $V_1$ | $\alpha_2$ | n |
|---|---|---|---|---|---|---|---|
| eq8 | 1.69566 | 18.58 | 1.898 | 11.308 | | | |
| eq26 | 1.70185 | 18.63 | 3.122 | 11.241 | | | |
| eq19 | 1.65183 | 22.25 | 48.183 | 11.279 | | 188.884 | |
| eq18 | 1.66414 | 22.36 | 0.925 | | 21.133 | 0 | |
| eq21 | 1.67467 | 22.45 | 3.855 | 11.278 | | 11.83 | |
| eq20 | 1.67714 | 22.47 | 3.468 | 11.278 | | 10.291 | |
| eq22 | 1.6785 | 22.48 | 3.482 | 11.277 | | 9.574 | |
| eq23 | 1.68202 | 22.51 | 3.521 | 11.277 | | 2.269 | |
| eq5 | 1.68475 | 22.53 | 2.679 | 11.274 | 19.822 | | |
| eq1 | 1.69224 | 22.59 | 1.917 | 11.288 | | | 1.011 |
| eq4 | 1.69377 | 22.61 | 1.866 | 11.295 | | 3.615 | |
| eq25 | 2.4134 | 23.52 | 4.921 | 11.089 | | | |
| eq3 | 1.67961 | 27.54 | 1.0873 | 11.3725 | 22.0115 | 0.3613 | |
| eq7 | 3.50368 | 28.74 | 2.008 | 10.9 | | | |
| eq28 | 4.26879 | 31.5 | 3.442 | 10.858 | | | |
| eq6 | 3.48153 | 32.69 | 2.178 | 10.906 | 10.099 | | |
| eq9 | 13.16382 | 47.27 | 5.147 | 10.168 | | | |
| eq24 | 106.5948 | 76.55 | 0.116 | | 7.145 | | |
| eq27 | 142.0577 | 80.57 | 0.134 | | 6.481 | | |

**Table 7 - LQRP fits to Munch-Petersen et al's 1993 data**

| EQ | SSE | AICc | $\alpha_1$ | $V_\infty$ | $V_1$ | $\alpha_2$ | n |
|---|---|---|---|---|---|---|---|
| eq3 | 0.1502 | -13.29 | 0.436 | 8.6199 | 9.3605 | 0.0764 | |
| eq18 | 0.47862 | 0.89 | 0.495 | | 10.324 | 0.003 | |
| eq6 | 0.59866 | 4.47 | 0.792 | 9.159 | 7.024 | | |
| eq7 | 0.80163 | 5.51 | 1.259 | 9.374 | | | |
| eq1 | 0.72345 | 7.5 | 1.33 | 9.394 | | | 1.237 |
| eq4 | 0.74461 | 7.96 | 0.732 | 9.451 | | 1.543 | |
| eq21 | 0.78879 | 8.88 | 0.721 | 9.424 | | 1.729 | |
| eq20 | 0.92797 | 11.48 | 1.159 | 9.518 | | 3.026 | |
| eq25 | 1.25015 | 12.62 | 3.074 | 9.57 | | | |
| eq22 | 1.00152 | 12.71 | 1.105 | 9.565 | | 4.085 | |
| eq26 | 1.29873 | 13.23 | 1.939 | 9.7 | | | |
| eq5 | 1.12152 | 14.52 | 2.571 | 9.627 | 4.984 | | |
| eq23 | 1.23238 | 16.02 | 1.67 | 9.683 | | 3.164 | |
| eq19 | 1.24215 | 16.15 | 3.848 | 9.601 | | 11.302 | |
| eq28 | 1.59368 | 16.5 | 2.148 | 9.381 | | | |
| eq8 | 1.64634 | 17.02 | 1.175 | 9.765 | | | |
| eq9 | 4.40849 | 32.78 | 1.922 | 8.869 | | | |
| eq24 | 30.12785 | 63.53 | 0.091 | | 3.904 | | |
| eq27 | 44.92134 | 69.92 | 0.094 | | 3.107 | | |

**Table 8 - Available Human Thymidine Kinase 1 data**

| dT | v | $E_0$ | ATP | year | figure | first author |
|---|---|---|---|---|---|---|
| 0.0917 | 0.012 | 306 | 6 | 2006 | 4a | Birringer |
| 0.249 | 0.0282 | 306 | 6 | 2006 | 4a | Birringer |
| 0.498 | 0.0709 | 306 | 6 | 2006 | 4a | Birringer |
| 0.747 | 0.0862 | 306 | 6 | 2006 | 4a | Birringer |
| 0.996 | 0.0883 | 306 | 6 | 2006 | 4a | Birringer |
| 1.99 | 0.116 | 306 | 6 | 2006 | 4a | Birringer |
| 3 | 0.133 | 306 | 6 | 2006 | 4a | Birringer |
| 4.49 | 0.127 | 306 | 6 | 2006 | 4a | Birringer |
| 5.99 | 0.143 | 306 | 6 | 2006 | 4a | Birringer |
| 0.0699 | 0.342 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 0.119 | 1.03 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 0.214 | 1.92 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 0.427 | 3.63 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 0.846 | 5.81 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 1.24 | 6.46 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 1.65 | 6.49 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 2.04 | 7.55 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 4.05 | 8.76 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 8.01 | 8.62 | 200 | 2.5 | 2004 | 4a | Frederiksen |
| 0.04535 | 0.907 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 0.096983 | 2.25 | 200 | 2.5 | 2000 | 2a | Berenstein |

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.197633 | 3.34 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 0.394156 | 6.07 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 0.802521 | 9.55 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 1.183486 | 12.9 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 1.587112 | 13.3 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 2.008547 | 14.1 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 4.047619 | 15.3 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 7.819905 | 16.5 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 16.1 | 16.9 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 24 | 16.4 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 40 | 16.7 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 79.9 | 17.6 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 120 | 16.1 | 200 | 2.5 | 2000 | 2a | Berenstein |
| 0.075862 | 1.32 | 200 | 2.5 | 2004 | 2a | Li |
| 0.035823 | 0.566 | 200 | 2.5 | 2004 | 2a | Li |
| 0.198658 | 2.96 | 200 | 2.5 | 2004 | 2a | Li |
| 0.314729 | 4.06 | 200 | 2.5 | 2004 | 2a | Li |
| 0.402344 | 5.15 | 200 | 2.5 | 2004 | 2a | Li |
| 0.806024 | 6.69 | 200 | 2.5 | 2004 | 2a | Li |
| 1.007353 | 8.22 | 200 | 2.5 | 2004 | 2a | Li |
| 1.202703 | 8.01 | 200 | 2.5 | 2004 | 2a | Li |
| 1.638507 | 8.34 | 200 | 2.5 | 2004 | 2a | Li |
| 2.01467 | 8.24 | 200 | 2.5 | 2004 | 2a | Li |
| 9.72 | 10.8 | 200 | 2.5 | 2004 | 2a | Li |
| 19.7 | 11.1 | 200 | 2.5 | 2004 | 2a | Li |
| 49.7 | 11.6 | 200 | 2.5 | 2004 | 2a | Li |
| 100 | 10.9 | 200 | 2.5 | 2004 | 2a | Li |
| 0.051474 | 0.489 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 0.097841 | 0.861 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 0.201282 | 1.57 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 0.39749 | 2.85 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 0.589425 | 3.79 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 1.005871 | 5.14 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 2.026393 | 6.91 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 3.045455 | 8.04 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 4.033816 | 8.35 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 5.081395 | 8.74 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 5.933333 | 8.9 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 8.107143 | 9.08 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 12.37845 | 9.42 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 19.91091 | 8.94 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 27.90274 | 9.18 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |
| 64.38849 | 8.95 | 80 | 2.5 | 1993 | 4 | Munch-Petersen |

## Additional files

**Additional file 1 – R script to generate Figure 2**
This file can be sourced into R to recreate Figure 2 and Table 2.

**Additional file 2 – Available TK1 Data**
This is a tab delimited version of Table 8. It is read in by Additional Files 3-6.

**Additional file 3 – R script to generate Figure 3**
This R script can be sourced into R to generate Figure 3 and Table 3 to 7.

**Additional file 4 – R script to generate parameter estimates of Eq. (29)**
This R script generates Eq. (29) parameter estimates for the data of Birringer et al.

**Additional file 5 – R script to normalize data**
This R script amplitude normalizes the data in Additional File 2.

**Additional file 6 – R script to generate Figure 4**
This R script generates Figure 4.

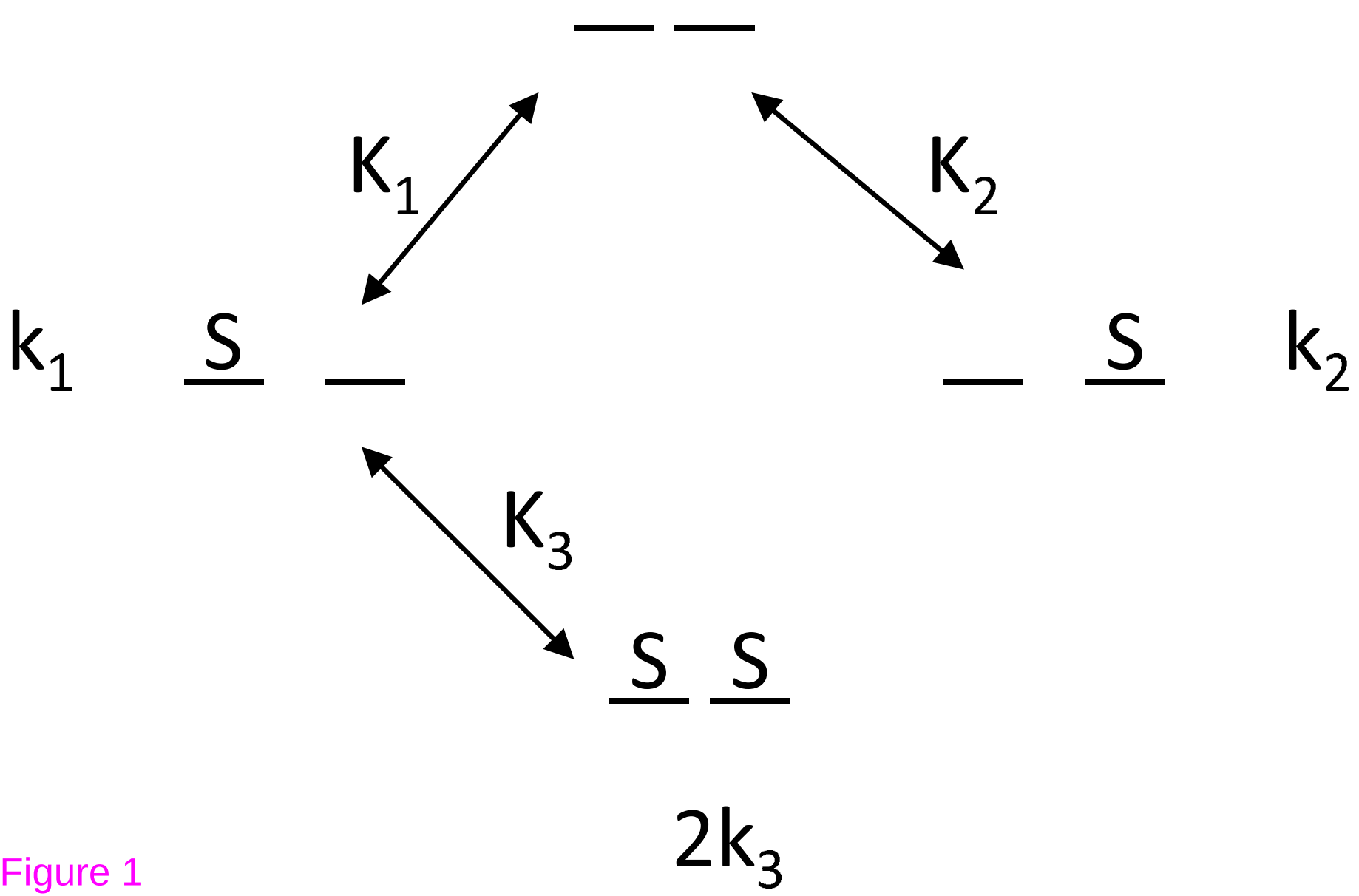


Figure 1

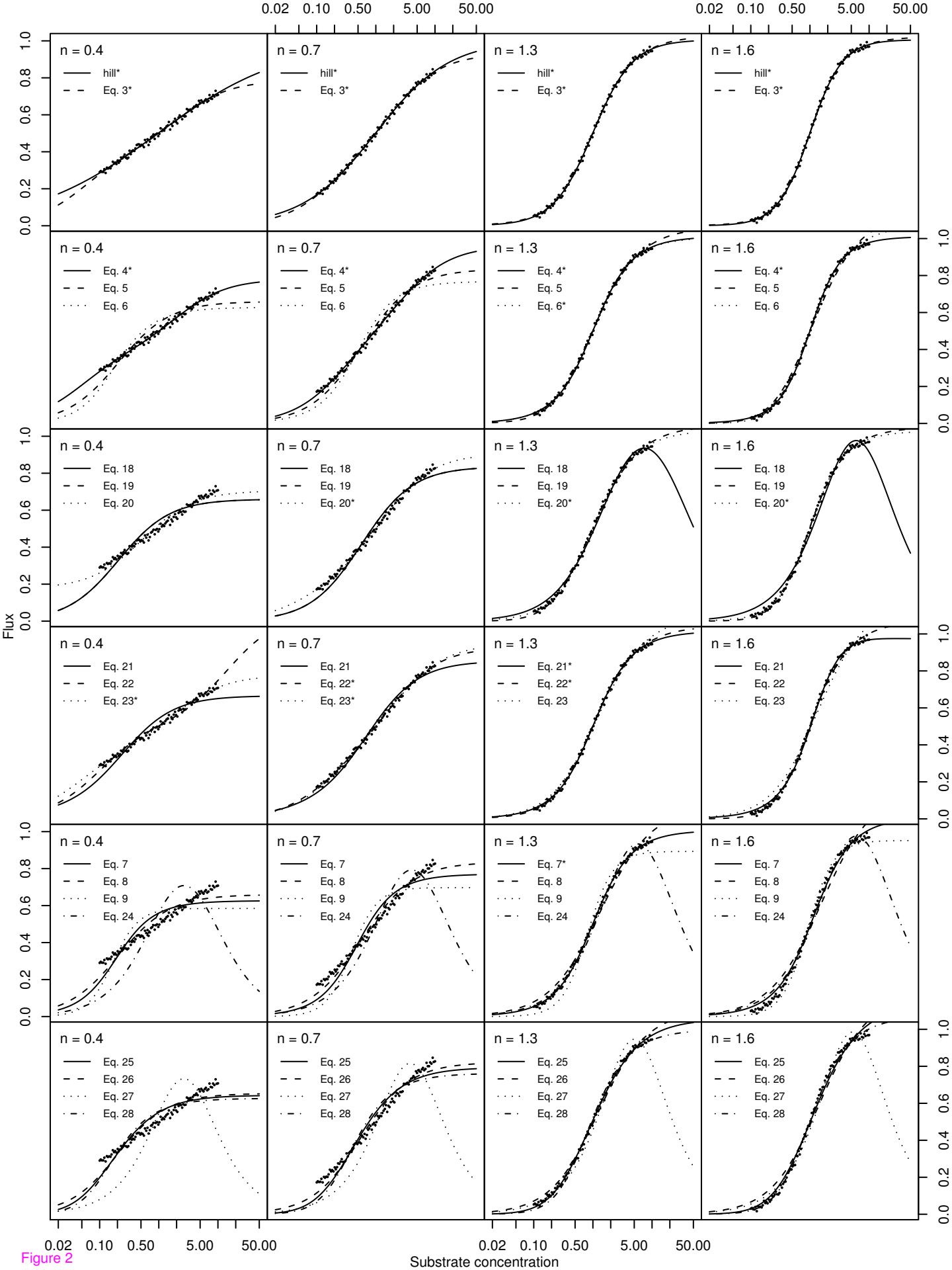
n = 0.4
hill*
Eq. 3*
n = 0.7
hill*
Eq. 3*
n = 1.3
hill*
Eq. 3*
n = 1.6
hill*
Eq. 3*
n = 0.4
Eq. 4*
Eq. 5
Eq. 6
n = 0.7
Eq. 4*
Eq. 5
Eq. 6
n = 1.3
Eq. 4*
Eq. 5
Eq. 6*
n = 1.6
Eq. 4*
Eq. 5
Eq. 6
n = 0.4
Eq. 18
Eq. 19
Eq. 20
n = 0.7
Eq. 18
Eq. 19
Eq. 20*
n = 1.3
Eq. 18
Eq. 19
Eq. 20*
n = 1.6
Eq. 18
Eq. 19
Eq. 20*
n = 0.4
Eq. 21
Eq. 22
Eq. 23*
n = 0.7
Eq. 21
Eq. 22*
Eq. 23*
n = 1.3
Eq. 21*
Eq. 22*
Eq. 23
n = 1.6
Eq. 21
Eq. 22
Eq. 23
n = 0.4
Eq. 7
Eq. 8
Eq. 9
Eq. 24
n = 0.7
Eq. 7
Eq. 8
Eq. 9
Eq. 24
n = 1.3
Eq. 7*
Eq. 8
Eq. 9
Eq. 24
n = 1.6
Eq. 7
Eq. 8
Eq. 9
Eq. 24
n = 0.4
Eq. 25
Eq. 26
Eq. 27
Eq. 28
n = 0.7
Eq. 25
Eq. 26
Eq. 27
Eq. 28
n = 1.3
Eq. 25
Eq. 26
Eq. 27
Eq. 28
n = 1.6
Eq. 25
Eq. 26
Eq. 27
Eq. 28
0.02
0.10
0.50
5.00
50.00
0.0
0.2
0.4
0.6
0.8
1.0
Flux
Substrate concentration

Figure 2

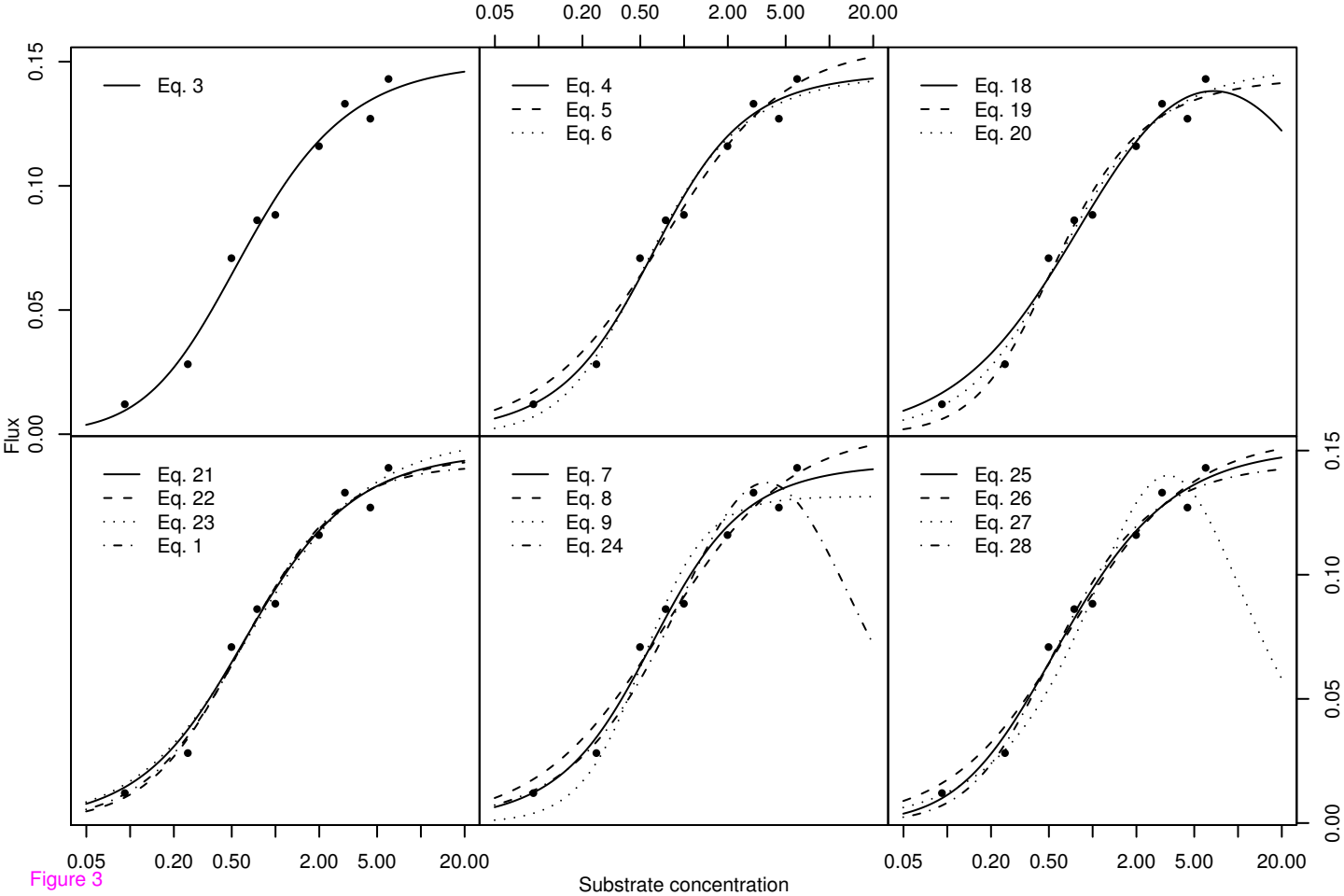
Eq. 3
Eq. 4
Eq. 5
Eq. 6
Eq. 18
Eq. 19
Eq. 20
Eq. 21
Eq. 22
Eq. 23
Eq. 1
Eq. 7
Eq. 8
Eq. 9
Eq. 24
Eq. 25
Eq. 26
Eq. 27
Eq. 28
Flux
0.00
0.05
0.10
0.15
0.05
0.20
0.50
2.00
5.00
20.00
Substrate concentration

Figure 3

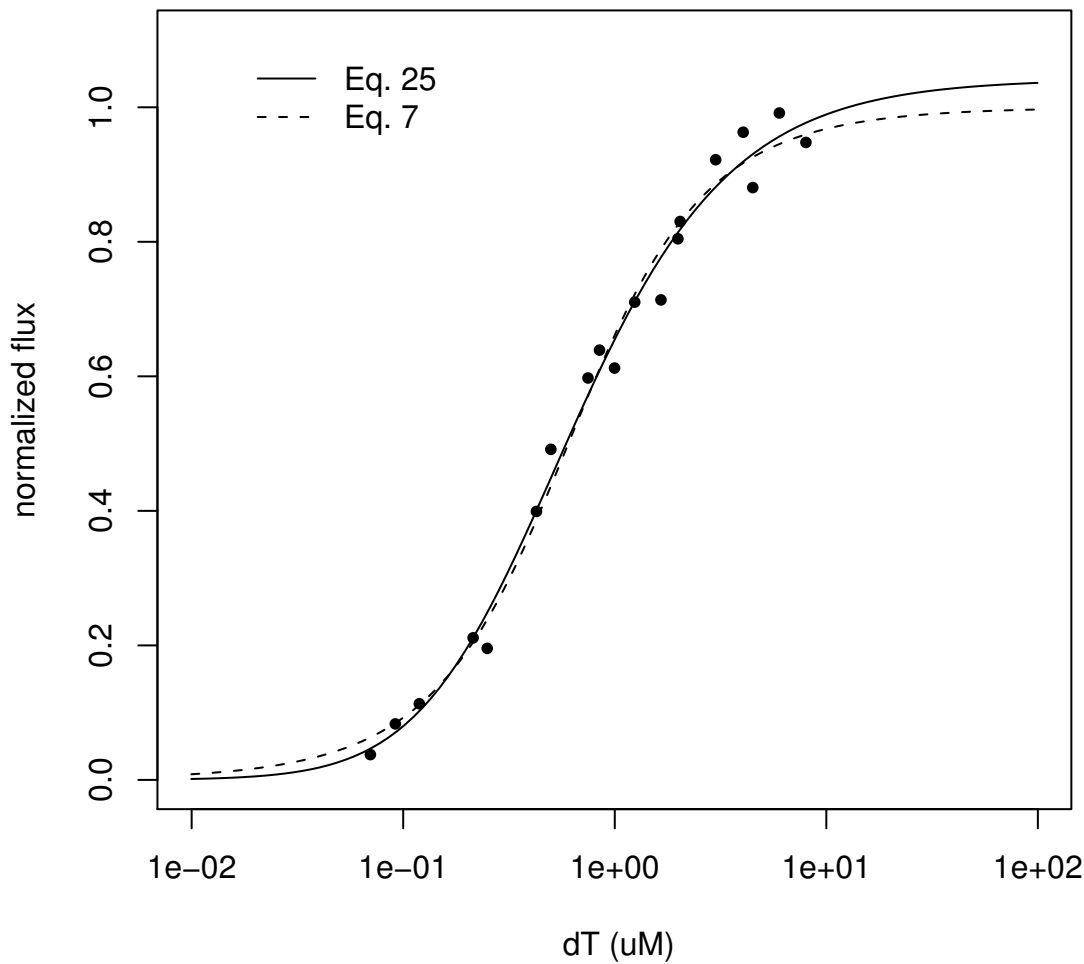
Eq. 25
Eq. 7
normalized flux
0.0
0.2
0.4
0.6
0.8
1.0
1e−02
1e−01
1e+00
1e+01
1e+02
dT (uM)

Figure 4

**Additional files provided with this submission:**

Additional file 1: addf1fithills.r, 7K
http://www.biomedcentral.com/imedia/1541234632221165/supp1.r
Additional file 2: addf2tk1.txt, 3K
http://www.biomedcentral.com/imedia/8264360982211658/supp2.txt
Additional file 3: addf3fitall.r, 7K
http://www.biomedcentral.com/imedia/7156695992211658/supp3.r
Additional file 4: addf4birreq29.r, 0K
http://www.biomedcentral.com/imedia/2884002422116584/supp4.r
Additional file 5: addf5mknorm.r, 2K
http://www.biomedcentral.com/imedia/1173268833221165/supp5.r
Additional file 6: addf6top2.r, 1K
http://www.biomedcentral.com/imedia/1807485967221165/supp6.r