PLOS COMPUTATIONAL BIOLOGY

Effect of magnitude and variability of energy of activation in multisite ultrasensitive biochemical processes

Leonila Lagunes[1], **Lee Bardwell**[1], **German A. Enciso**[1,2]*

1

2

*

OPEN ACCESS

Citation:

Editor:

Received:

Accepted:

Published:

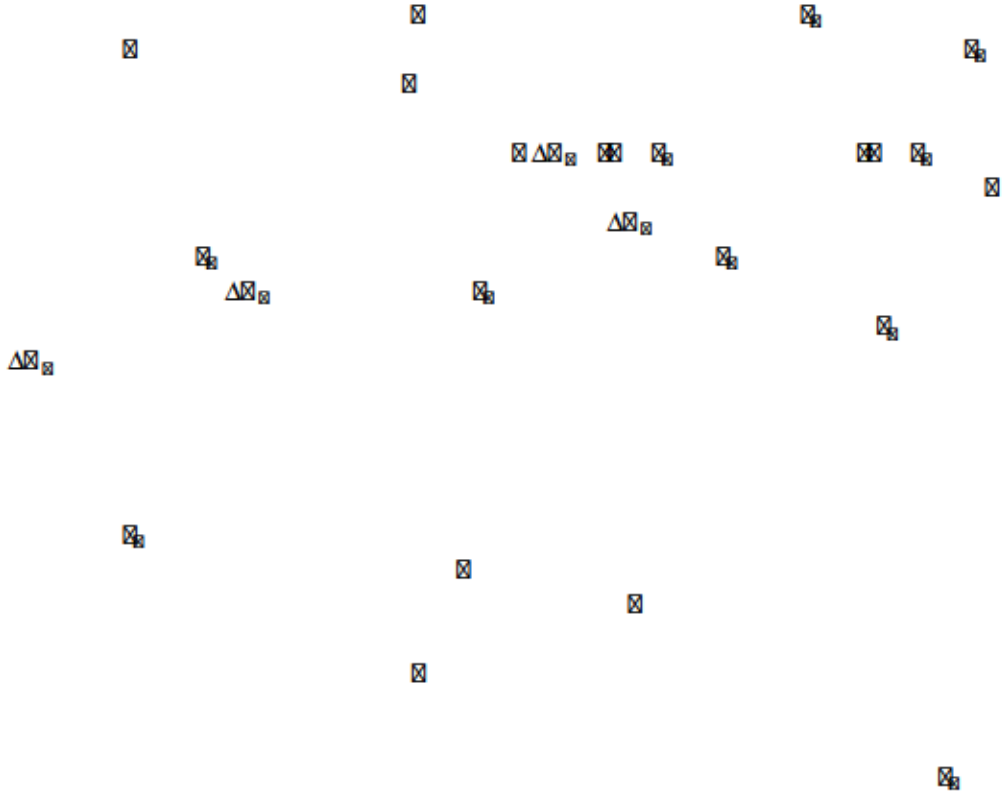
Data Availability Statement:

Funding:

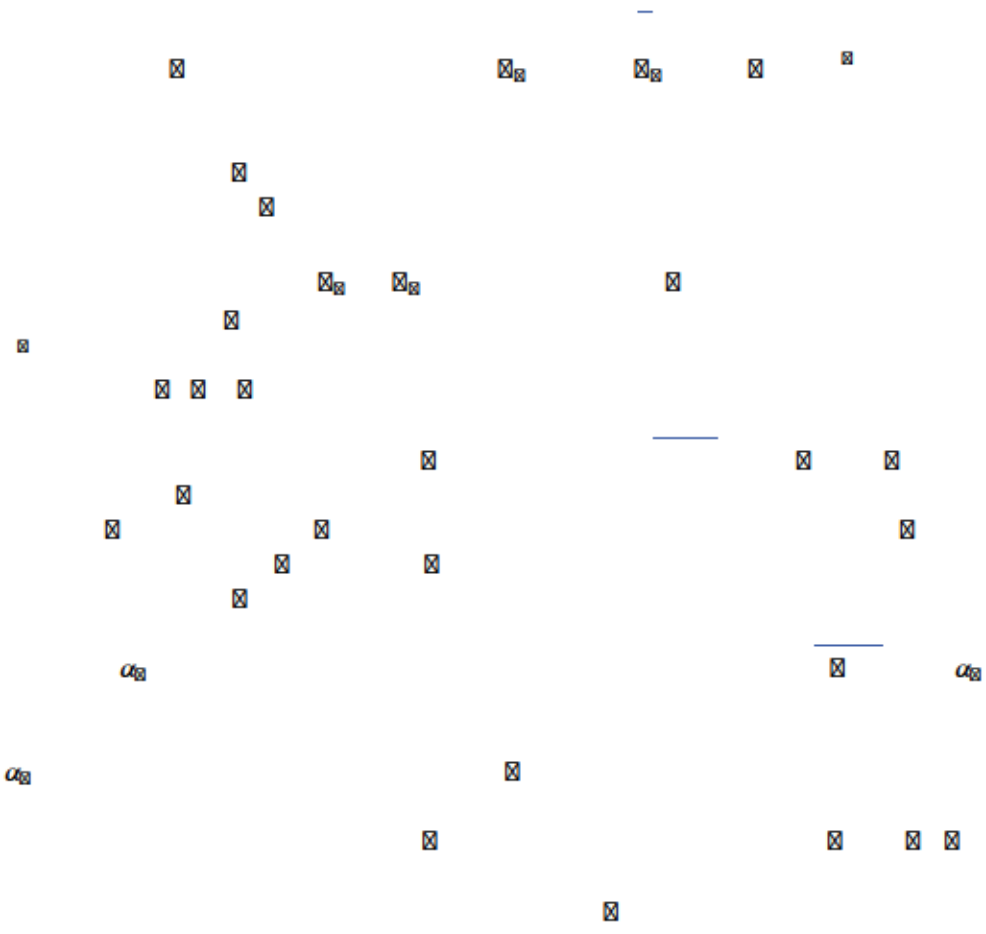
Abstract

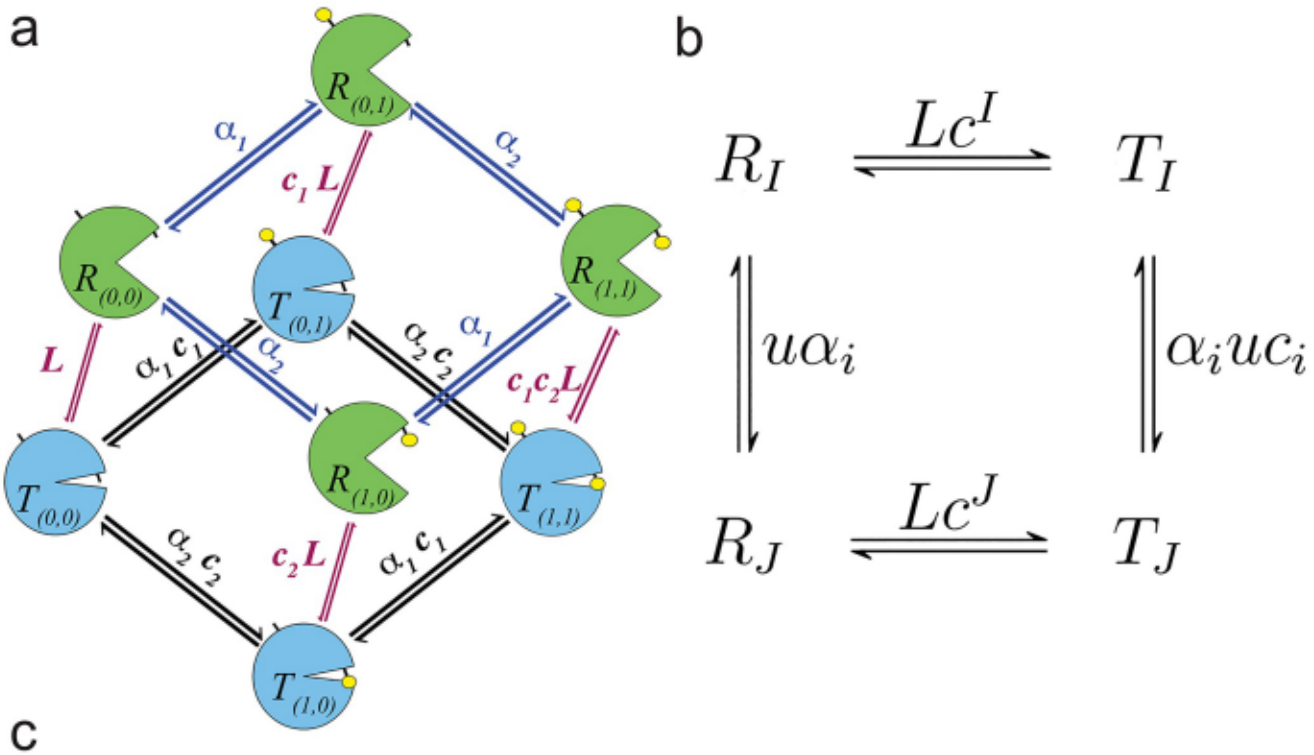
Competing interests:

Introduction



Results



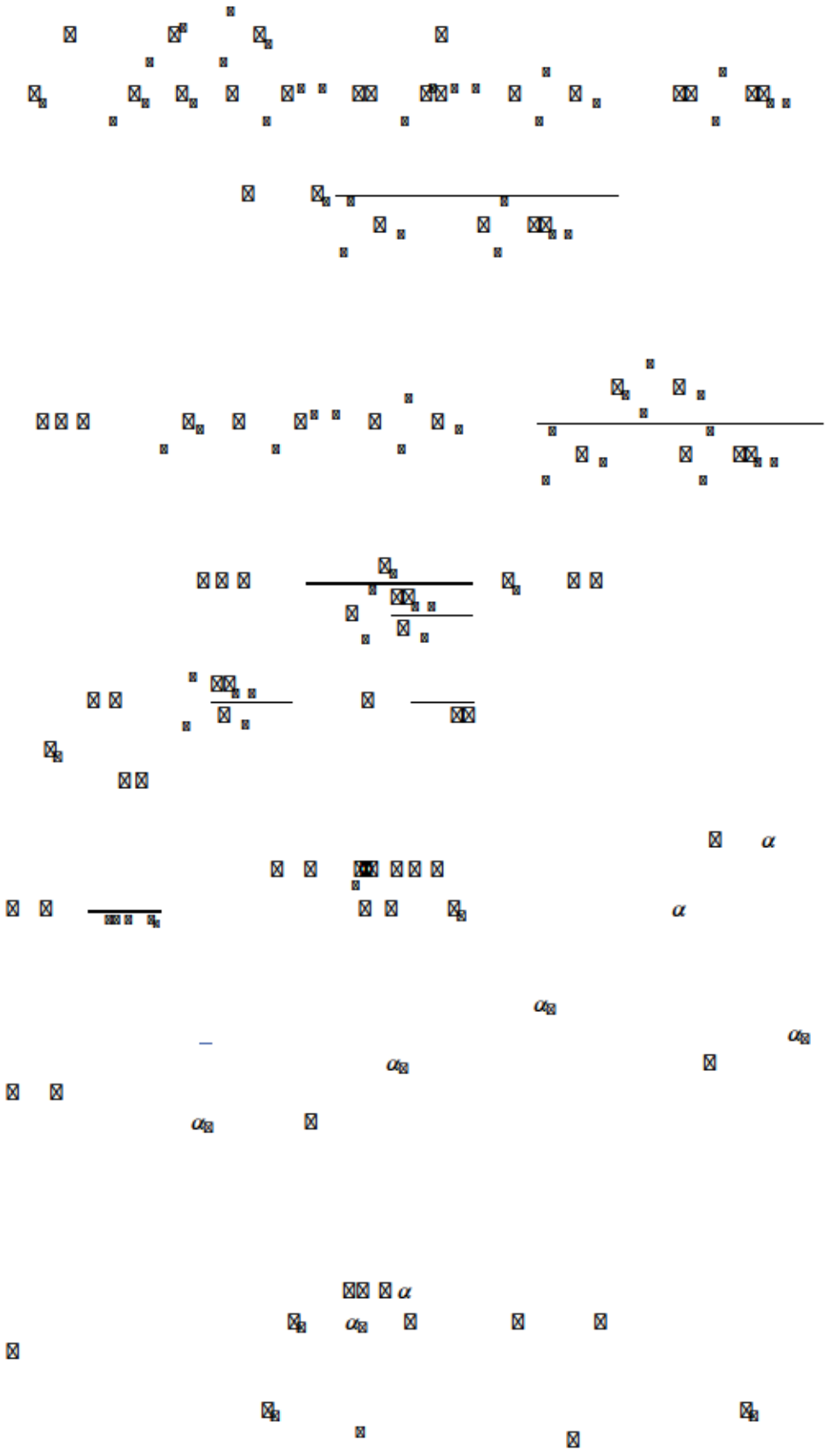


c

State	Statistical Weight	State	Statistical Weight
$R_{(0,0)}$	1	$T_{(0,0)}$	L
$R_{(1,0)}$	α_2	$T_{(1,0)}$	$L\alpha_2 c_2$
$R_{(0,1)}$	α_1	$T_{(0,1)}$	$L\alpha_1 c_1$
$R_{(1,1)}$	$\alpha_1\alpha_2$	$T_{(1,1)}$	$L\alpha_1\alpha_2 c_1 c_2$

Fig 1. Generalized MWC allosteric model. (a) The figure shows the eight possible states of a target molecule/receptor regulated by the MWC mechanism and containing two sites for ligand binding or post translational modification (i.e., $n = 2$). The four states shown in blue (with closed 'mouth') are the tense, inactive states, while the four states shown in green (with open 'mouth') are the relaxed, active states. Modification/ligand binding is indicated by the presence of absence of a small yellow ball. The L, α and c parameters are explained in the text. (b) Chemical reaction network demonstrating the possible modified-forms of a receptor with n sites, where I is the index vector for the modified-form and J is the index vector after adding one more modification at site i. (c) Table of statistical weights for each state possible with $n = 2$.

https://doi.org/10.1371/journal.pcbi.1007966.g001



parameters α_i were chosen to be identical to each other, $\alpha_i = \bar{\alpha}$, here the value of $\bar{\alpha}$ does not affect the Hill coefficient.

We calculated the Hill coefficient H by solving for EC_{90} and EC_{10} with a standard numerical solver. Here, we solved for u such that $f(u, c, \alpha) - \beta f^{\infty}(c) = 0$ for both $\beta = 10\%$ and 90%. With both EC_{10} and EC_{90}, we can calculate H as

$$H = \frac{ln(81)}{ln\left(\frac{EC_{90}}{EC_{10}}\right)} \tag{2}$$

derived in [27]. $H > 1$ implies the dose response curve is ultrasensitive, while $H = 1$ implies there is no ultrasensitivity, and $H < 1$ shows negative ultrasensitivity. One can also think of $H > 1$ showing that the dose response has a good switch [28]; the larger the value of H the more ultrasensitive the dose response curve.

Fig 2a displays the dose response curves in this system for $n = 2, 4, 8$, $c_i = 0.01$, $L = 1000$, and $\alpha_i = \bar{\alpha} = 1$. These functions show that when all the sites contribute equally, the Hill

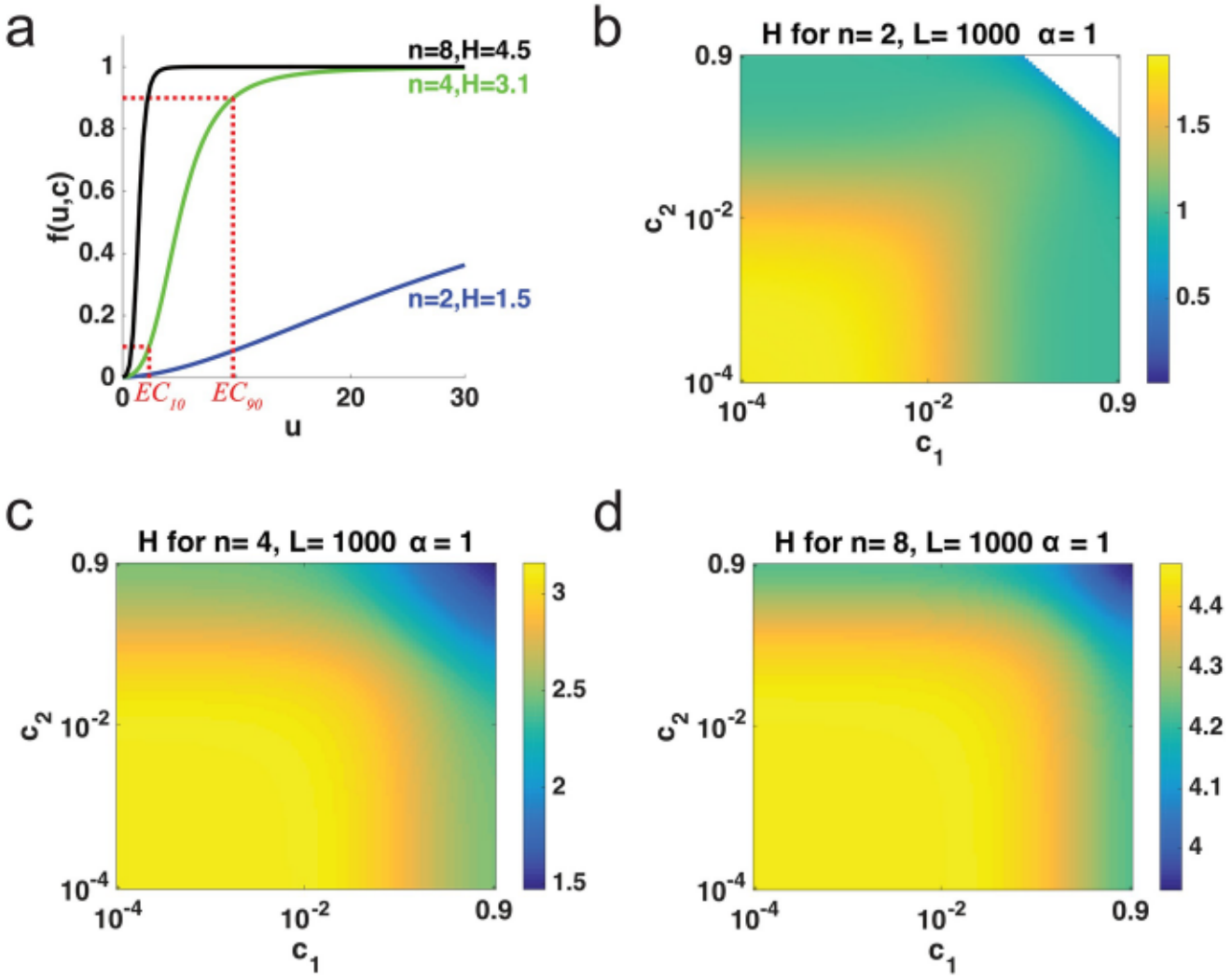
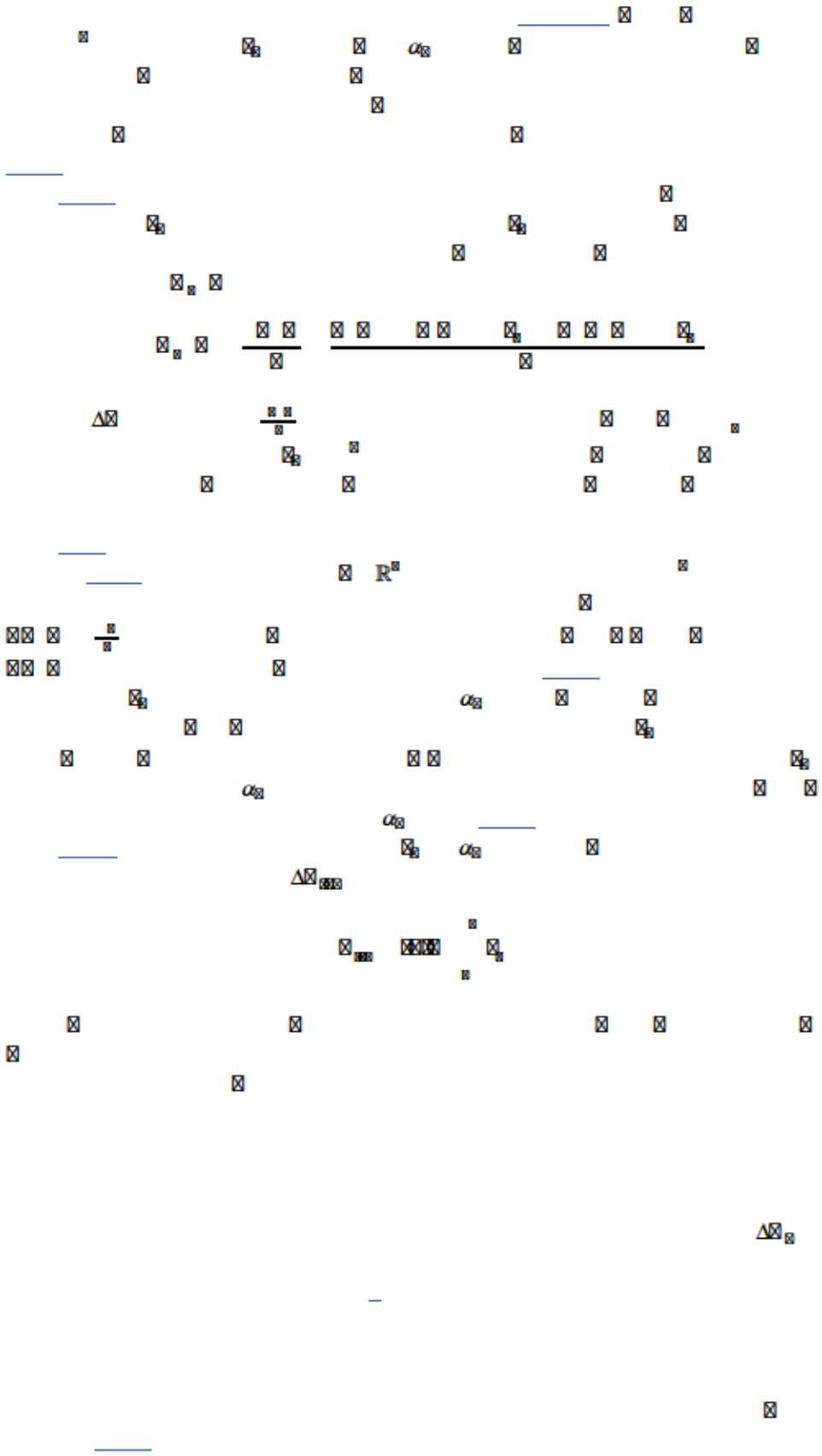


Fig 2. Ultrasensitivity of MWC system. (a) Dose response curve, $f(u, c, \alpha)$, when $n = 2, 4$, and 8 for increasing u with $c_i = 0.01$, $\alpha_i = \bar{\alpha} = 1$ and $L = 1000$. (b-d) Heat maps for H when $c_1, c_2 \in [10^{-4}, 0.9]$ with $L = 1000$ and $\alpha_i = \bar{\alpha} = 1$ and (b) $n = 2$, (c) $n = 4$, $c_i = 0.01$ for $i \geq 3$, similarly with (d) $n = 8$. White indicates undefined H values.

https://doi.org/10.1371/journal.pcbi.1007966.g002



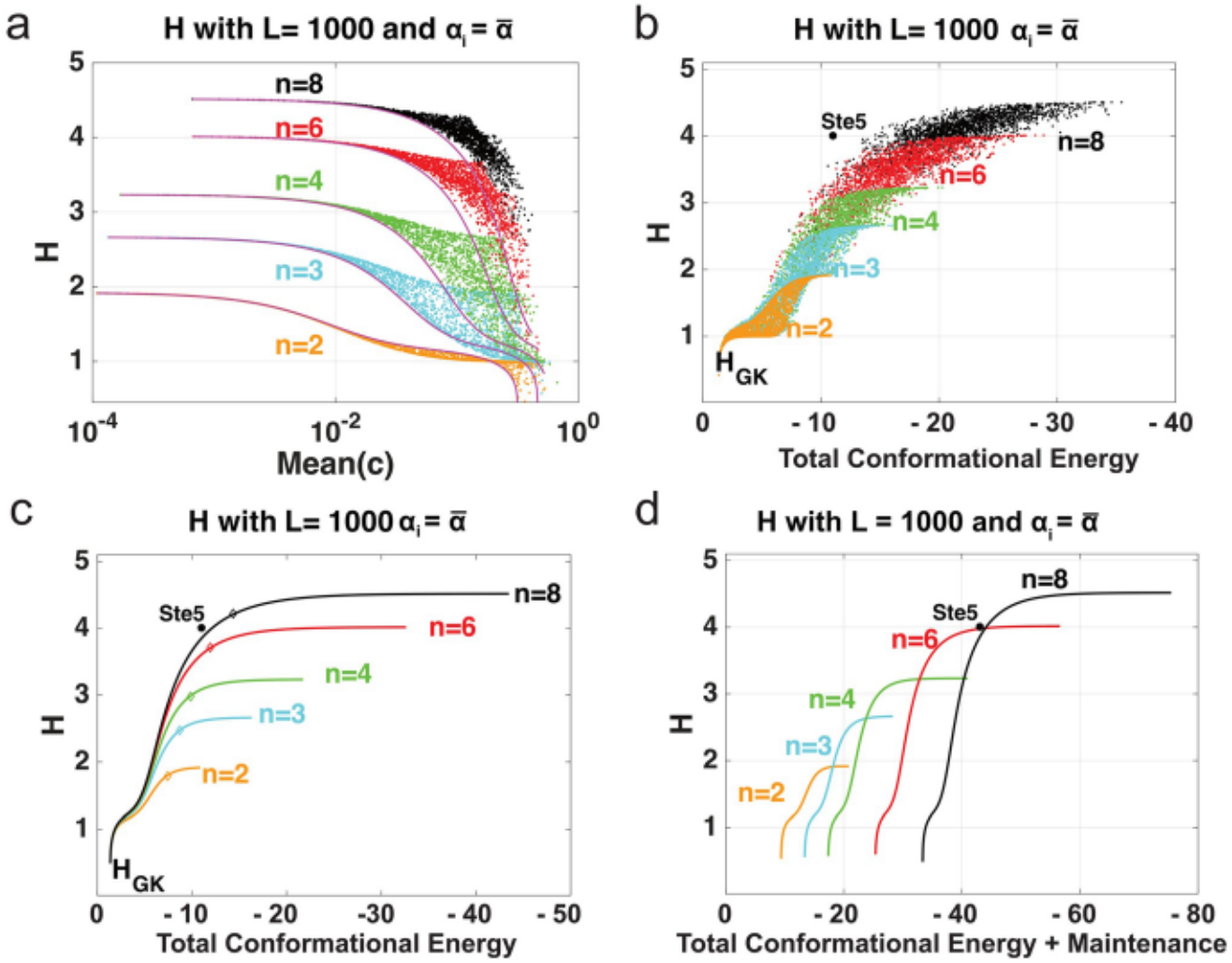
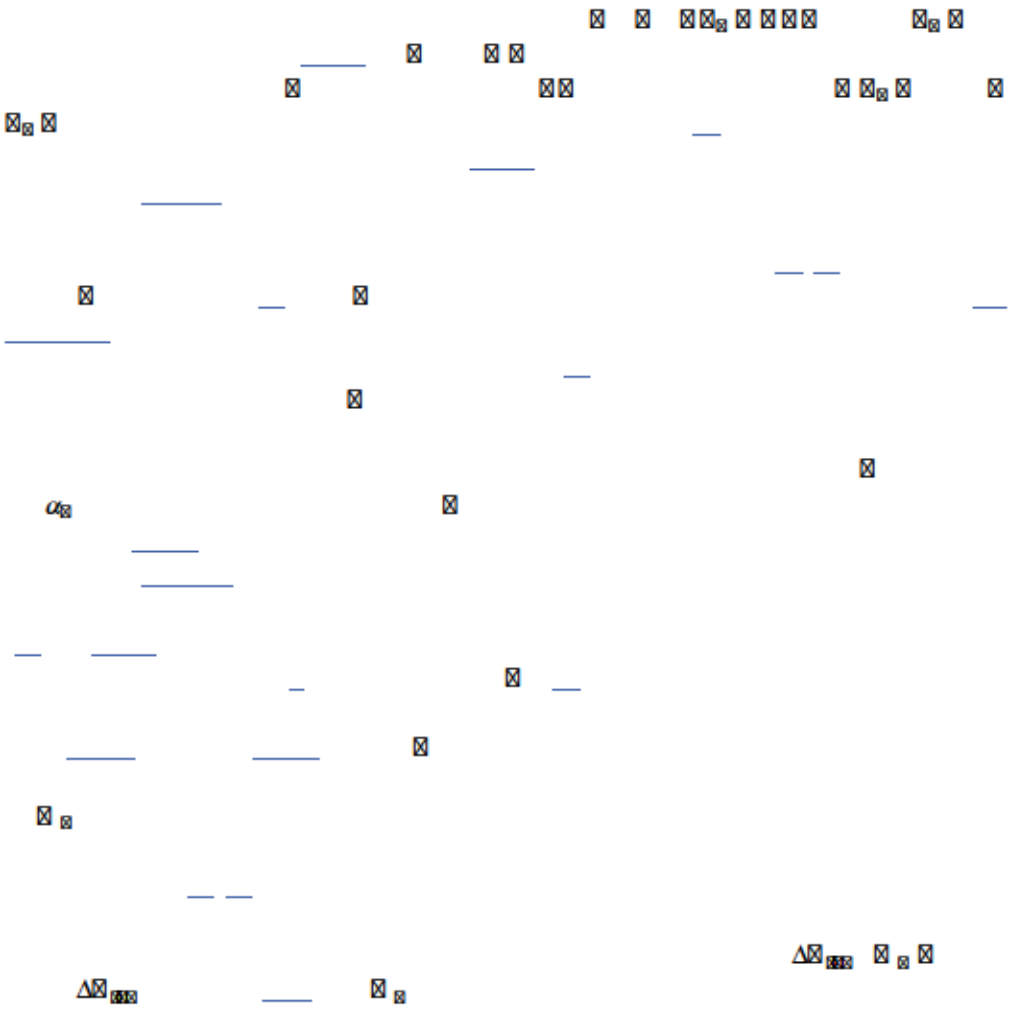


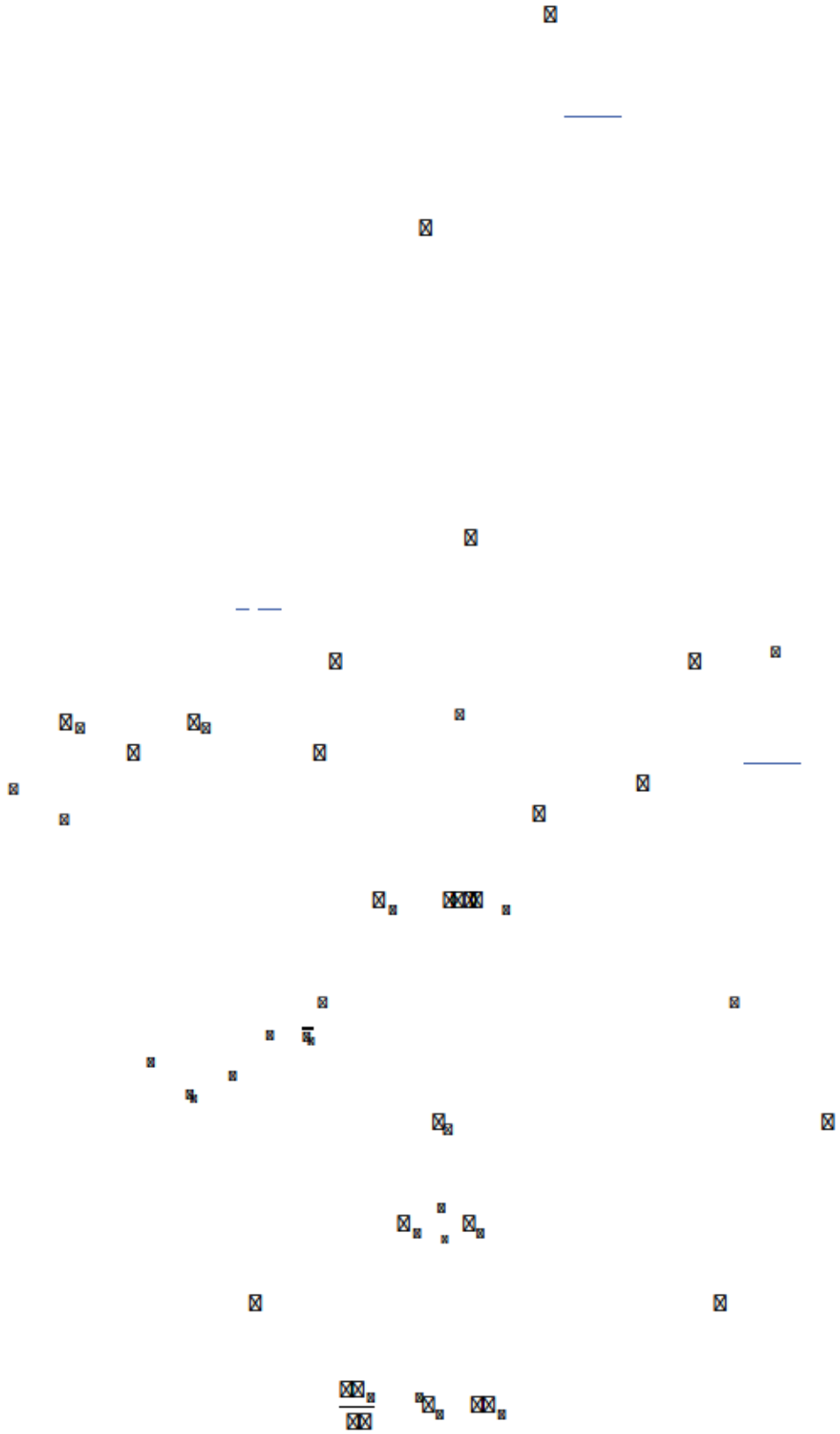
Fig 3. Activation parameters and H in MWC. (a) Scatter plot for H when c_i are independently and logarithmically chosen from the interval $[10^{-4}, 0.9]$ (dots) and when c_i are all identical (solid line), $L = 1000$, and $\alpha_i = \bar{\alpha}$ for $n = 2, 3, 4, 6, 8$. (b) Scatter plot for H for increasing total conformational free energy contribution (Eq 3) when $c_i = c \in [10^{-4}, 0.9]$, $\alpha_i = \bar{\alpha}$, and $L = 1000$ for $n = 2, 3, 4, 6, 8$. Asterisk is the approximated H for Ste5 from [24]. (c) Scatter plot for H for increasing total conformational free energy contribution (Eq 3) when $c_i = \bar{c} \in [10^{-4}, 0.9]$, $\alpha_i = \bar{\alpha}$, and $L = 1000$ for $n = 2, 3, 4, 6, 8$. Diamonds represent the knee of the curve. (d) H for increasing total conformational free energy contribution where $c_i = \bar{c} \in [10^{-4}, 0.9]$, $\alpha_i = \bar{\alpha}$ and $L = 1000$ and with a maintenance cost of 4 kcal/mol per site where $n = 2, 3, 4, 6, 8$. The Ste5 data point is added for illustration purposes with the same maintenance cost for each of the 8 phosphorylation sites.

https://doi.org/10.1371/journal.pcbi.1007966.g003

additional mutations of this type arise and become fixed by natural selection, the molecule will move to the top right of the cloud; here the conformational free energies will be approximately balanced and have magnitudes of approximately -2 to -4 kcal/mol. At this point, substantial improvement to ultrasensitivity (i.e., an increase of the Hill number by greater than 0.5 units) can only arise if the molecule evolves an additional site.

To view this more clearly, consider Fig 3c, which shows ultrasensitivity for increasing values of total conformational free energy contribution where parameter values have been set to $c_i = \bar{c}$ and $\alpha_i = \bar{\alpha} = 1$ and fixed n and L. In other words, when each c_i is the same, meaning the conformational free energy contribution is the same in all sites, we can see that ultrasensitivity generally increases and eventually levels off as the conformational free energy contribution





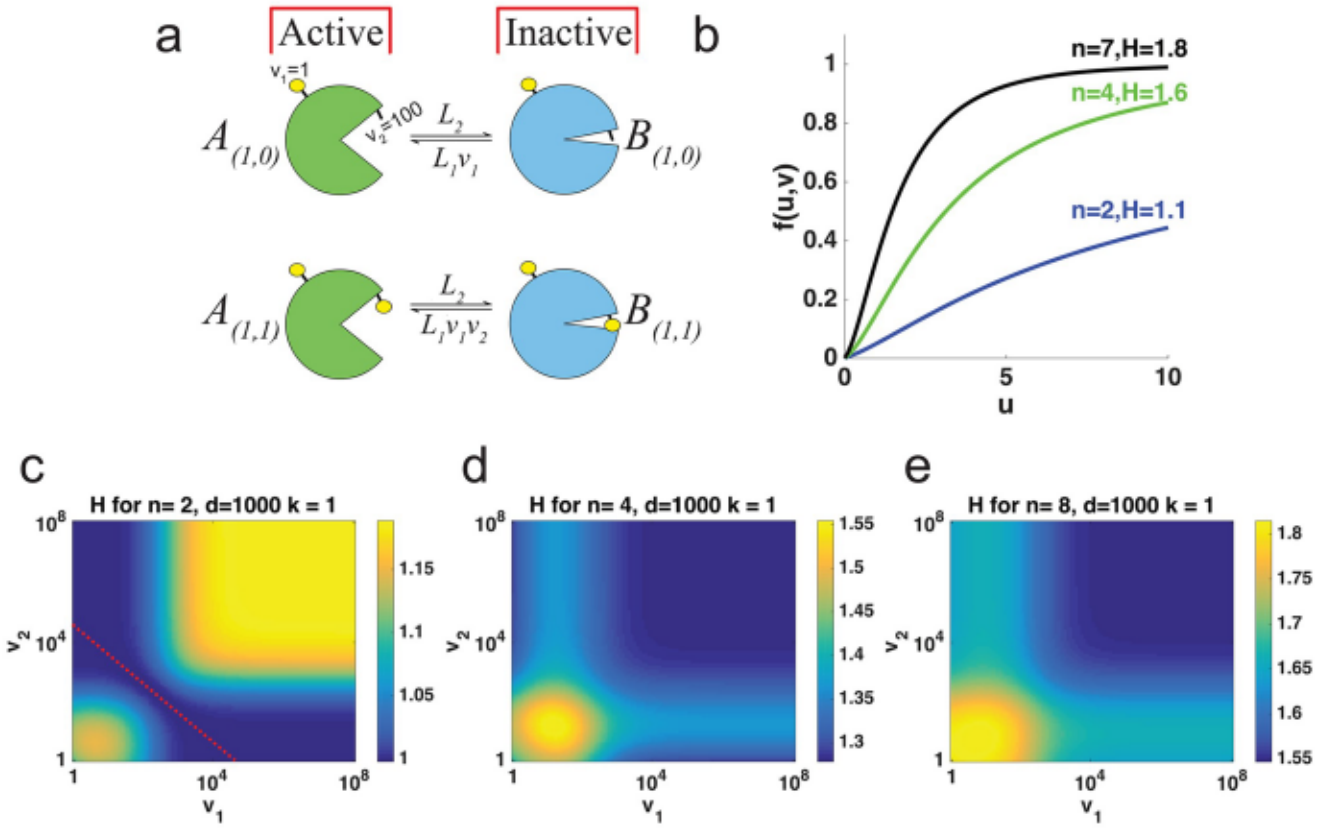


Fig 4. Independent Multisite Modification Model. (a) Target molecule in modified-form I can be in the inactive state B_I or active A_I state. (b) Dose response curve, $f(u, v)$ when $n = 2, 5, 7$ for increasing kinase concentration u with $v_1 = 100$ and $d = 1000$ (c-e) Heat maps for H when $v_1, v_2 \in [10, 10^8]$ with $d = 1000$, and (c) $n = 2$, dashed line used to denote region where $d = \sqrt{v_1 v_2}$. (d) $n = 4$, $v_i = 100$ for all $i \geq 3$, similarly with (e) $n = 7$.

https://doi.org/10.1371/journal.pcbi.1007966.g004

with conservation of mass equation for the target in modified-form I:

$$S_I = A_I + B_I.$$

We allow this reaction to reach equilibrium by assuming that this activation/deactivation reaction is much faster than protein modification. This is a reasonable assumption in the case of protein phosphorylation. Solving for steady state of (4),

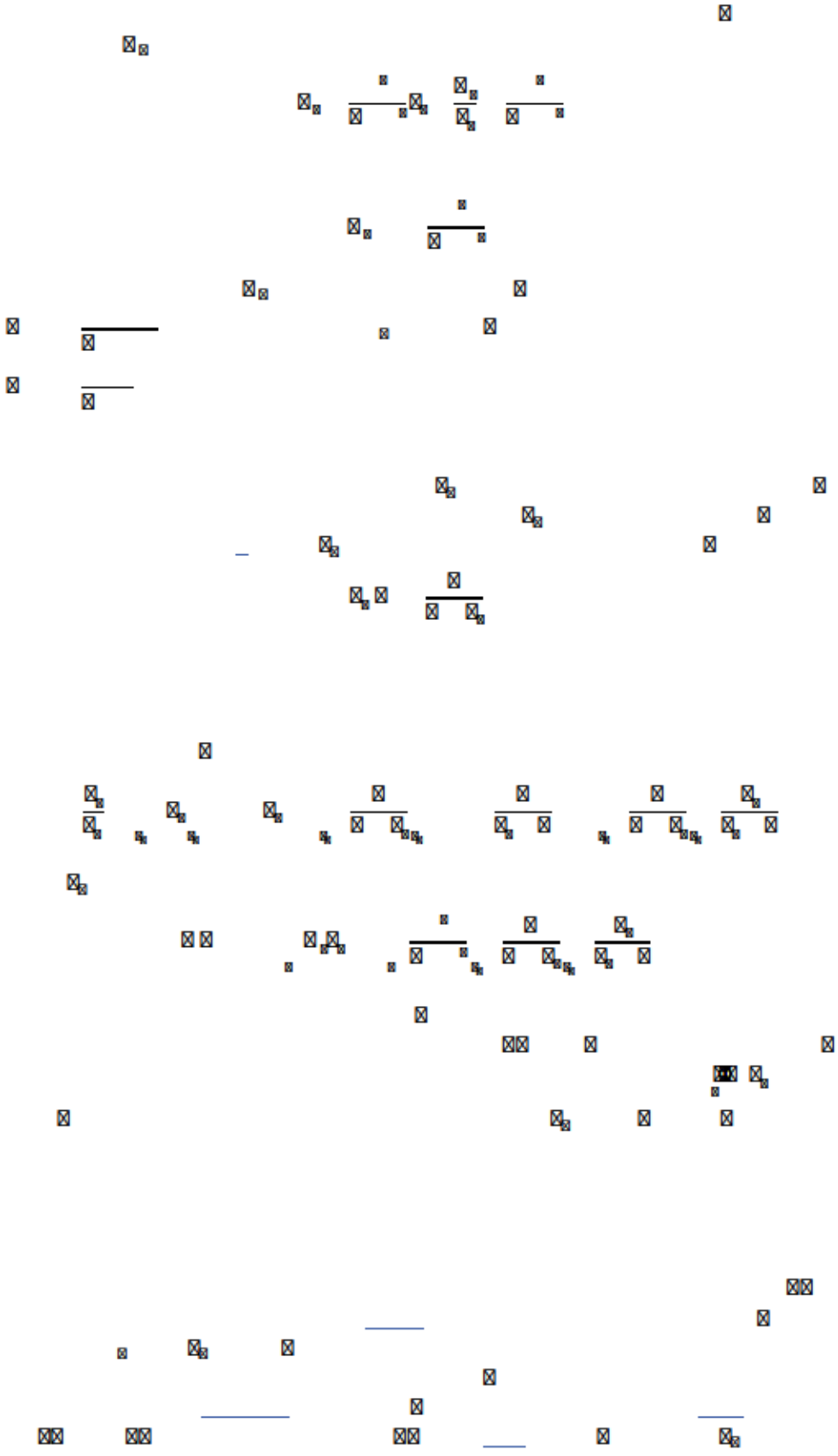
$$0 \quad = v^I(S_I - A_I) - dA_I = v^I S_I - A_I(v^I + d),$$

that is

$$A_I(v^I + d) \quad = v^I S_I$$

and

$$A_I \quad = \frac{v^I}{v^I + d} S_I$$



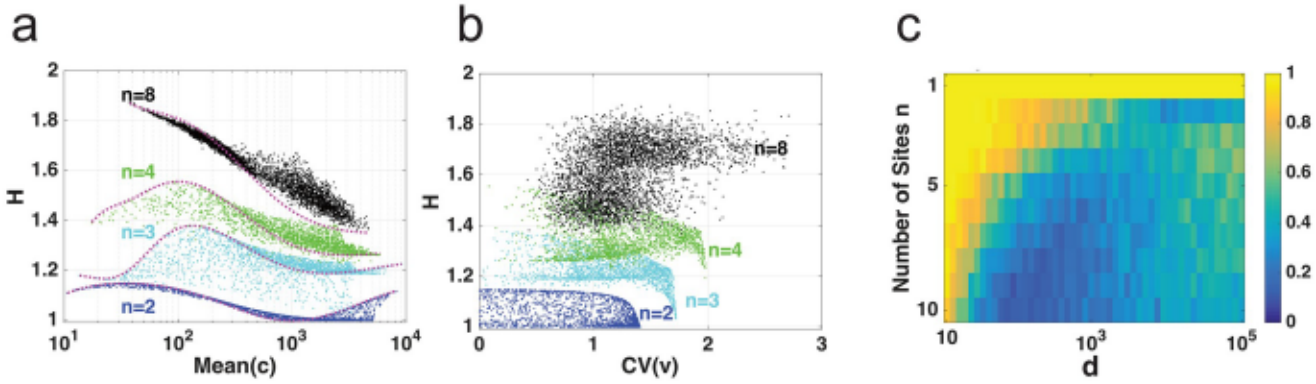


Fig 5. Activation parameters and H in independent model. (a) Scatter plot for H when $v_i \in [10, 10^4]$ (asterisks) and when $v_i = \bar{v}$ for $i = 1, 2, \cdots, n$ (solid curve) for different values of n. (b) H vs $CV(v)$ for different values of n. (c) Heat map showing the proportion of times H increased with increasing v_i as a function of n and d for $v_i \in [10, 10^4]$ and $k_i = 1$ with 1000 simulations of randomly chosen $v_i \in [10, 10^4]$.

https://doi.org/10.1371/journal.pcbi.1007966.g005

parameters v_1 and v_2 were sampled with values in $[1, 10^8]$ logarithmically and each $v_i = 100$ for $i >= 3$ for $n = 2$, 4, and 8. This implies that H does not increase monotonically with increasing v_i, and there is a local minimum for low values of v_i.

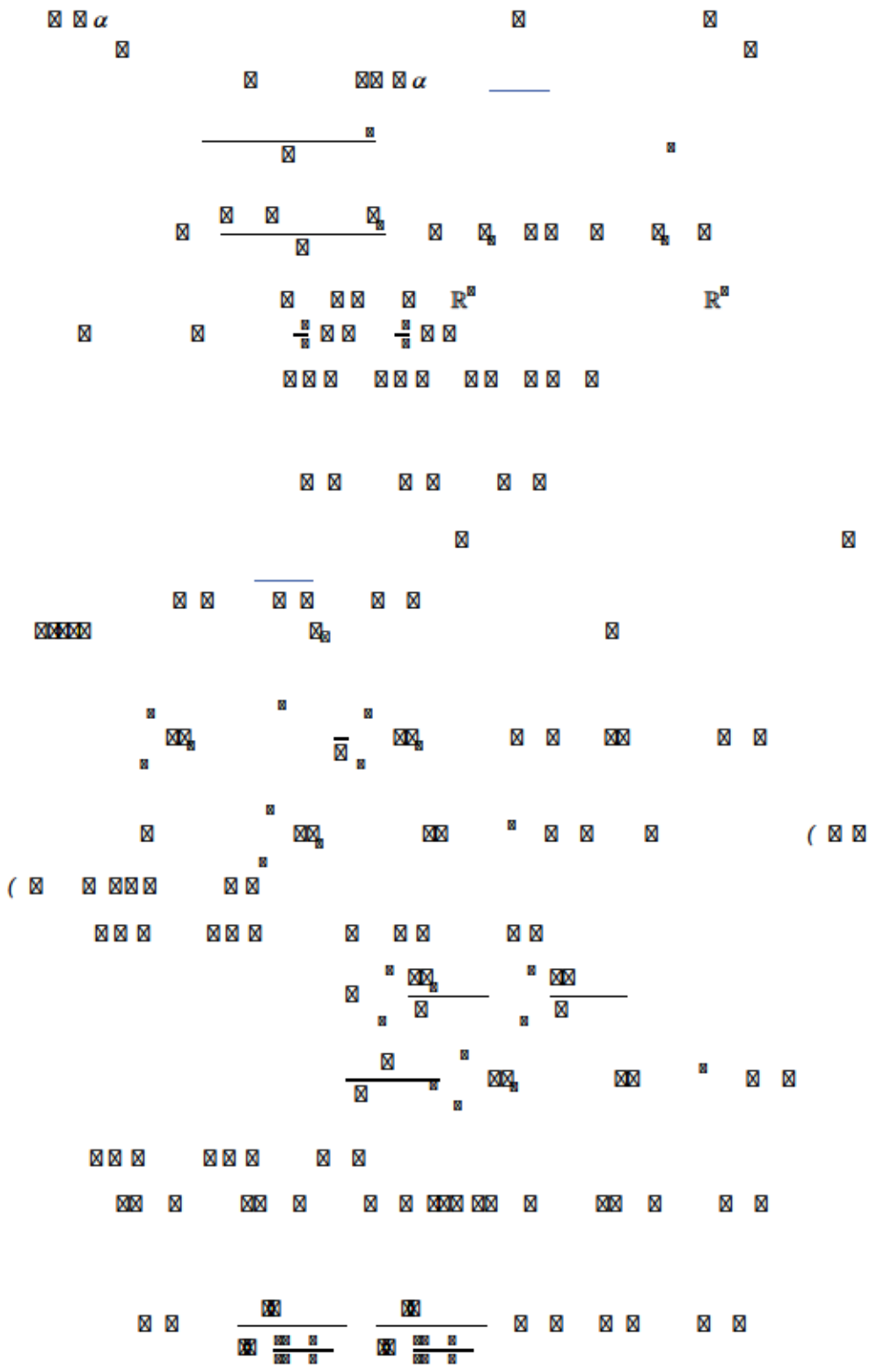
To determine the effect the variability between parameters v_i has on H, we varied parameters v_i, measured H and compared to when all parameters v_i are equal, similar to the MWC system. In Fig 5a, we sampled a vector with entries from $[1, 10^4]$, logarithmically. This v has an arithmetic mean $\bar{v}$ and coefficient of variation, $CV = \frac{sd(v)}{\bar{v}}$. For each sample, there is a second vector, $\hat{v} = (\bar{v}, \bar{v}, \cdots, \bar{v})$ such that $CV(\hat{v}) = 0$. After calculating H for each case, we can see in Fig 5a, that when there is no variation between v_i (solid line), $k_i = 1$ and $d = 1000$, H can increase or decrease depending on the mean of v for $n = 2$, 3, 4 and 8. Here, we can see that any variation between the v_i may affect H (asterisks).

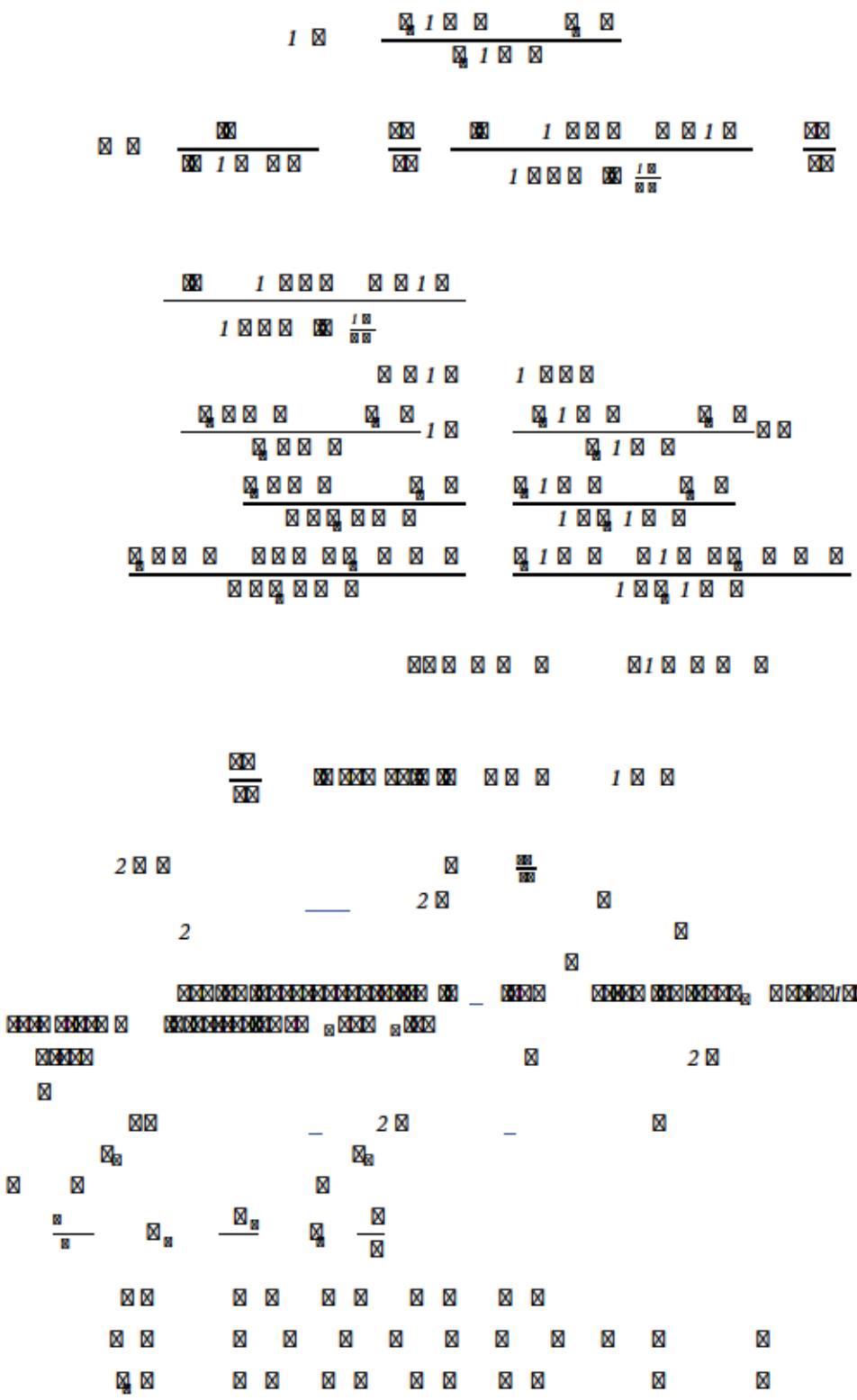
In Fig 5b, we show the same data from Fig 5a but plotting $CV(v)$ vs H. Here we see that $CV(v)$ has some effect on H, regardless of n. This is particularly interesting since, contrary to MWC, the variation between v_i affects H. We also see that there are values of $\bar{v}$ where H increases and values where it decreases. How often is H increasing with increasing v_1?

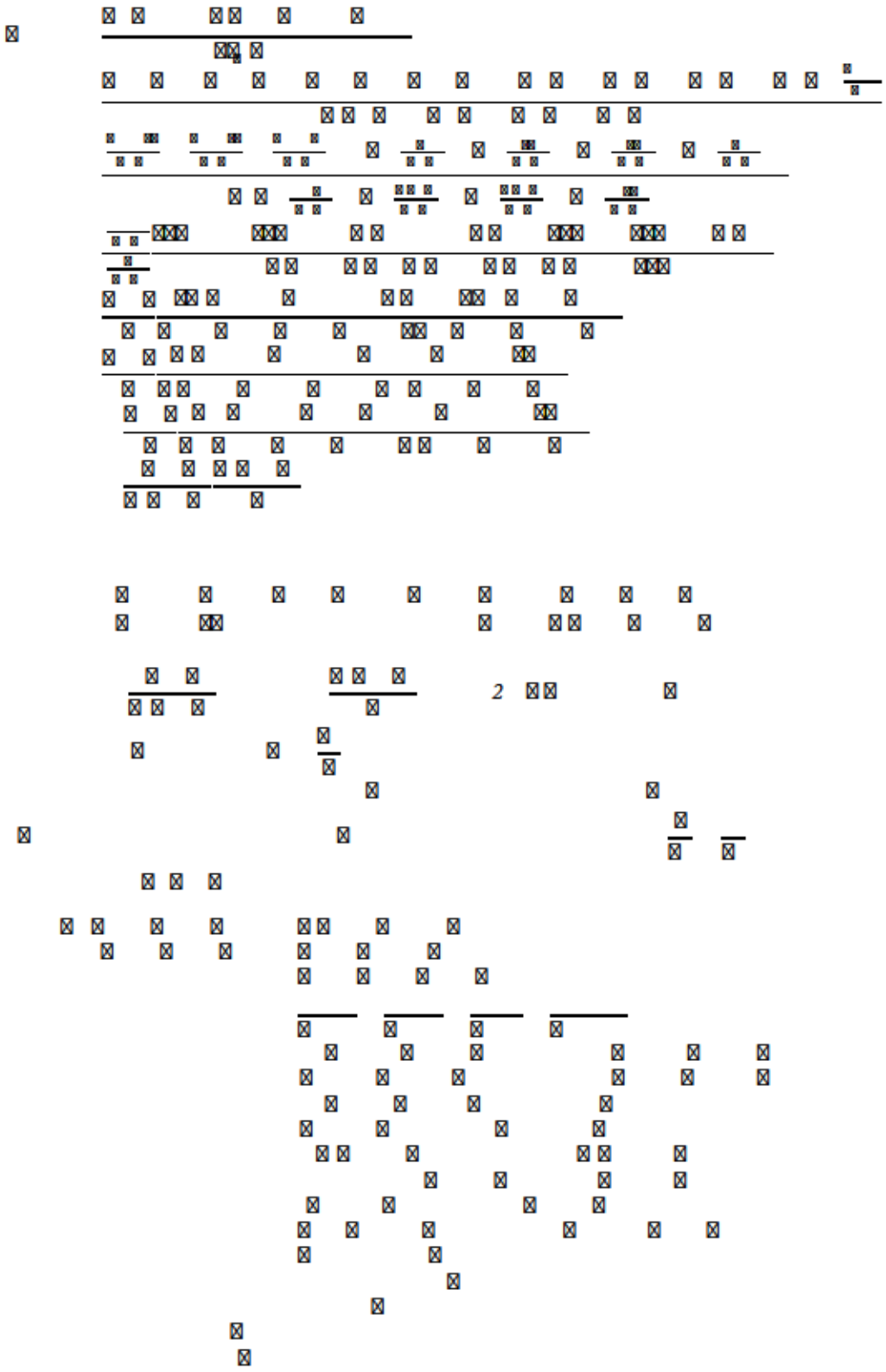
In Fig 5c, similar to S3 Fig panel e, we provide the proportion of simulations where H increases with increasing v_1 based on n and d. The proportion was found in a similar fashion as in the MWC system. Here, we logarithmically sampled $v_i \in [10, 10^4]$ and $k_i = 1$.

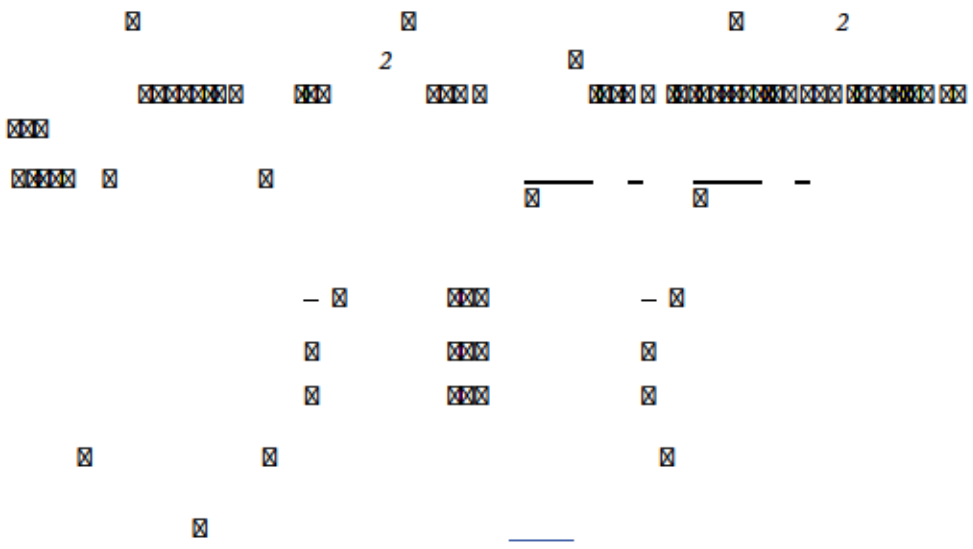
The computational and analytical results described in the section below titled "Independent System Mathematical Analysis" suggest that that $d > \sqrt{v_1 v_2}$ is a biologically reasonable assumption that will give dose response functions where the effect of two modifications is significantly different than the effect of a single modification. Similarly, $d < \sqrt{v_1 v_2}$ gives dose response functions where the effect of a single modification has a similar effect as two modifications, termed "1+" regime. In this "1+" regime we see H increasing on v. When $d = \sqrt{v_1 v_2}$, we have a dose response function where the effect of one modification is approximately 50% of the effect of two modifications, with no ultrasensitivity ($H \approx 1$). We can also see that if d is slightly past the 50% of max activation, H can be maximized by increasing the free activation of energy v.

To summarize, in this section we show that (1) ultrasensitivity increases under specific parameter regimes and (2) may depend on the variability between the activation parameters.



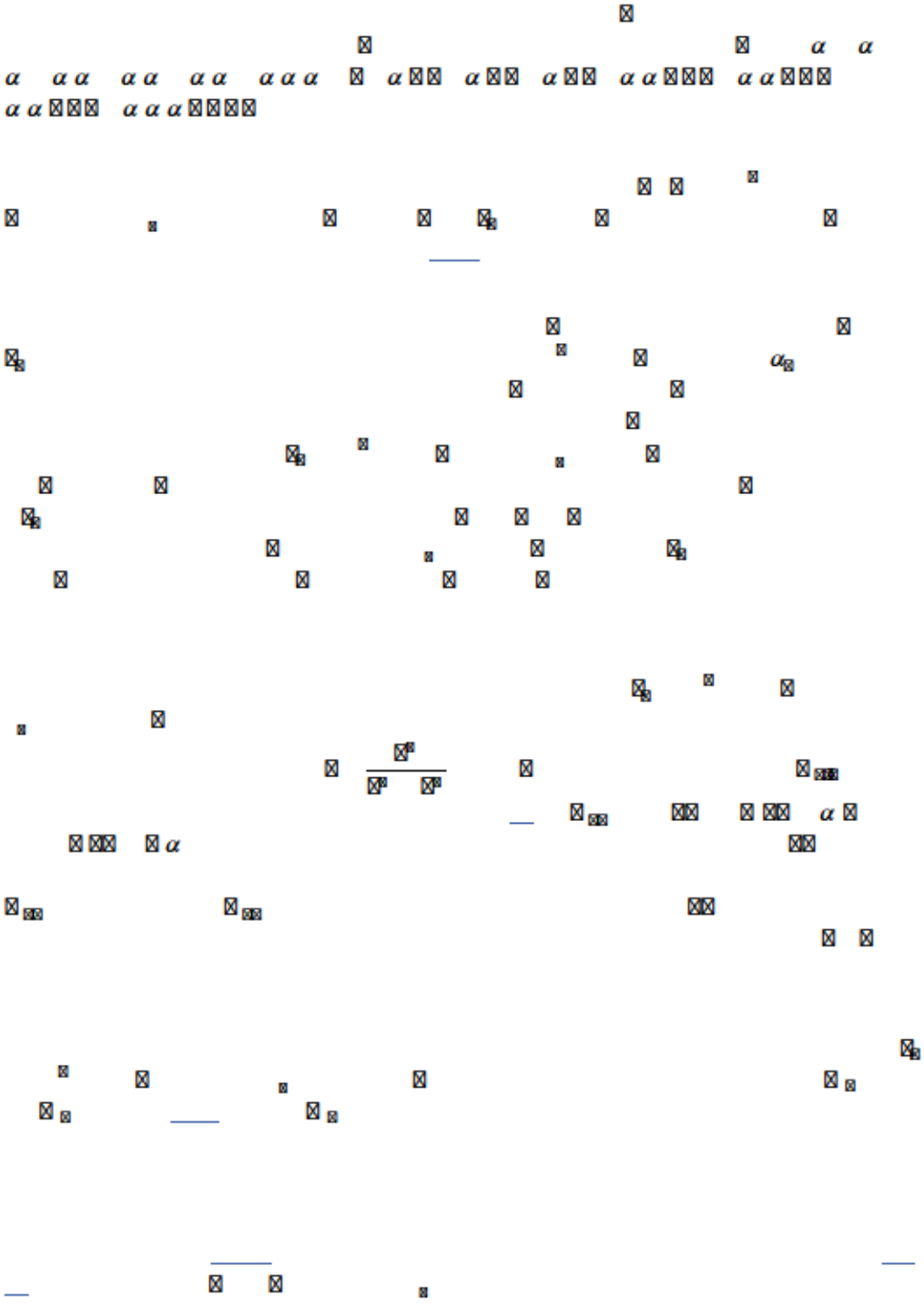






Discussion

Supporting information



Acknowledgments

Author Contributions

References

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.

13.
14.
15.
16.
17.
18.
19.
20.
21.
22.
23.
24.
25.
26.
27.
28.
29.
30.
31.
32.
33.
34.
35.
36.

37.
38.
39.
40.
41.
42.
43.
44.
45.
46.
47.
48.
49.
50.
51.
52.
53.
54.
55.