Electronic Supplementary Material (ESI) for Molecular BioSystems.

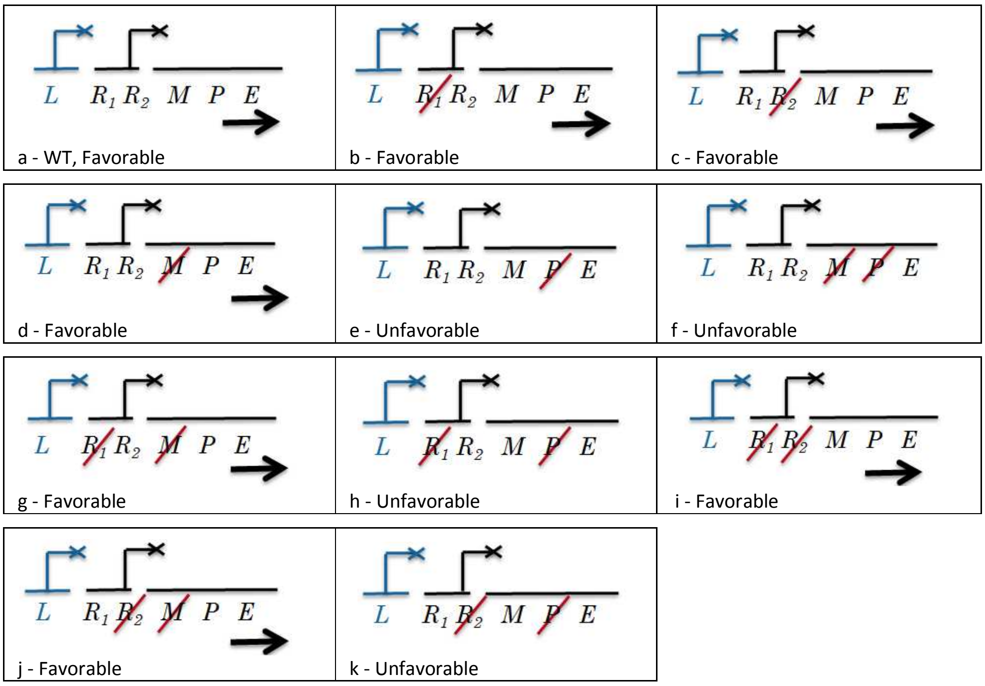

# SI Appendix: Mathematical modeling of the *apo* and *holo* transcriptional regulation in *Escherichia coli*

Fernando J. Álvarez-Vásquez, Julio A. Freyre-González, Yalbi I. Balderas-Martínez, Mónica I. Delgado-Carrillo, Julio Collado-Vides

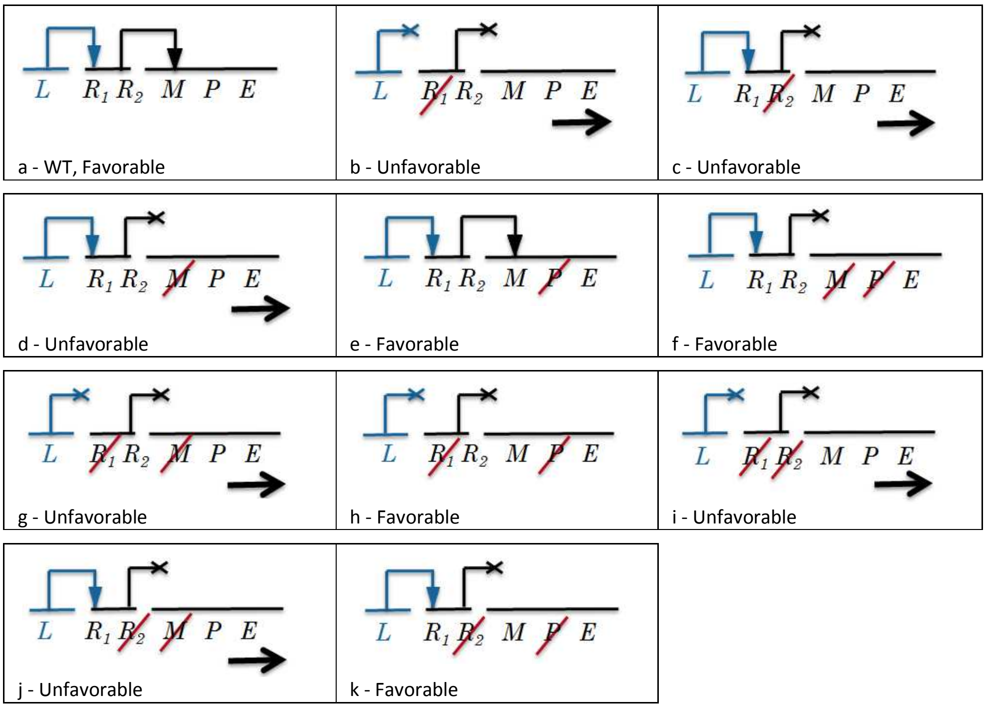
## Contents

# 1.- SI Figures

## Fig. S1




## Fig. S2



**Figs. S1-2. Regulation by *TrpR* of repressible system with negative control during high (Fig S1) and low demand (Fig S2).** The DNA can mutate (diagonal red line) in the modulator (*M*), promoter (*P*), and/or in the regulator site, $R_1$ if the mutation occurs in the TF-ligand domain or in $R_2$ if the mutation occurs in the TF-DNA binding domain. The horizontal arrow represents the gene expression of the structural gene (*E*). A dotted blue line starting from $R_2$ and ending in an arrowhead indicates interaction of the TF with the DNA; if the blue line ends in an X, it represents no TF-DNA interaction with the operon. Fig. S1: High demand; *a*) wild type, *b*-*e*) four single mutants, *f*-*k*) six double mutants. Fig. S2: Low demand; *a*-*k*) similar to Figs. 4a.

**Fig. S3**

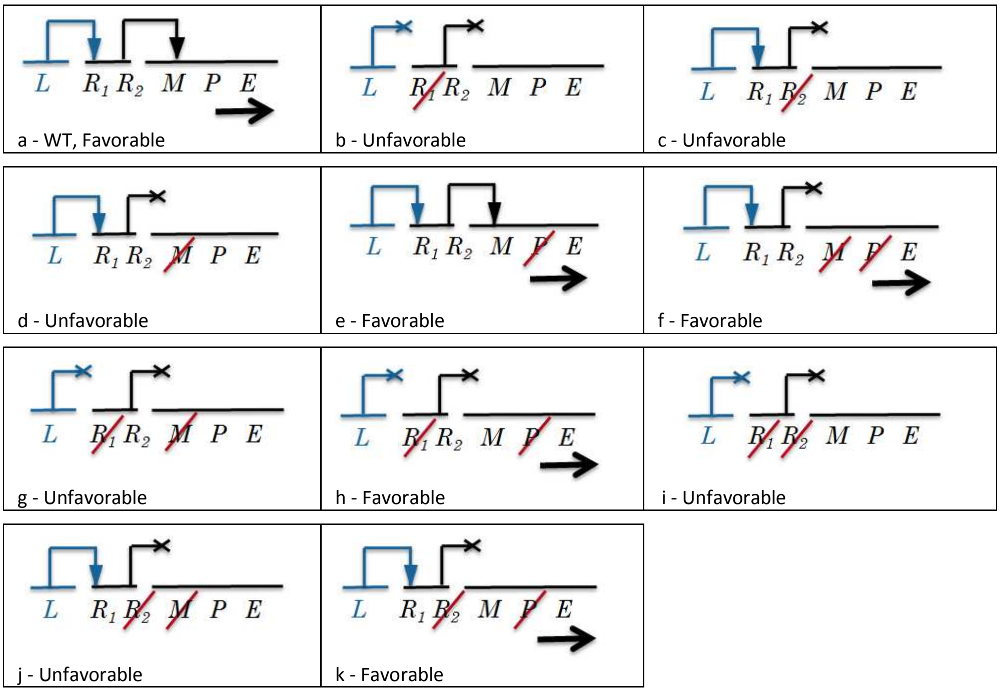
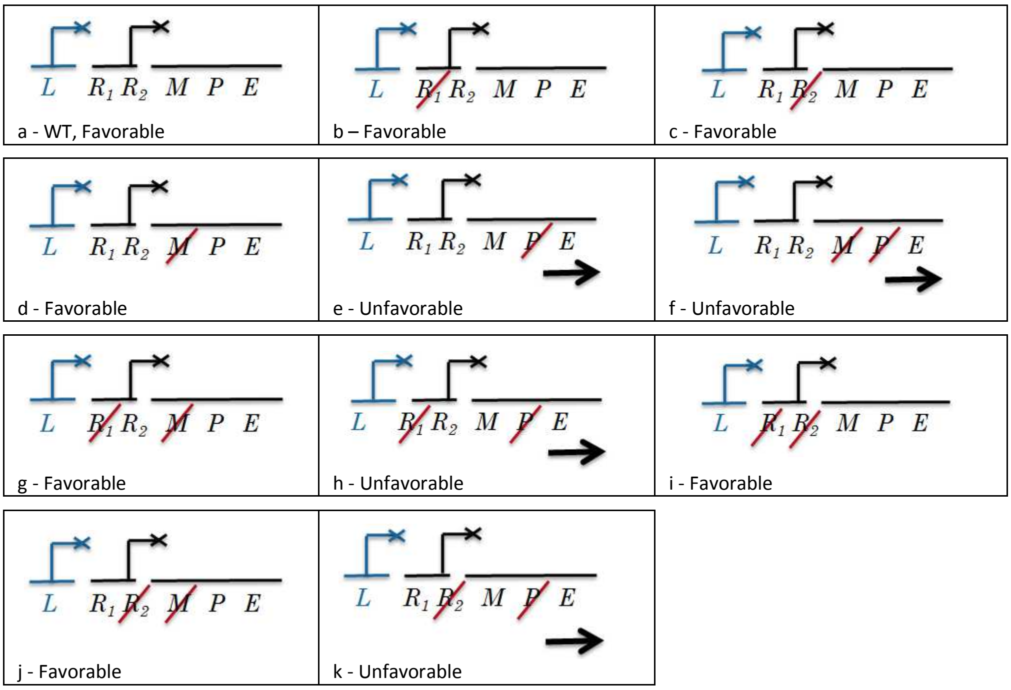



**Fig. S4**




**Figs. S3-4. Regulation by *MalT* of inducible system with positive control during high (Fig. S3) and low demand (Fig. S4).** For explanation of symbols see legend for Figs. S1-2.

**Fig. S5**

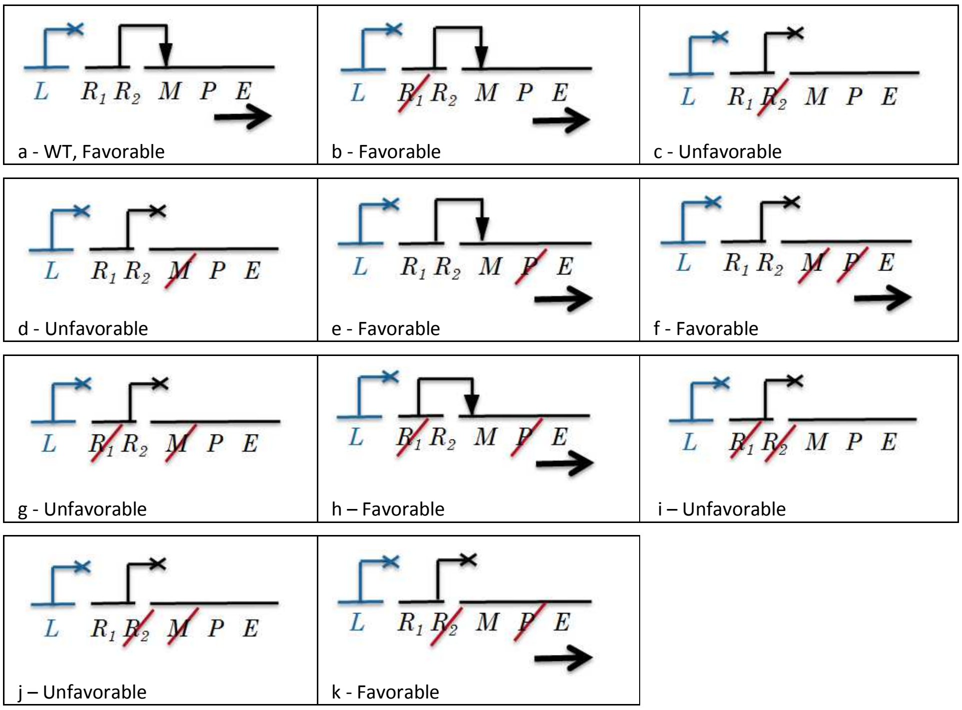
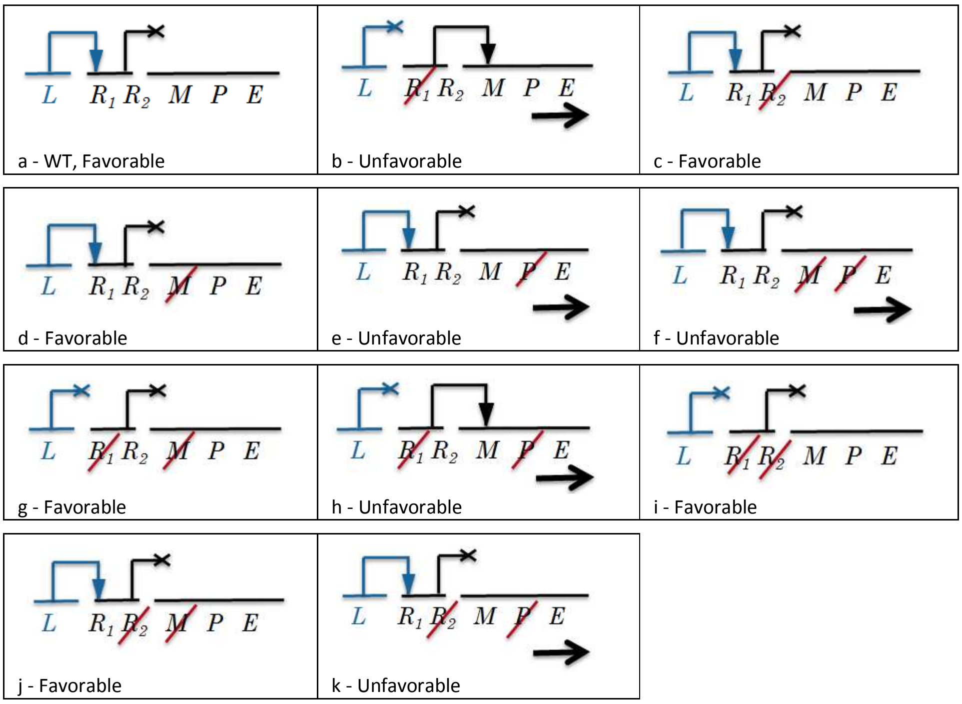
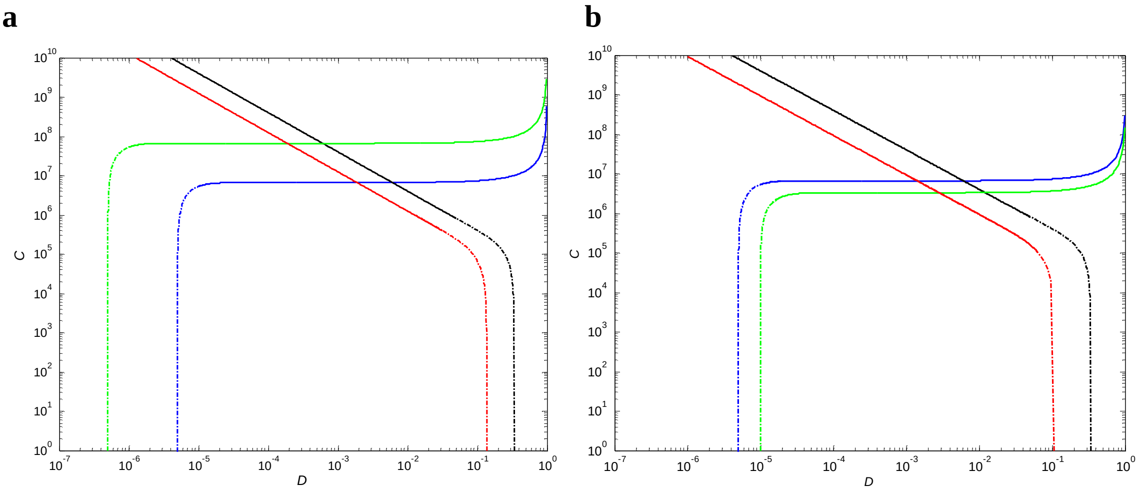


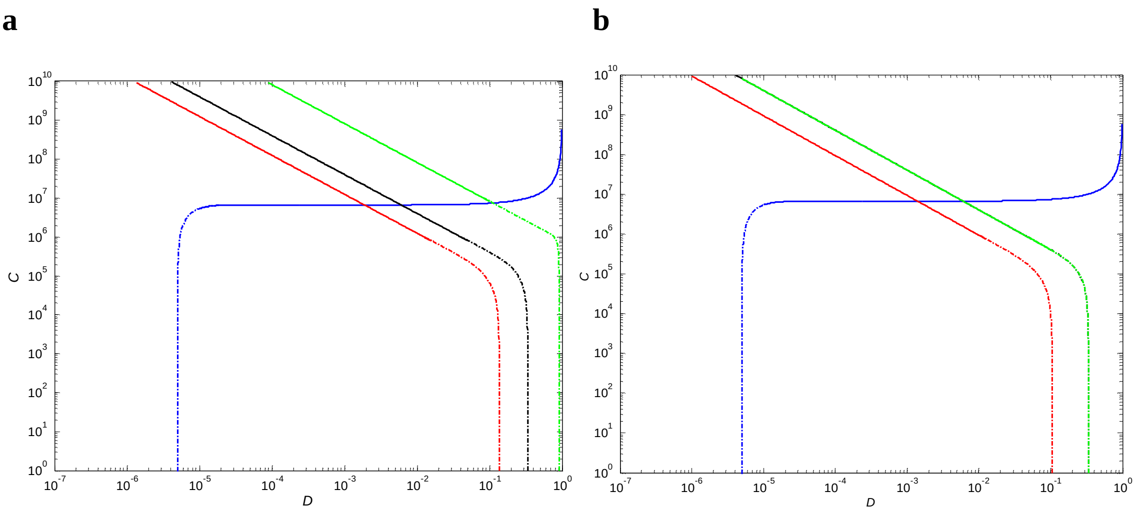

**Fig. S6**



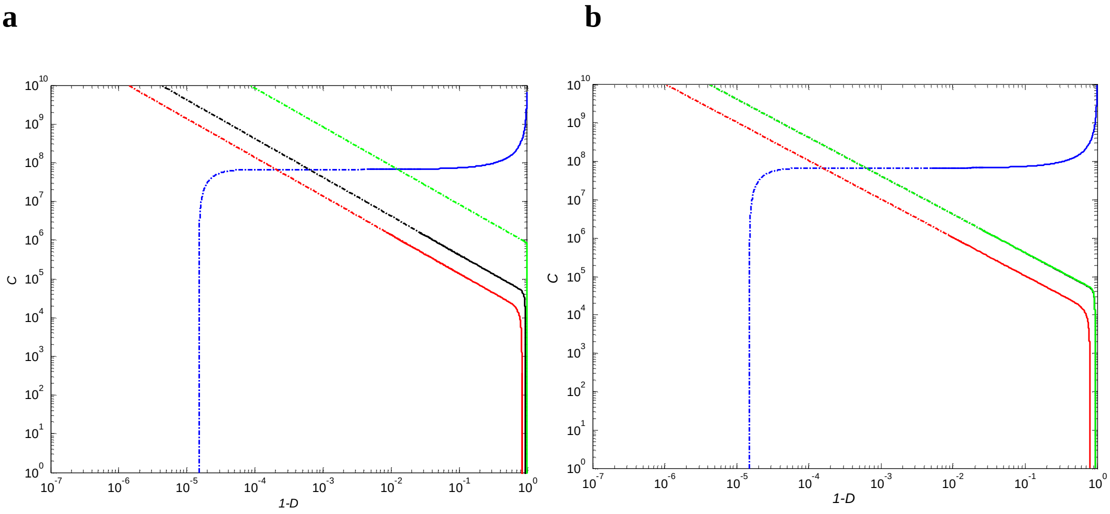

**Figs. S5-6. Regulation by *Cbl* of positive regulation during high (Fig. S5) and low (Fig. S6) demand.** For explanation of symbols see legend for Figs. S1-2.



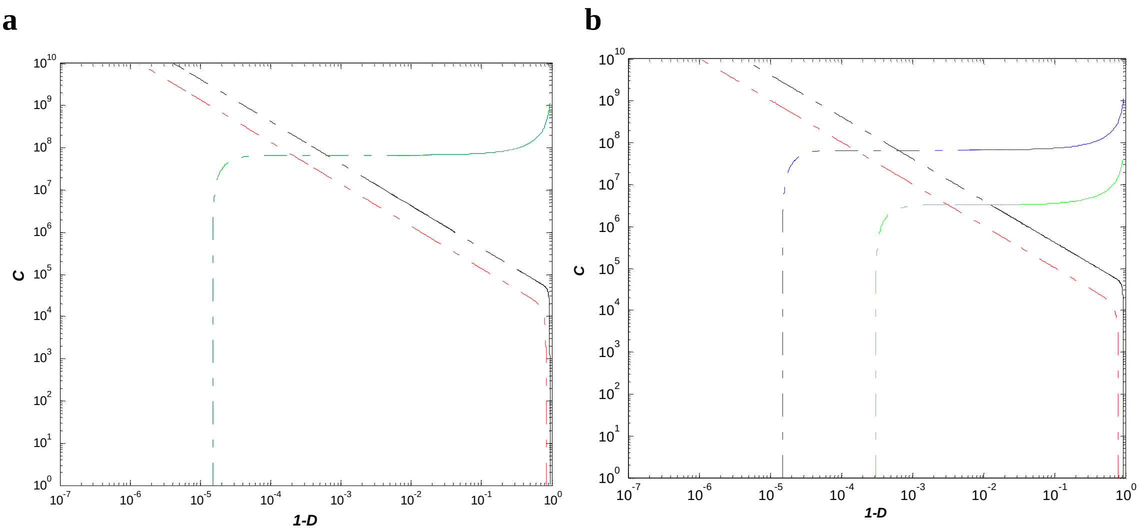

**Figs. S7. *LacI* threshold for selection of the wild-type regulatory mechanism**. The thresholds are represented as functions of the cycle time and the demand for gene expression with negative mode of regulation. The demand (*D*) and the total cycle (*C*) are represented on a logarithmic scale. The thresholds are for the promoter (*p*) in blue, modulator (*m*) in black, TF-effector regulatory section ($r_1$) in green, and TF-DNA regulatory section ($r_2$) in red. The solid and dotted line intervals for each curve represent the low- and high-C asymptotes, respectively, where the root finding method was implemented. Curves when: *a)* ω = 1; *b*) ω = 20.



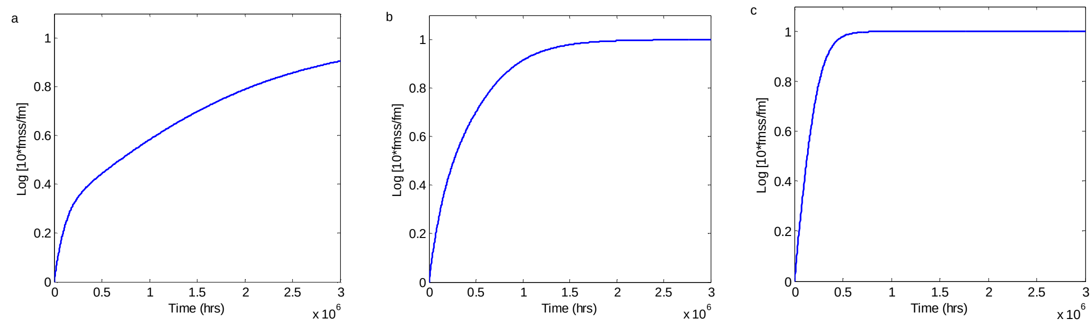

**Figs. S8. *TrpR* threshold for selection of the wild-type regulatory mechanism**. Descriptions as in Figs. S7. Curves when: *a)* ω = 1; *b*) ω = 20, the curves for $X_{r_1}/X_w$ and $X_m/X_w$ are superimposed.



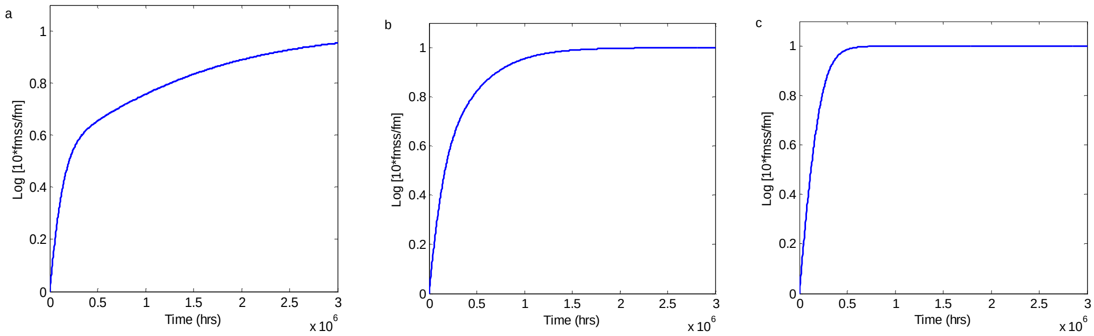

**Figs. S9. *MalT* threshold for selection of the wild-type regulatory mechanism**. Thresholds are represented as functions of the cycle time and the demand for gene expression with positive mode of regulation. The demand (1-*D*) and the total cycle (*C*) are represented on a logarithmic scale. The thresholds presented are for the promoter ($p$) in blue, modulator ($m$) in black, TF-effector regulatory section ($r_1$) in green, and TF-DNA regulatory section ($r_2$) in red. The solid and dotted line intervals for each curve represent the low- and high-C asymptotes, respectively, where the root finding method was implemented. Curves when: *a)* ω = 1; *b*) ω = 20, the curves for $X_{r_1}/X_w$ and $X_m/X_w$ are superimposed.



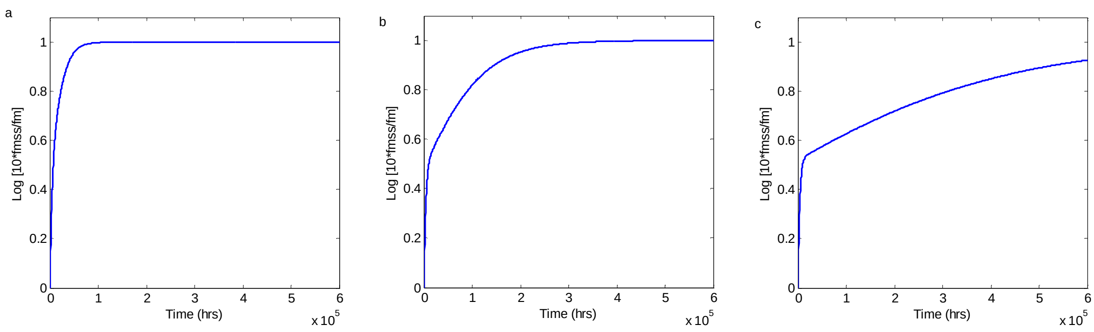

**Figs. S10. *Cbl* threshold for selection of the wild-type regulatory mechanism**. Descriptions as in Figs. S9. Curves when: *a)* ω = 1, the curves for $X_{r_1}/X_w$ and $X_p/X_w$ are superimposed; *b*) ω = 20.



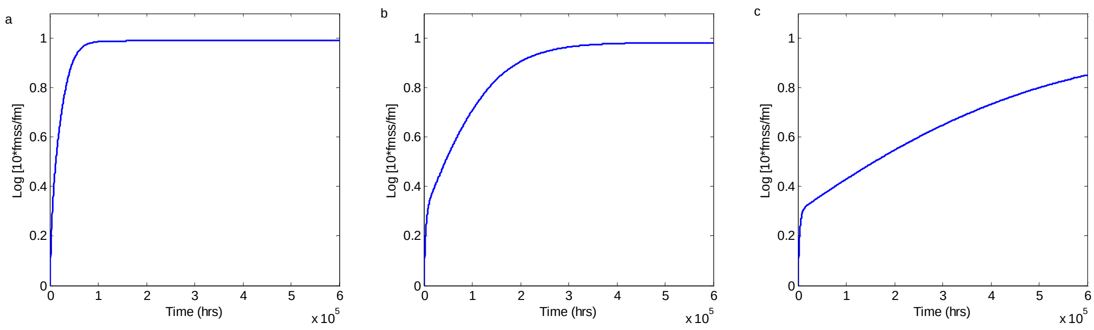

**Figs. S11. *LacI* enrichment of the wild-type regulatory mechanism over time.** The wild-type enrichment was calculated as a ratio with respect to the mutant populations $X_m$, $X_p$, $X_{r1}$, and $X_{r2}$. The recursive ratio $f_m = X_w / (X_m + X_p + X_{r1} + X_{r2})$ was calculated from Eqs. S33-S34 and Eqs. S31-S32 to calculate $f_m^{SS}(C)$ and $f_m(t + C)$, respectively. The normalized log ratio is enclosed between [0,1] and is given by $10\times[X_w / X_w / (X_m + X_p + X_{r1} + X_{r2})] / [X_w / X_w / (X_m + X_p + X_{r1} + X_{r2})]^{SS}$ or $10\, f_m^{SS} / f_m$. For all cases the cycle time is $C = 3000$. All enrichments were calculated with $\omega = 20$. *a*) $D = 0.0000316$; *b*) $D = 0.0001$; *c*) $D = 0.000316$.




**Figs. S12. *TrpR* enrichment of the wild-type regulatory mechanism over time.** Procedure as in Figs. S11. For all cases $C = 3000$. *a*) $D = 0.0000316$; *b*) $D = 0.0001$; *c*) $D = 0.000316$.



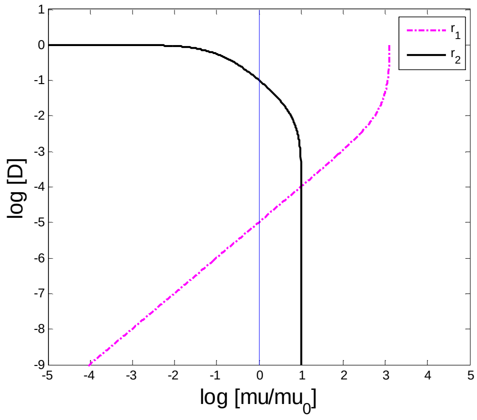

**Figs. S13. *MalT* enrichment of the wild-type regulatory mechanism over time.** Procedure as in Figs. S11. For all cases $C$ = 300. *a*) $D$ = 0.937; *b*) $D$ = 0.985; *c*) $D$ = 0.996.




**Figs. S14. *Cbl* enrichment of the wild-type regulatory mechanism over time.** Procedure as in Figs. S11. For all cases $C$ = 300. *a*) $D$ = 0.937; *b*) $D$ = 0.985; *c*) $D$ = 0.996.

**Figs S15-a.-** *mu* (μ)

*LacI*

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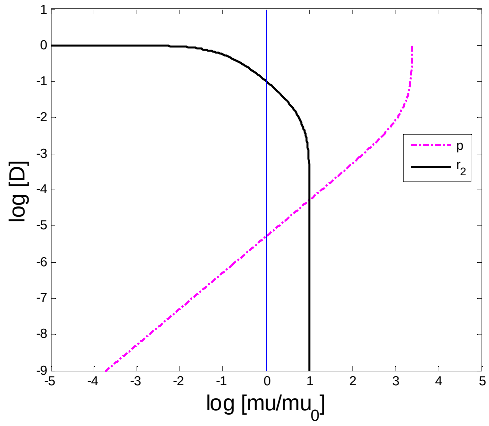
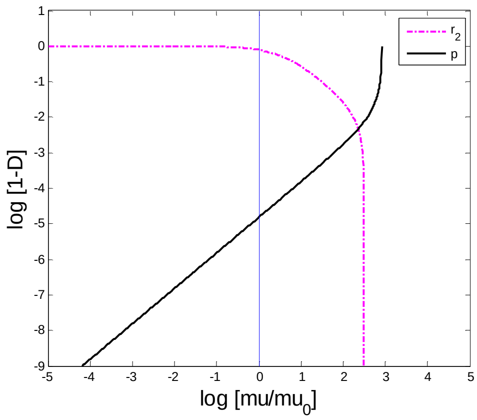



*MalT*

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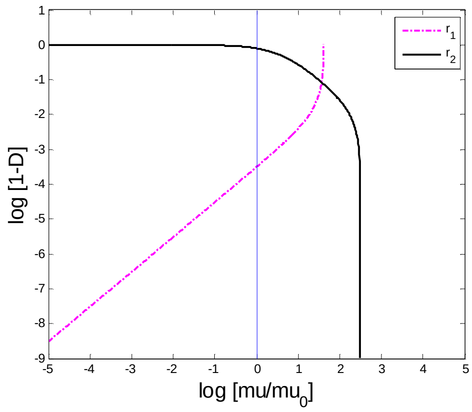
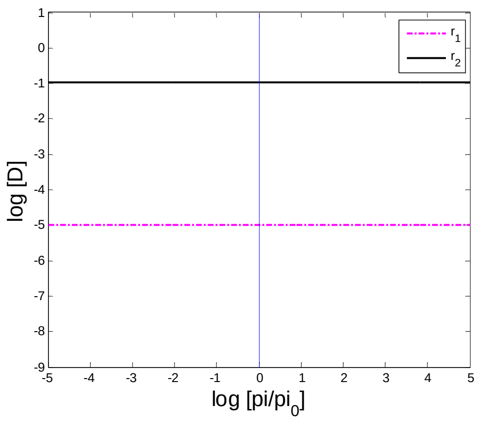



**Figs. S15-b.-** *pi* (π)

*LacI*

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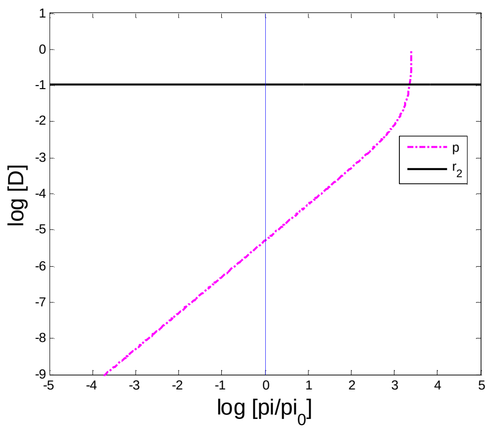
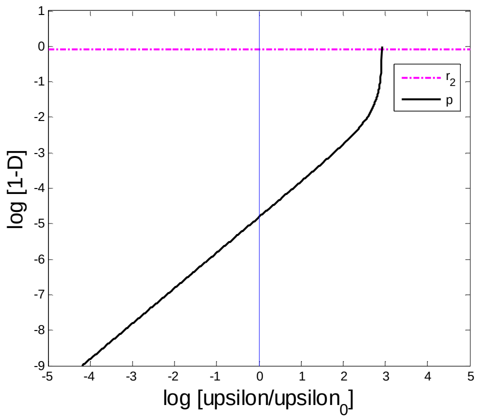
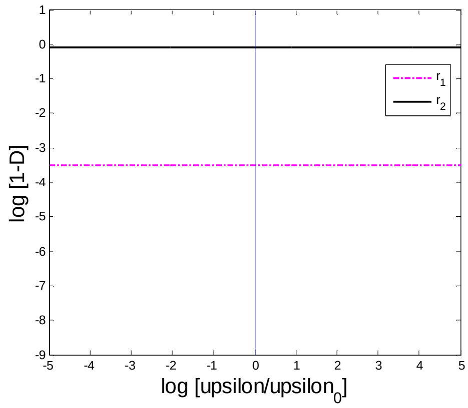


**Figs. S15-c.-** *upsilon* (υ)

*MalT*

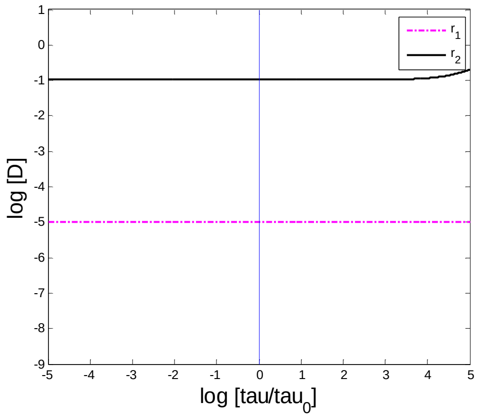



*Cbl.*




**Figs S15-d.-** *tau* (τ)

*LacI*

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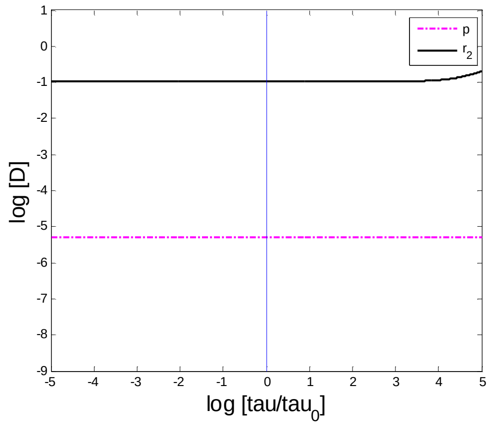


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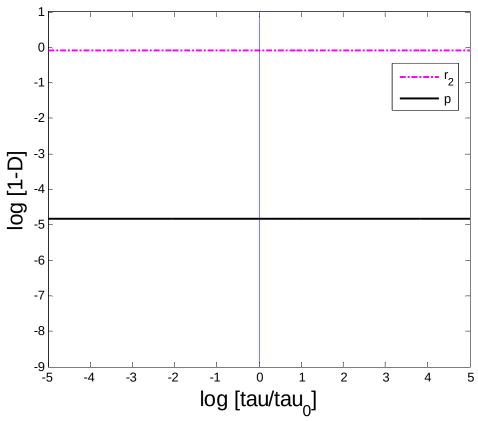


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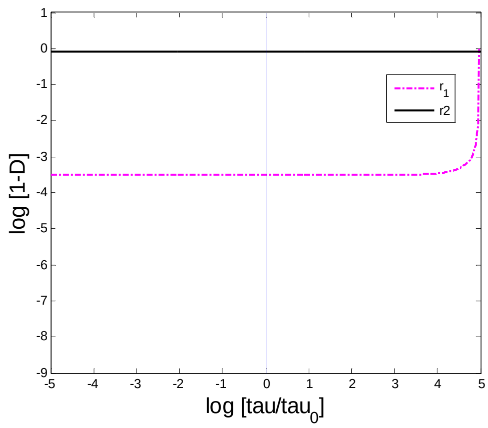
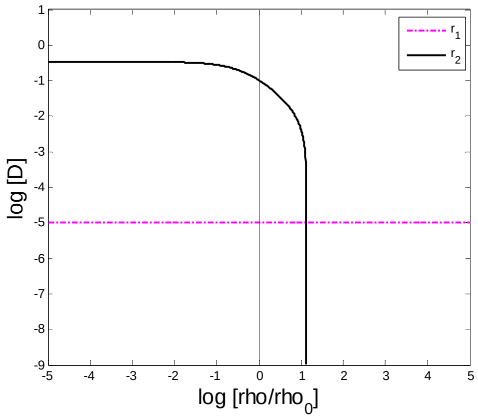


**Figs S15-e.-** *rho* (ρ)

*LacI*

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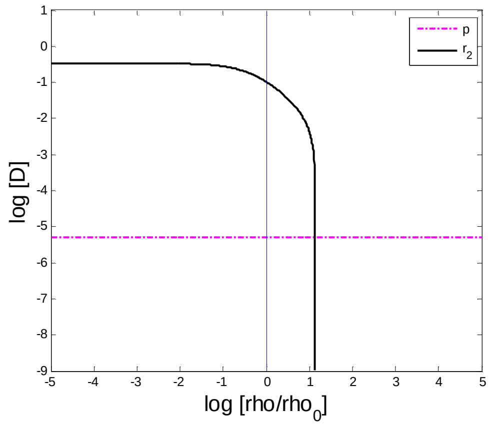


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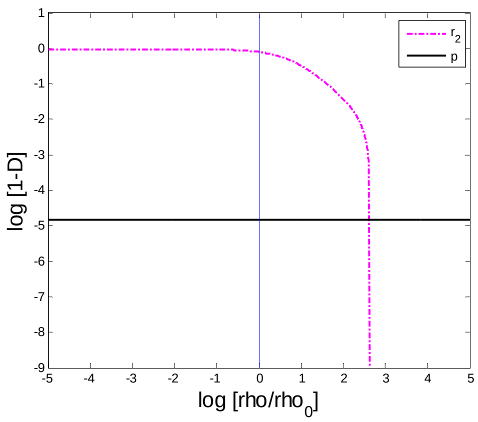


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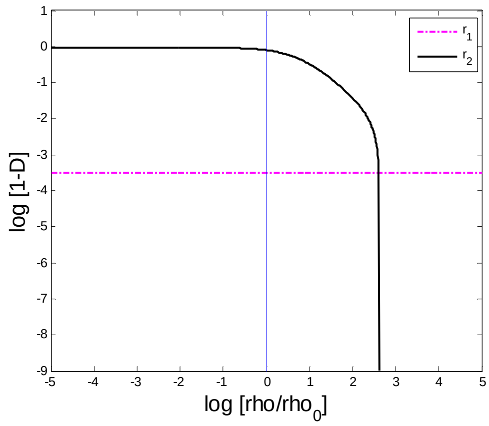
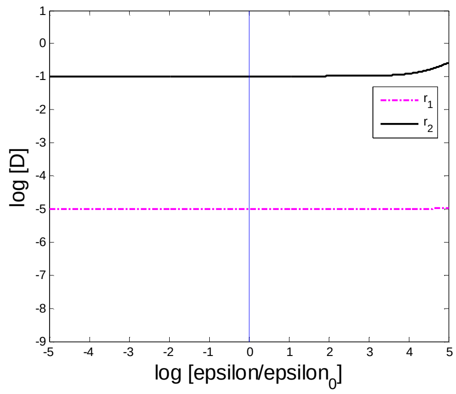



**Figs S15-f.-** *epsilon* (ε)

*LacI*

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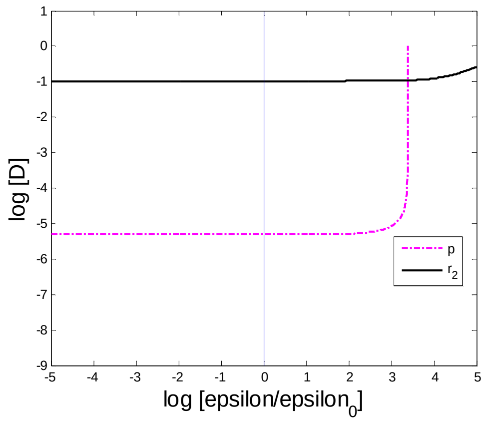

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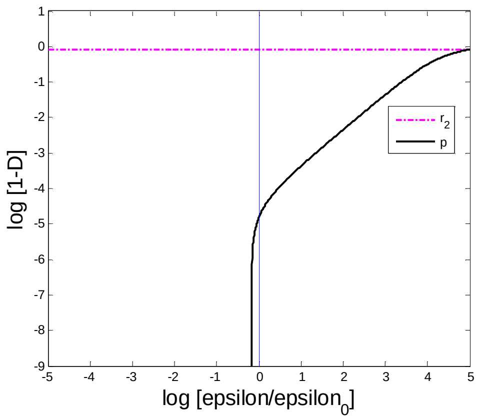


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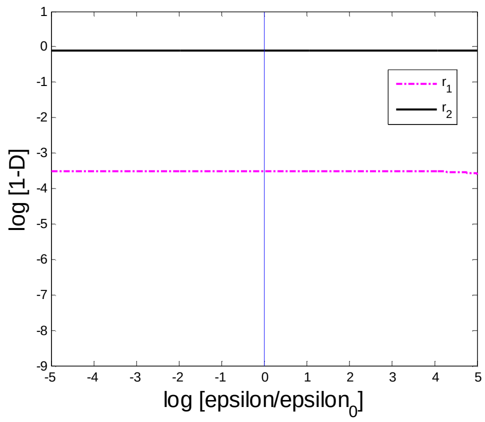
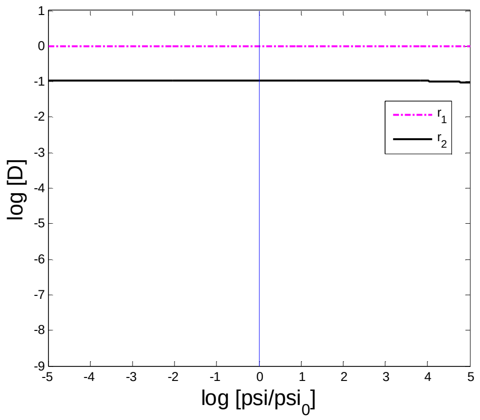



**Figs S15-g.-** *psi* ($\psi$)

*LacI*

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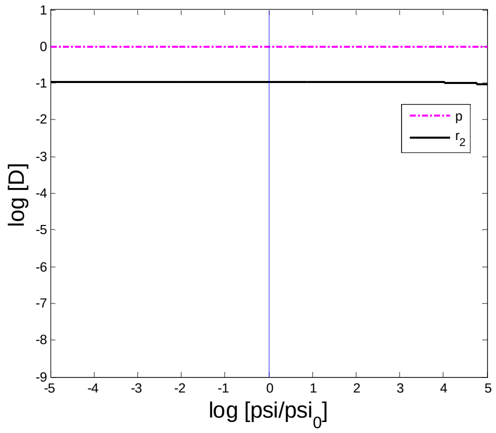


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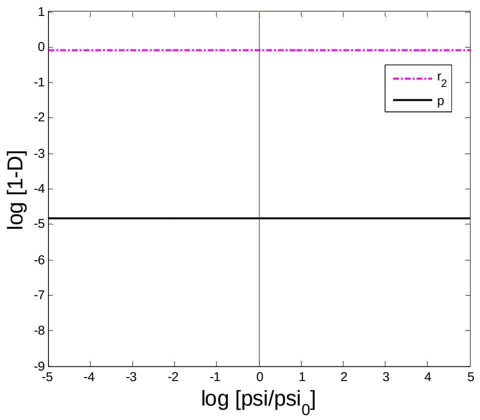


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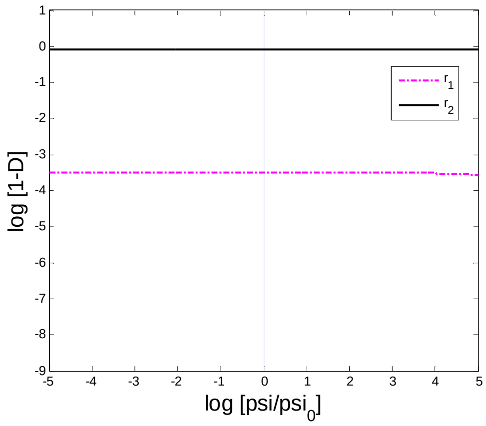
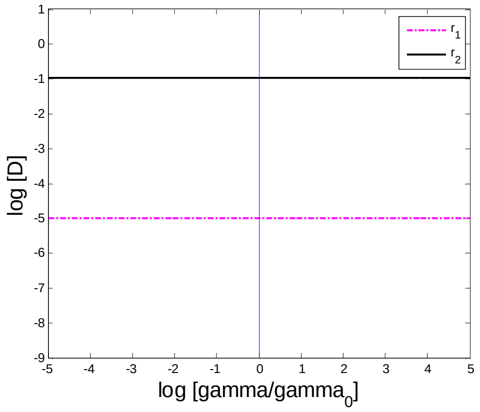


**Figs S15-h.-** *gamma* (γ)

*LacI*

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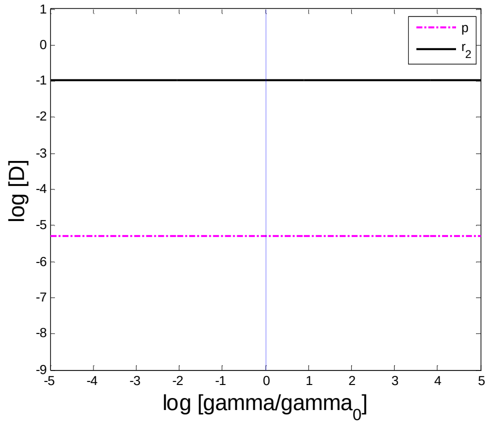


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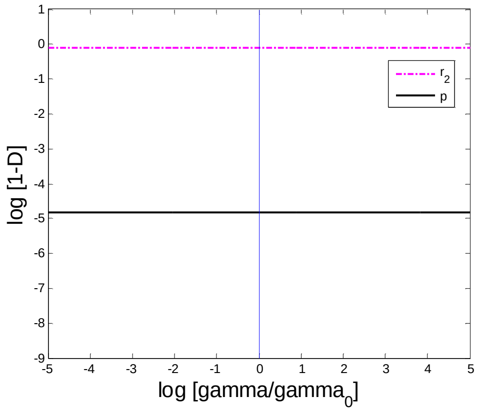


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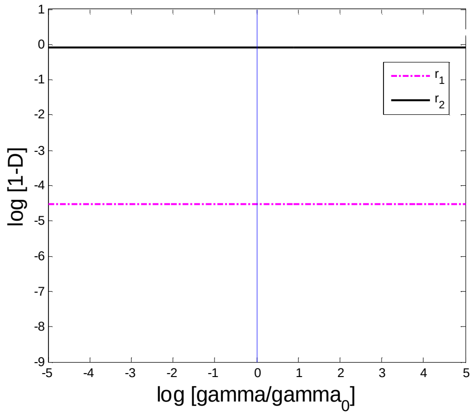
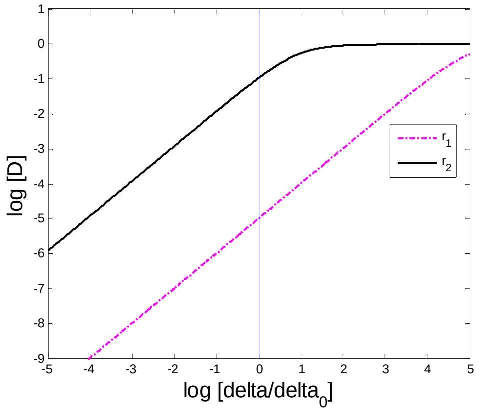



**Figs S15-i.-** *delta* (δ)

*LacI*

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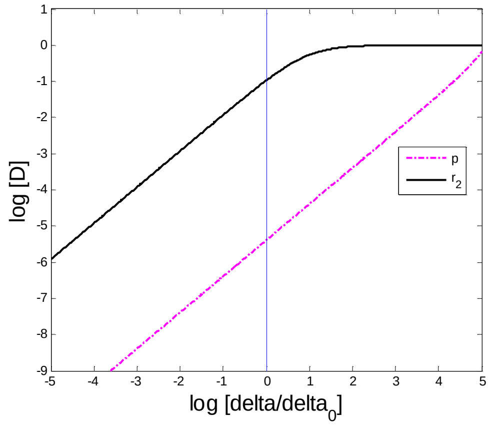
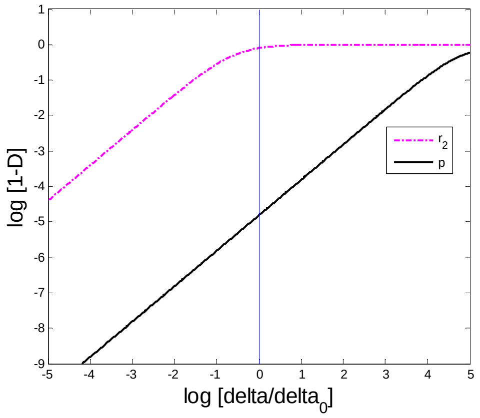
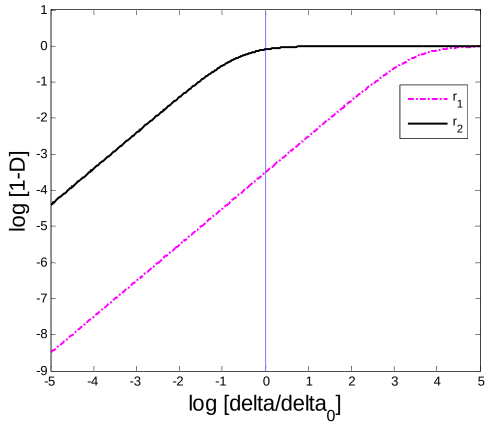



*MalT*

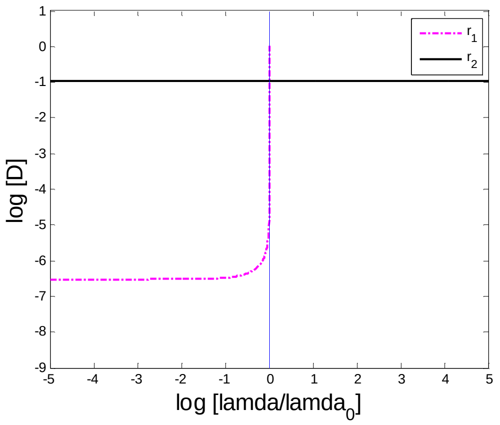
*Cbl*




**Figs S15-j.-** *lambda* (λ)

*LacI*

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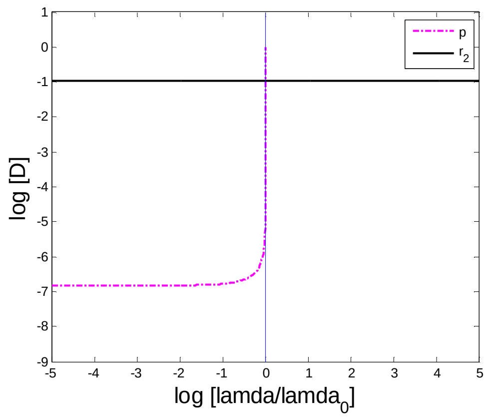


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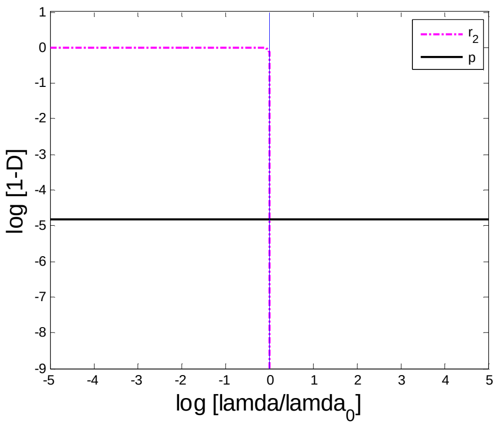


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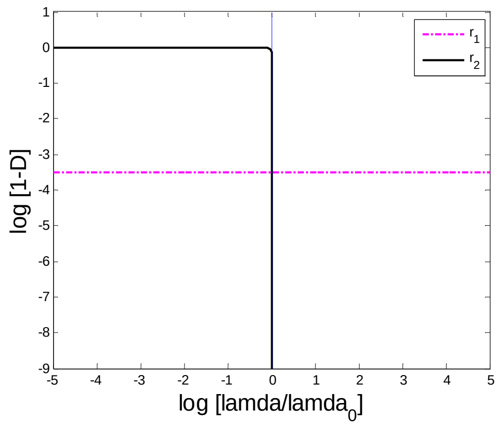
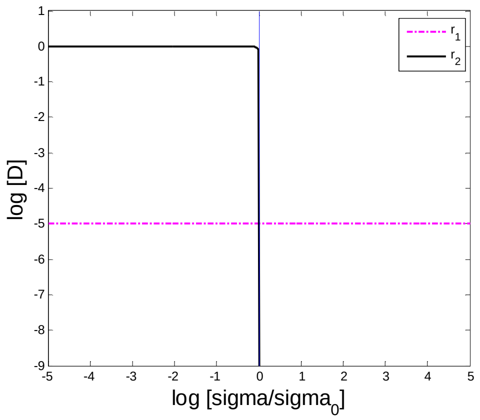


**Figs S15-k.-** *sigma* (σ)

*LacI*

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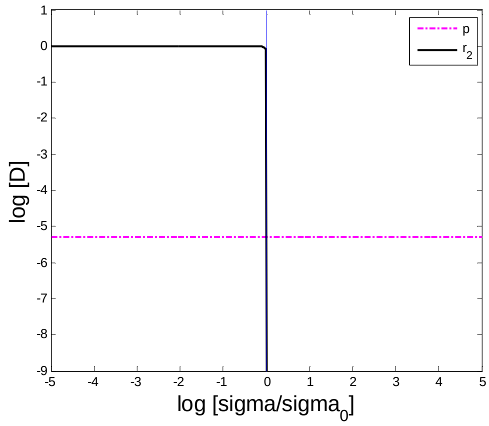


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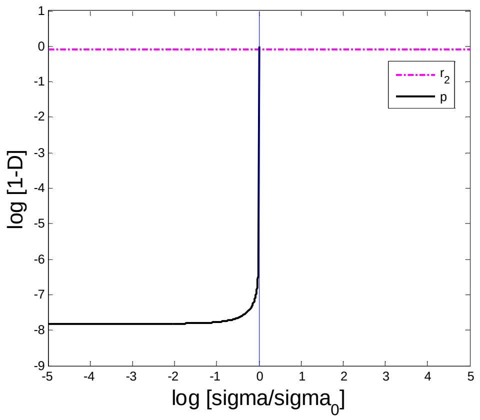


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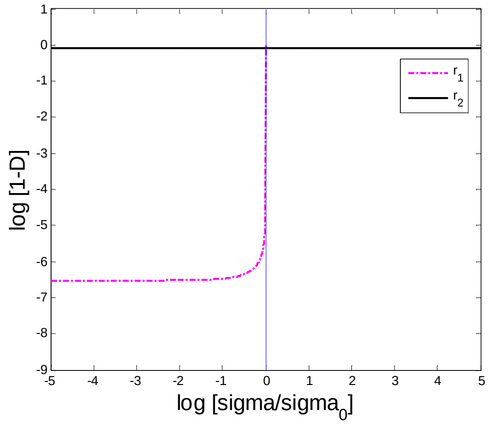
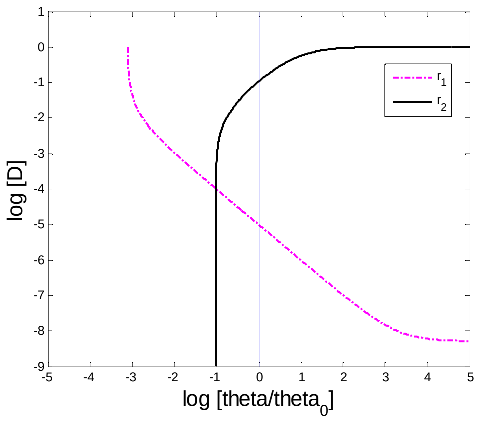



**Figs S15-l.-** *theta* (θ)

*LacI*

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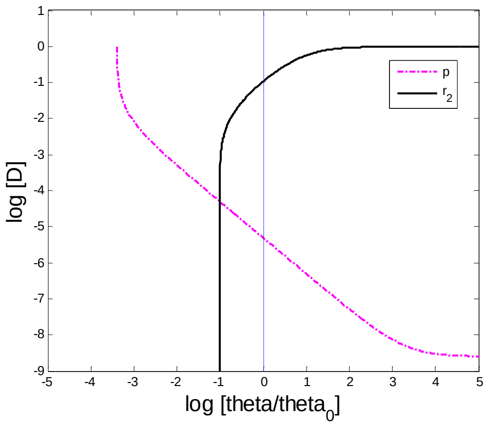

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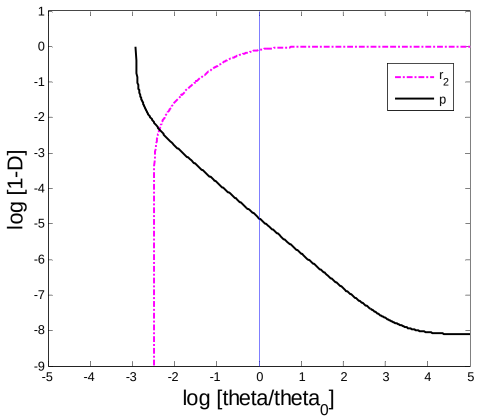


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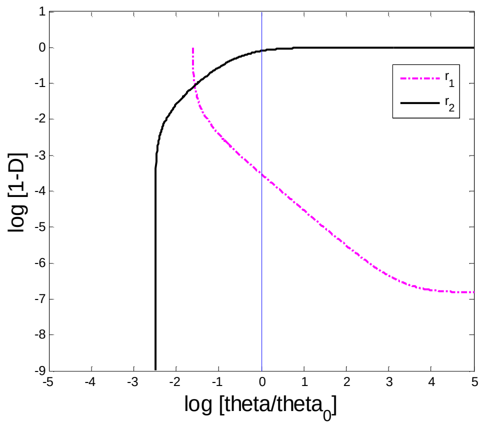
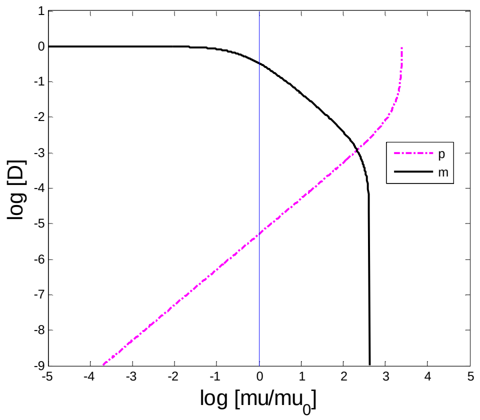


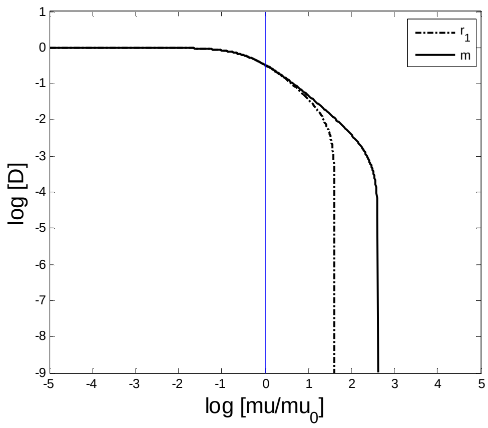

**Figs. S15. Influence of the constituent parameters on the values of the wild-type $D_{min}$ and $D_{max}$.** Each model parameter is varied around its nominal value, and the resulting lower ($D_{min}$) and upper ($D_{max}$) values are calculated. Solid lines correspond to the $D_{max}$, magenta dashed-dotted lines correspond to the $D_{min}$. Mutation rate $\omega$ = 20 was used along these analysis. The axes are represented in decimal logarithmic scale. Mutation rates: *a*) mu ($\mu$); *b*) pi ($\pi$); *c*) upsilon ($\upsilon$); *d*) tau ($\tau$); *e*) rho ($\rho$); *f*) epsilon ($\varepsilon$); *g*) psi ($\psi$). Growth rates: *h*) gamma ($\gamma$); *i*) delta ($\delta$); *j*) lambda ($\lambda$); *k*) sigma ($\sigma$). Criterion of selection: *l*) theta ($\theta$).

**Figs. S16-a.-** *mu* (μ)

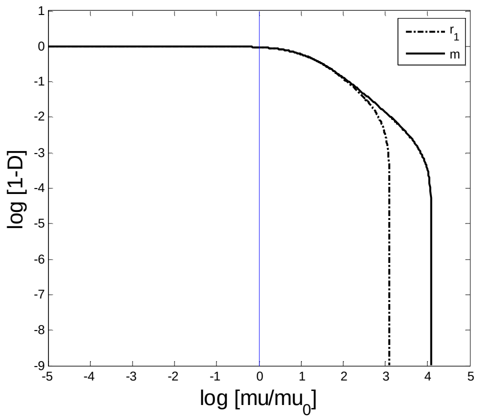
*LacI*



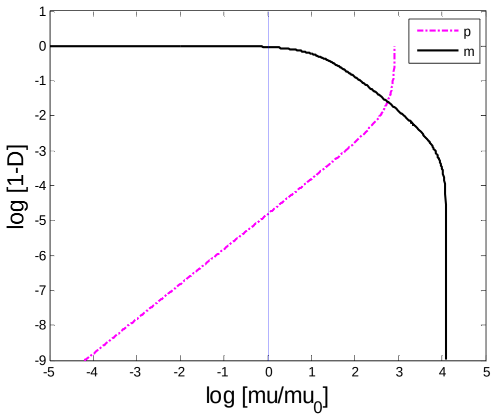

*TrpR*




*MalT*

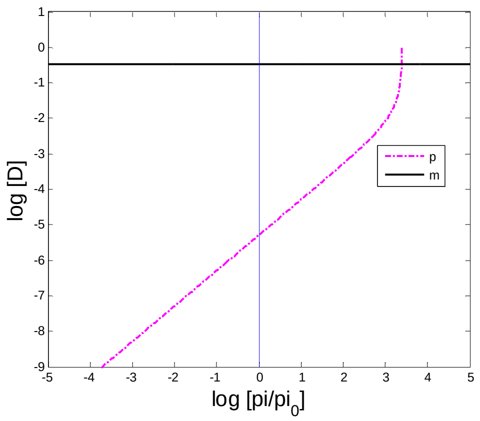



*Cbl*

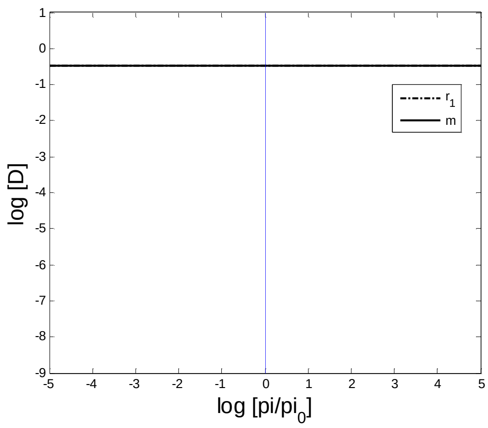



**Figs. S16-b.-** *pi* (π)

*LacI*

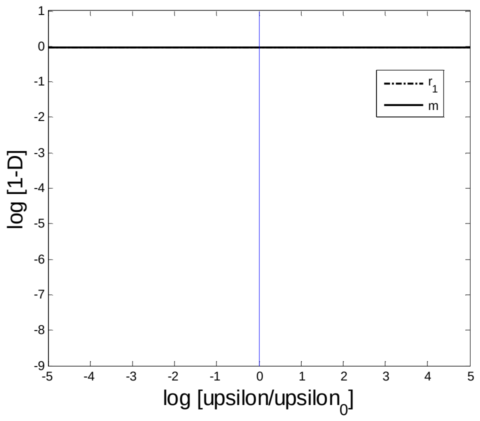



*TrpR*

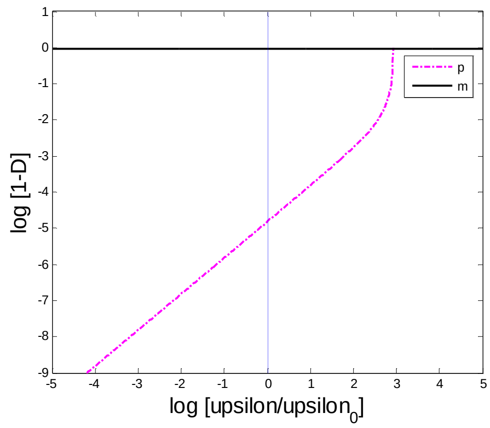


**Figs. S16-c.-** *upsilon* (υ)

*MalT*

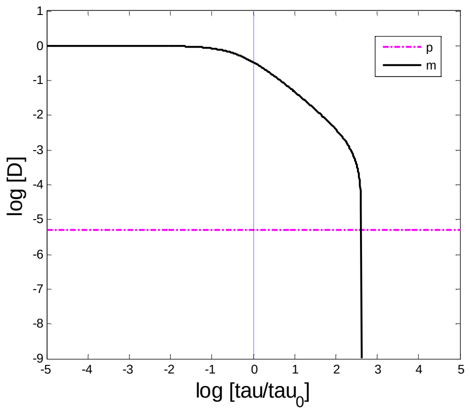



*Cbl.*

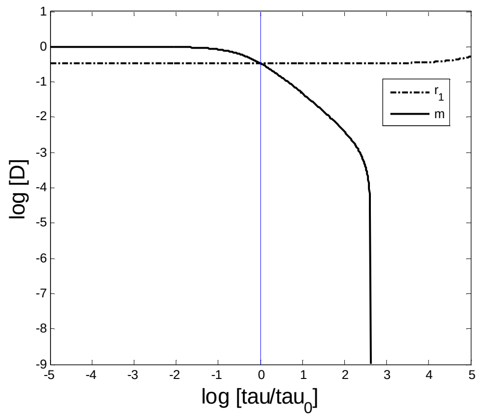



**Figs. S16-d.-** *tau* (τ)

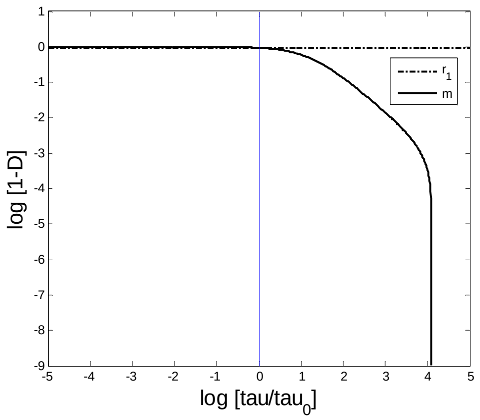
*LacI*



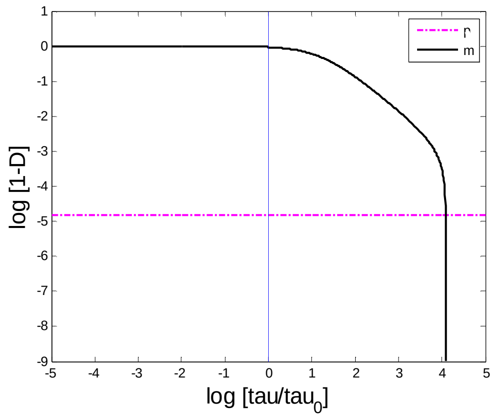

*TrpR*




*MalT*

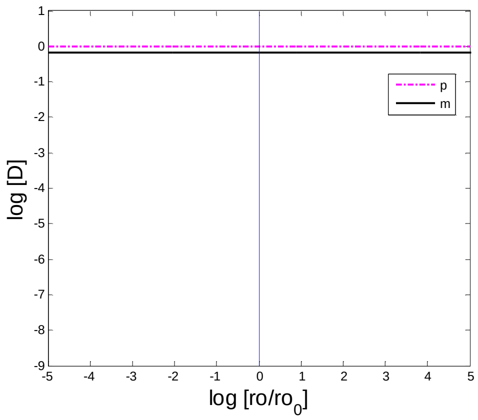



*Cbl*

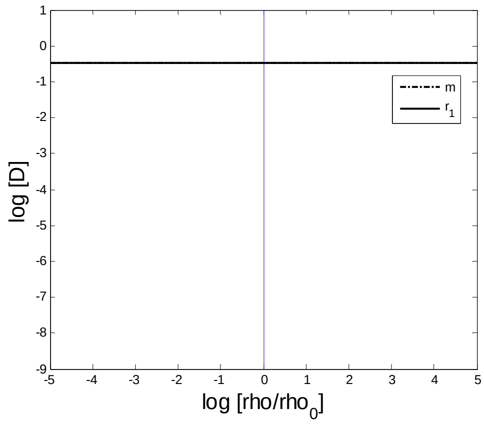


**Figs. S16-e.-** *rho* (ρ)

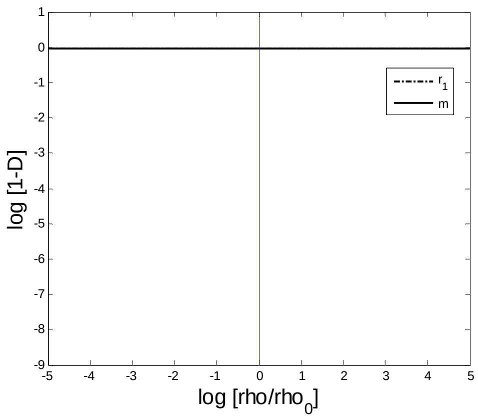
*LacI*



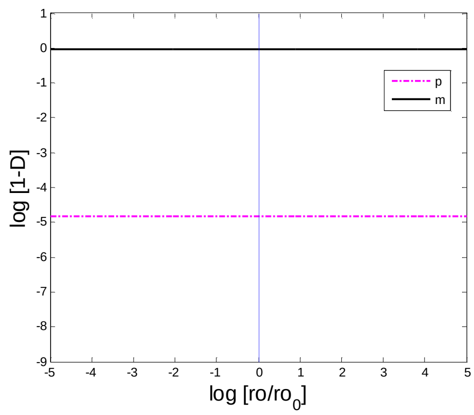

*TrpR*




*MalT*

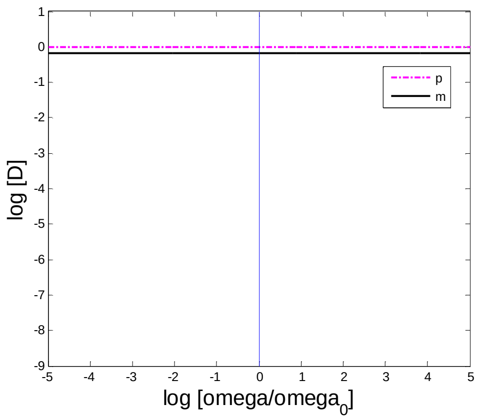



*Cbl*

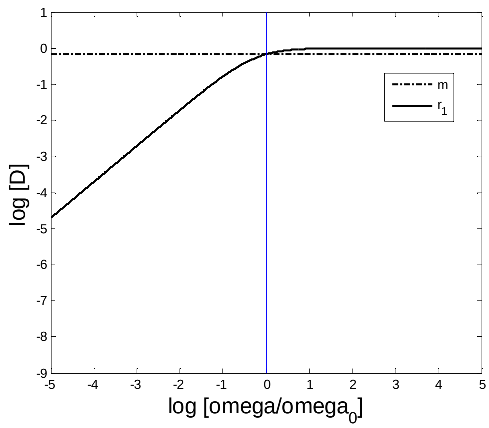



**Figs. S16-f.-** *omega* (ω)

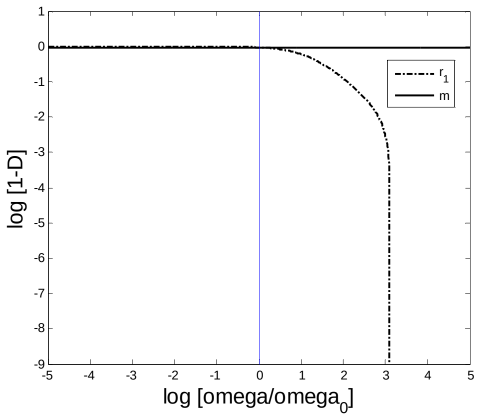
*LacI*



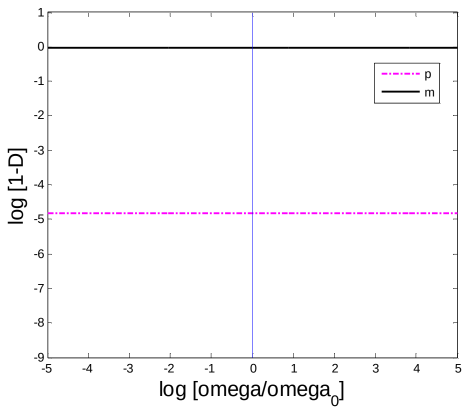

*TrpR*



*MalT*

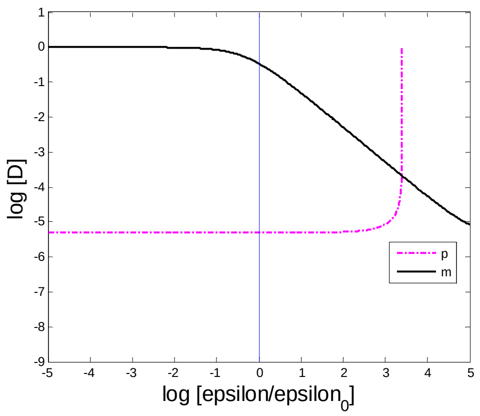



*Cbl*

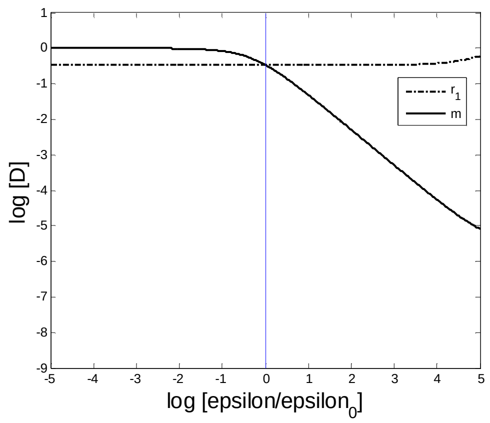



**Figs. S16-g.-** *epsilon* (ε)

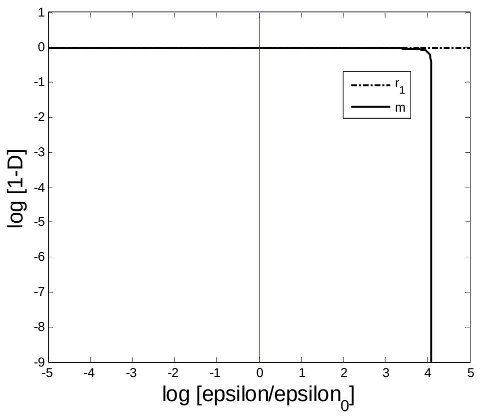
*LacI*



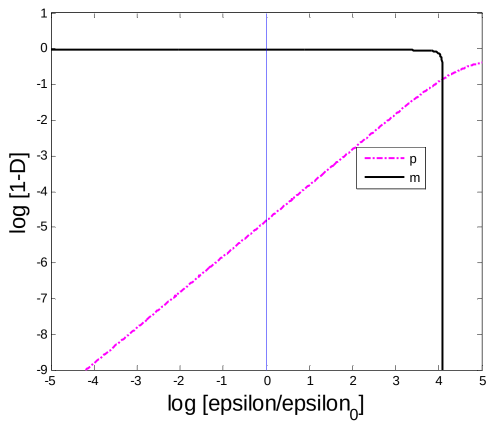

*TrpR*




*MalT*

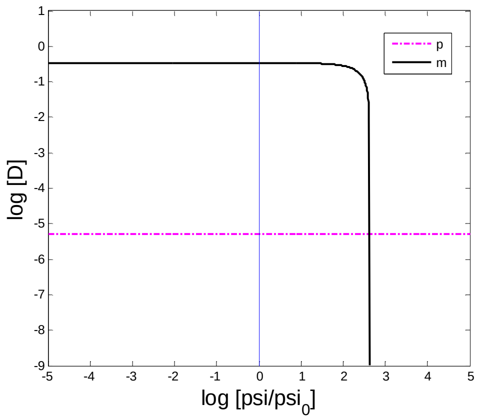



*Cbl*

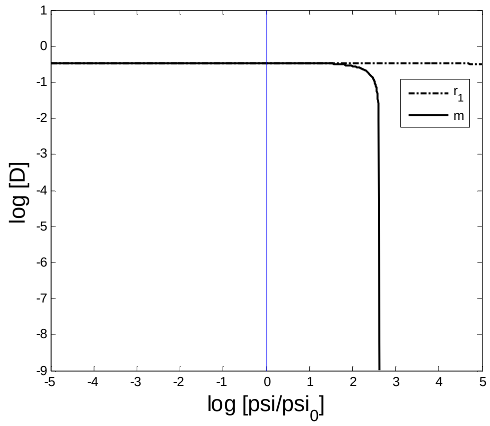


**Figs. S16-h.-** *psi* (ψ)

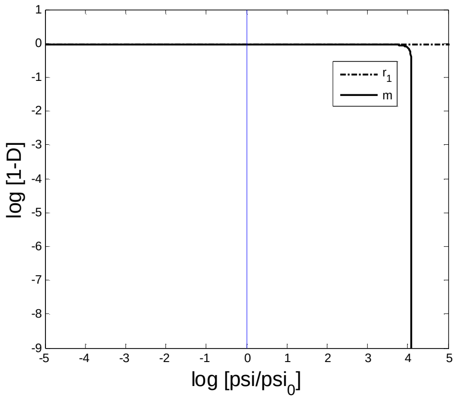
*LacI*



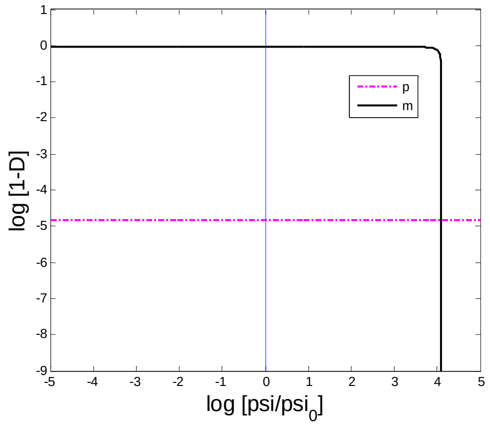

*TrpR*




*MalT*

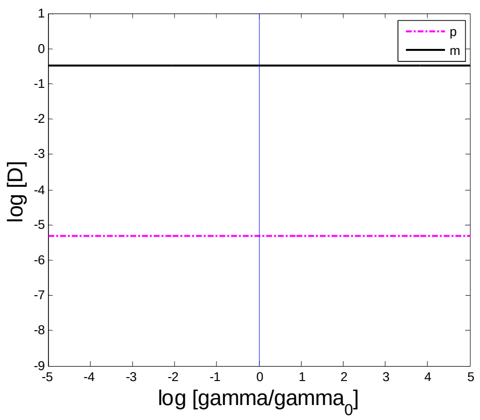



*Cbl*

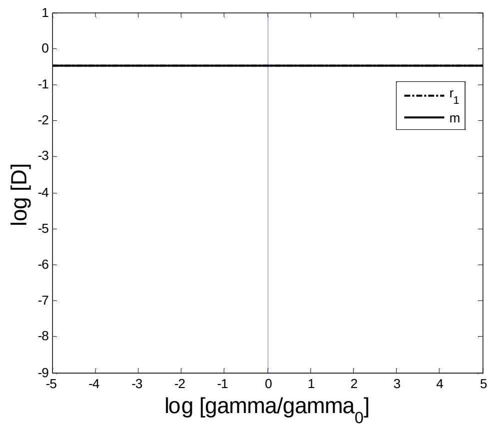



**Figs. S16-i.-** *gamma* (γ)

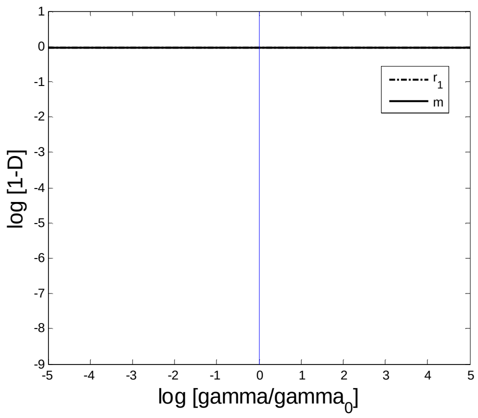
*LacI*



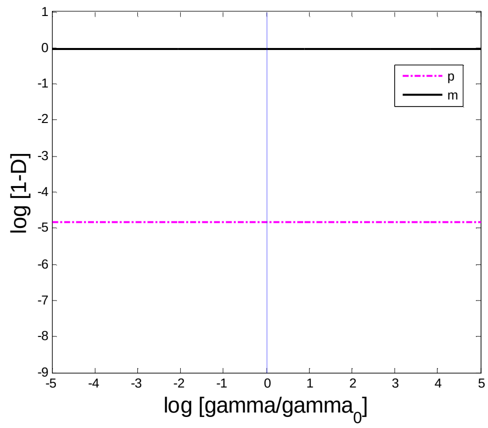

*TrpR*



*MalT*

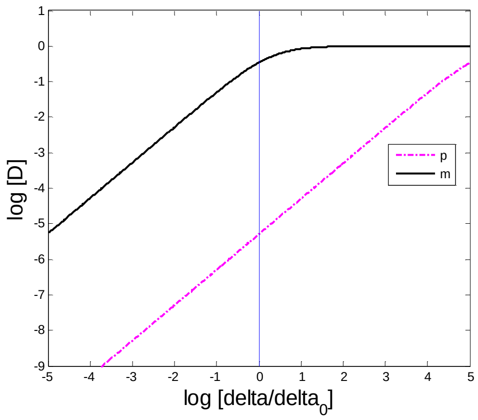



*Cbl*

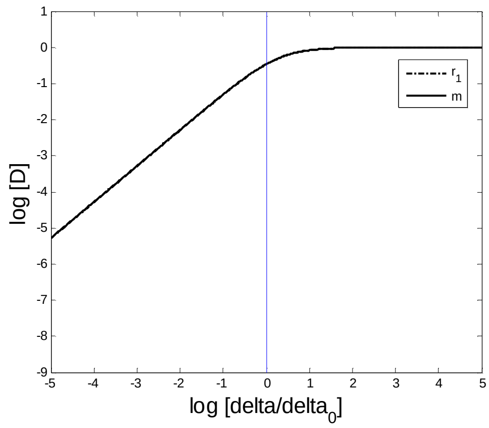



**Figs. S16-j.-** *delta* (δ)

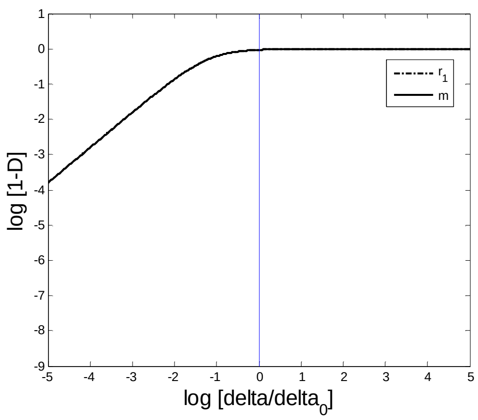
*LacI*



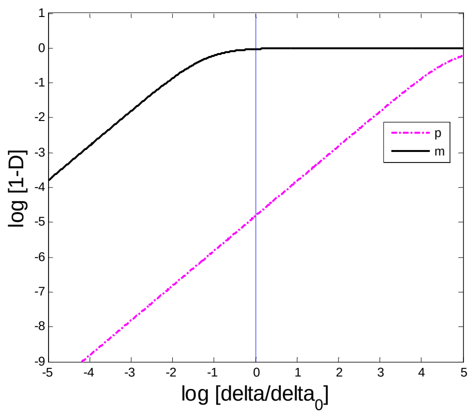

*TrpR*




*MalT*

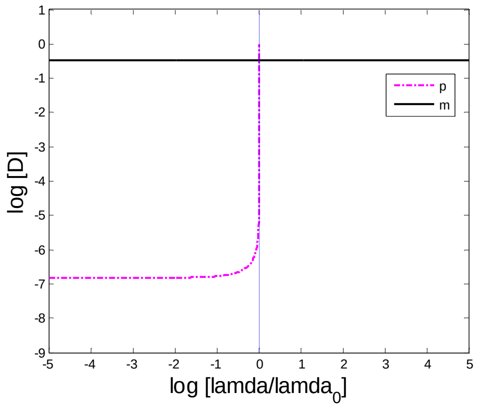



*Cbl*

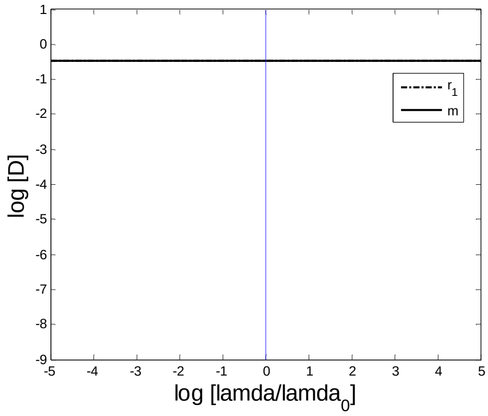


**Figs. S16-k.-** *lambda* (λ)

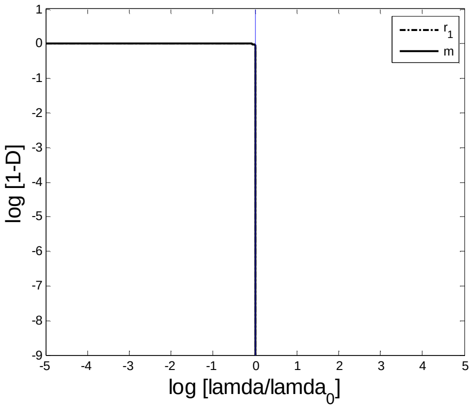
*LacI*



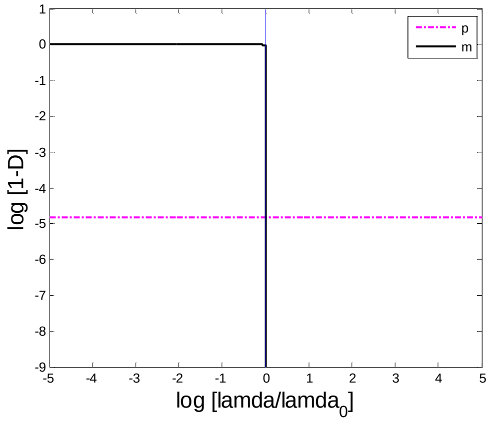

*TrpR*




*MalT*

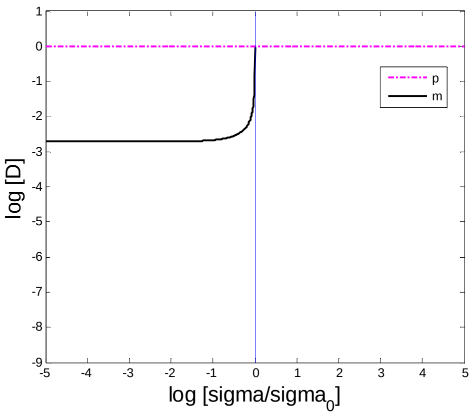



*Cbl*

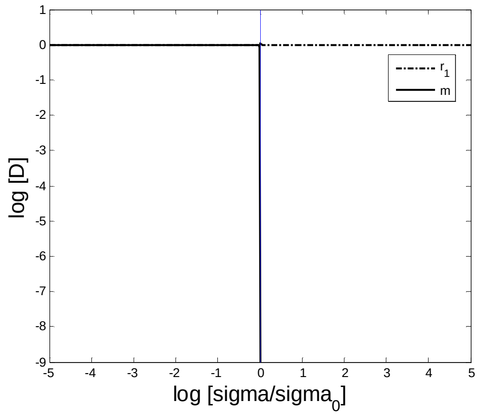



**Figs. S16-l.-** *sigma* (σ)

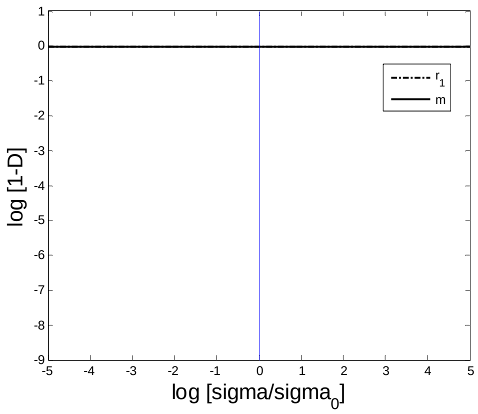
*LacI*



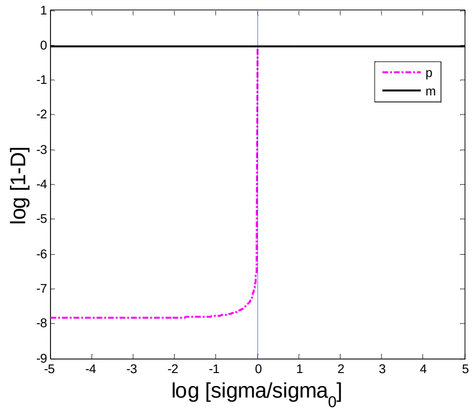

*TrpR*



*MalT*

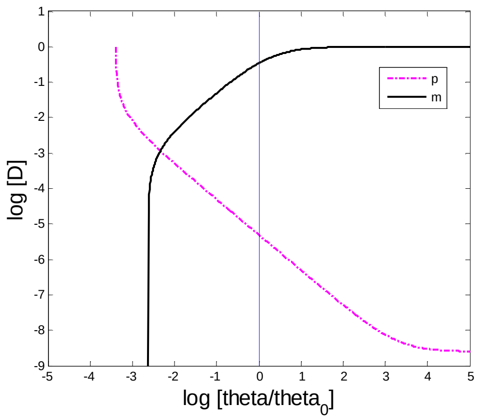



*Cbl*

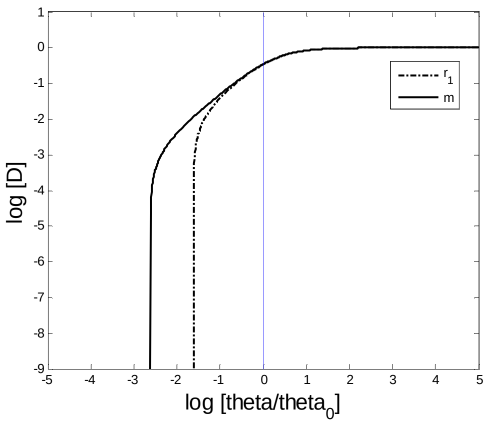



**Figs. S16-m.-** *theta* (θ)

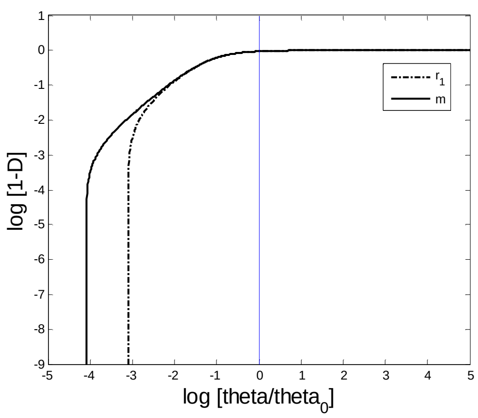
*LacI*



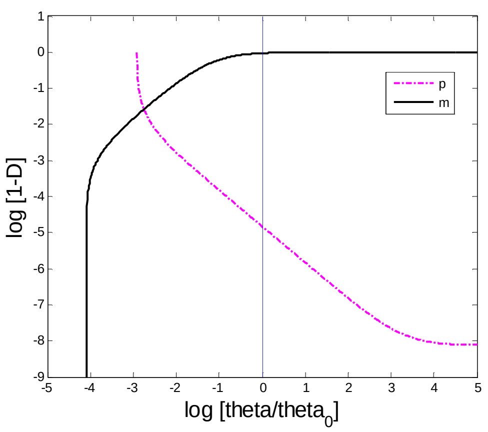

*TrpR*




*MalT*




*Cbl*



**Figs. S16a-m. Influence of the constituent parameters on the values for the $D_{min}$ and $D_{max}$ for the thresholds of selection not surrounding the wild-type region.** Each model parameter is varied around its nominal value, and the resulting thresholds are calculated. Solid and dashed-dotted black lines correspond to thresholds surrounding the wild-type $D_{max}$ threshold. Magenta dashed-dotted line corresponds to the thresholds surrounding the wild-type $D_{min}$ threshold region. Mutation rate $\omega = 20$ was used along these analysis. The axes are represented in decimal logarithmic scale. Mutation rates: *a*) mu ($\mu$); *b*) pi ($\pi$); *c*) upsilon ($\upsilon$); *d*) tau ($\tau$); *e*) rho ($\rho$); *f*) omega ($\omega$); *g*) epsilon ($\varepsilon$); *h*) psi ($\psi$). Growth rates: *i*) gamma ($\gamma$); *j*) delta ($\delta$); *k*) lambda ($\lambda$); *l*) sigma ($\sigma$). Criterion of selection: *m*) theta ($\theta$).

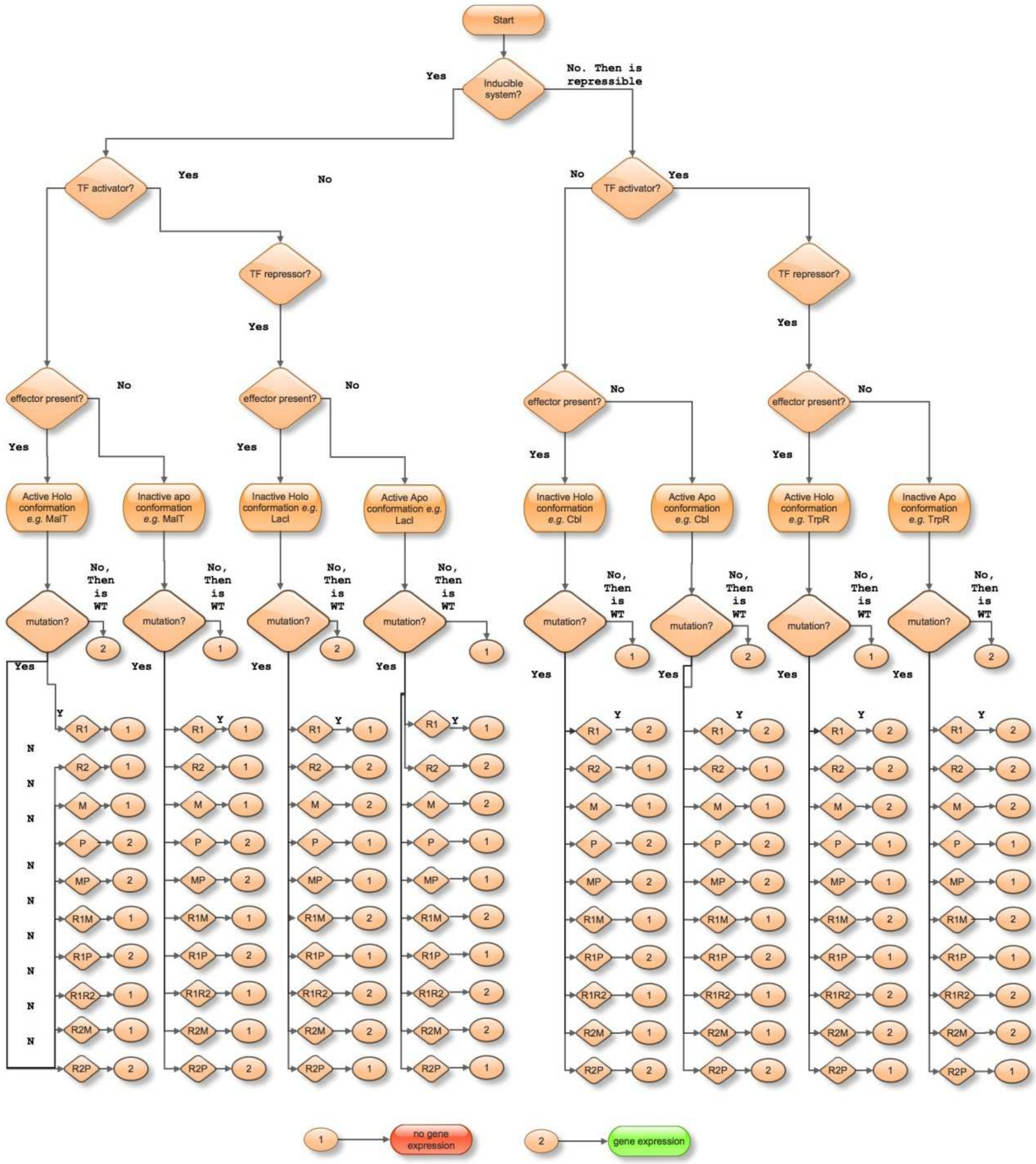



**Fig. S17. General flow chart of wild-type and mutant populations.** The output for the expression or non-expression for the structural genes is summarized here for all the TFs and conditions contemplated in Figs. 41-2 and S1-6.

# 2.- SI Tables

| | | Negative regulation | | | | Positive regulation | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | High demand | | Low demand | | High demand | | Low demand | |
| | | *Holo* Inactive | *Apo* Inactive | *Holo* Active | *Apo* Active | *Holo* Active | *Apo* Active | *Holo* Inactive | *Apo* Inactive |
| Fig. 2 key | Mutation | *LacI* | *TrpR* | *TrpR* | *LacI* | *MalT* | *Cbl* | *Cbl* | *MalT* |
| *b* | $m_{r_1w}$ | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω |
| *c* | $m_{r_2w}$ | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) |
| *d* | $m_{mw}$ | μ τ ε | μ τ ε | μ τ ψ | μ τ ψ | μ τ ε ψ | μ τ ε ψ | μ τ | μ τ |
| *e* | $m_{pw}$ | μ π ε | μ π ε | μ π | μ π | μ υ ε | μ υ ε | μ υ | μ υ |
| *f1* | $m_{d_1m}$ | μ π ε | μ π ε | μ π ε | μ π ε | μ υ | μ υ | μ υ | μ υ |
| *f2* | $m_{d_1p}$ | μ τ | μ τ | μ τ ψ | μ τ ψ | μ υ ε ψ | μ τ ε ψ | μ τ ε | μ τ ε |
| *g1* | $m_{d_6r_1}$ | μ τ ψ | μ τ ε | μ τ ε | μ τ ψ | μ τ | μ τ ε ψ | μ τ ε ψ | μ τ |
| *g2* | $m_{d_6m}$ | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω |
| *h1* | $m_{d_3r_1}$ | μ π | μ π ε | μ π ε | μ π | μ υ | μ υ ε | μ υ ε | μ υ |
| *h2* | $m_{d_3p}$ | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω |
| *i1* | $m_{d_2r_1}$ | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) |
| *i2* | $m_{d_2r_2}$ | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω | μ ω |
| *j1* | $m_{d_5r_2}$ | μ τ ε | μ τ ε | μ τ ε | μ τ ε | μ τ | μ τ | μ τ | μ τ |
| *j2* | $m_{d_5m}$ | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) |
| *k1* | $m_{d_4r_2}$ | μ π ε | μ π ε | μ π ε | μ π ε | μ υ | μ υ | μ υ | μ υ |
| *k2* | $m_{d_4p}$ | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) | μ (ω+ρ) |

**Table S1. TF mutation rate parameters for the mutant populations.** The mutation rate *i* and *j* subscripts ($m_{ij}$) can take the symbols {$w$, $m$, $p$, $r_1$, $r_2$, $d_1$, $d_2$, $d_3$, $d_4$, $d_5$, $d_6$} according to the origin (*j*) and destination (*i*) of the mutation. The first column corresponds to the key for Fig. 2.

| | Negative regulation | | | | Positive regulation | | | |
|---|---|---|---|---|---|---|---|---|
| | High demand | | Low demand | | High demand | | Low demand | |
| | *Holo* Inactive | *Apo* Inactive | *Holo* Active | *Apo* Active | *Holo* Active | *Apo* Active | *Holo* Inactive | *Apo* Inactive |
| | *LacI* | *TrpR* | *TrpR* | *LacI* | *MalT* | *Cbl* | *Cbl* | *MalT* |
| $g_w$ | $\gamma$ | $\gamma$ | $\gamma\delta$ | $\gamma\delta$ | $\gamma\delta$ | $\gamma\delta$ | $\gamma$ | $\gamma$ |
| $g_{r_1}$ | $\gamma\lambda$ | $\gamma$[1] | $\gamma\delta\sigma$ | $\gamma\delta$[1] | $\gamma\delta\lambda$ | $\gamma\delta$[1] | $\gamma\sigma$[1] | $\gamma$[1] |
| $g_{r_2}$ | $\gamma$[1] | $\gamma$[1] | $\gamma\delta\sigma$ | $\gamma\delta\sigma$ | $\gamma\delta\lambda$ | $\gamma\delta\lambda$ | $\gamma$ | $\gamma$[1] |
| $g_m$ | $\gamma$[1] | $\gamma$[1] | $\gamma\delta\sigma$ | $\gamma\delta\sigma$ | $\gamma\delta\lambda$ | $\gamma\delta\lambda$ | $\gamma$ | $\gamma$[1] |
| $g_p$ | $\gamma\lambda$ | $\gamma\lambda$ | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\sigma$[1] | $\gamma\sigma$ |
| $g_{d_1}$ | $\gamma\lambda$ | $\gamma\lambda$ | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\sigma$[1] | $\gamma\sigma$ |
| $g_{d_2}$ | $\gamma$[1] | $\gamma$[1] | $\gamma\delta\sigma$ | $\gamma\delta\sigma$ | $\gamma\delta\lambda$ | $\gamma\delta\lambda$ | $\gamma$ | $\gamma$[1] |
| $g_{d_3}$ | $\gamma\lambda$ | $\gamma\lambda$ | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\sigma$[1] | $\gamma\sigma$ |
| $g_{d_4}$ | $\gamma\lambda$ | $\gamma\lambda$ | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\delta$[1] | $\gamma\sigma$[1] | $\gamma\sigma$ |
| $g_{d_5}$ | $\gamma$[1] | $\gamma$[1] | $\gamma\delta\sigma$ | $\gamma\delta\sigma$ | $\gamma\delta\lambda$ | $\gamma\delta\lambda$ | $\gamma$ | $\gamma$[1] |
| $g_{d_6}$ | $\gamma$[1] | $\gamma$[1] | $\gamma\delta\sigma$ | $\gamma\delta\sigma$ | $\gamma\delta\lambda$ | $\gamma\delta\lambda$ | $\gamma$ | $\gamma$[1] |
| Single | 2/4 | 3/4 | 3/4 | 2/4 | 1/4 | 2/4 | 2/4 | 3/4 |
| Double | 3/6 | 3/6 | 3/6 | 3/6 | 3/6 | 3/6 | 3/6 | 3/6 |
| Total | 5/10 | 6/10 | 6/10 | 5/10 | 4/10 | 5/10 | 5/10 | 6/10 |

[1] Favorable mutant

**Table S2. TF growth rate parameters for the wild-type and mutant populations.** By definition, the *holo* and *apo* active conformations correspond at the TF, exerting their function over their DNA target. The last three rows compile the fractions of favorable single mutants from Figs. 3, S1-6 during high demand. Please note that these fractions do not include the favorable wild-type population during low demand.

| Mutation rates (*base*$^{-1}$ *generation*$^{-1}$) | Negative | Positive |
|---|---|---|
| μ | $6\times10^{-10}$ | $6\times10^{-10}$ |
| π | 10 | - |
| υ | - | 1 |
| τ | 20 | 20 |
| ρ | 60 | 60 |
| ω | 1, 20, 40 | 1, 20, 40 |
| ε | 1 | 1 |
| ψ | 0.1 | 0.1 |
| Growth rate (*generation hour*$^{-1}$) | | |
| γ | 1 | 1 |
| δ | 0.0125 | 0.0125 |
| λ | 0.97 | - |
| λ | - | 0.97 |
| σ | 0.999 | - |
| σ | - | 0.999 |

**Table S3. Numeric values for the mutation and growth rate parameters defined in Table 1.**

| | | 1 | | | | | 2 | | | | | 3 | | | | | 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *m* | *p* | $r_1$ | $r_2$ | | *m* | *p* | $r_1$ | $r_2$ | | *m* | *p* | $r_1$ | $r_2$ | | *m* | *p* | $r_1$ | $r_2$ |
| ***LacI*** | *A* | | + | − | − | + | | + | − | − | − | | − | − | − | − | | | | | |
| ***LacI*** | *B* | U | + | − | + | + | | + | − | + | − | | − | − | + | − | | | | | |
| ***LacI*** | *C* | F | + | + | + | + | F | + | + | + | − | | − | + | + | − | | | | | |
| ***TrpR*** | *A* | U | + | − | + | + | | + | − | + | − | | + | − | − | − | | − | − | − | − |
| ***TrpR*** | *B* | F | + | + | + | + | F | + | + | − | + | | − | + | + | − | | − | + | − | − |
| ***MalT*** | *A* | | − | − | − | − | | − | − | + | − | | + | − | + | − | F | + | − | + | + |
| ***MalT*** | *B* | | − | + | − | − | | − | + | + | − | U | + | + | + | − | F | + | + | + | + |
| ***Cbl*** | *A* | | − | − | + | − | | + | − | + | − | F | + | − | + | + | | | | | |
| ***Cbl*** | *A* | | − | + | − | − | | + | + | − | − | F | + | + | − | + | | | | | |
| ***Cbl*** | *B* | | − | + | + | − | U | + | + | + | − | F | + | + | + | + | | | | | |

**Table S4. Expression of wild-type and single mutant populations contemplated for the sectors of the threshold of selection when omega equals one**. The second column and first row indicate the *i* and *j* alpha-numbers for the different $C_{i,j}$ regions from Figs. 4a, 5a, 6a, and 7a. The favorable (F) or not favorable (U) single populations were obtained from Figs. 3, S1-6 during high demand. Positive and negative signs mean functional and non-functional sectors of the DNA or the TF. Modulator (*m*), promoter (*p*), effector-TF sector ($r_1$), or DNA-TF sector ($r_2$).

| | | 1 | | | | | 2 | | | | | 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $m$ | $p$ | $r_1$ | $r_2$ | | $m$ | $p$ | $r_1$ | $r_2$ | | $m$ | $p$ | $r_1$ | $r_2$ |
| ***LacI*** | *A* | | + | − | − | + | | + | − | − | − | | − | − | − | − |
| | *B* | U | + | + | − | + | | + | + | − | − | | − | + | − | − |
| | *C* | F | + | + | + | + | F | + | + | + | − | | − | + | + | − |
| ***TrpR*** | *A* | U | + | − | + | + | | + | − | + | − | | − | − | − | − |
| | *B* | F | + | + | + | + | F | + | + | + | − | | − | + | − | − |
| ***MalT*** | *A* | | − | − | − | − | | + | − | − | − | F | + | − | + | + |
| | *B* | | − | + | − | − | U | + | + | + | − | F | + | + | + | + |
| ***Cbl*** | *A* | | − | − | − | − | | + | − | − | − | | + | − | − | + |
| | *B* | | − | + | − | − | | + | + | − | − | F | + | + | − | + |
| | *C* | | − | + | + | − | U | + | + | + | − | F | + | + | + | + |

**Table S5. Expression of wild-type and single mutant populations contemplated for the sectors of the threshold of selection when omega equals twenty.** Descriptions as in Table S4.

| | | 1 | | | | | 2 | | | | | 3 | | | | | 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $m$ | $p$ | $r_1$ | $r_2$ | | $m$ | $p$ | $r_1$ | $r_2$ | | $m$ | $p$ | $r_1$ | $r_2$ | | $m$ | $p$ | $r_1$ | $r_2$ |
| ***LacI*** | *A* | | + | − | − | + | | + | − | − | − | | − | − | − | − | | | | | |
| | *B* | U | + | + | − | + | | + | + | − | − | | − | + | − | − | | | | | |
| | *C* | F | + | + | + | + | F | + | + | + | − | | − | + | + | − | | | | | |
| ***TrpR*** | *A* | U | + | − | + | + | | + | − | + | − | | + | − | − | − | | − | − | − | − |
| | *B* | F | + | + | + | + | F | + | + | + | − | | + | + | − | − | | − | + | − | − |
| ***MalT*** | *A* | | − | − | − | − | | + | − | − | − | | + | − | + | − | F | + | − | + | + |
| | *B* | | − | + | − | − | | + | + | − | − | U | + | + | + | − | F | + | + | + | + |
| ***Cbl*** | *A* | | − | − | − | − | | + | − | − | − | | + | − | − | + | | | | | |
| | *B* | | − | + | − | − | | + | + | − | − | F | + | + | − | + | | | | | |
| | *C* | | − | + | + | − | U | + | + | + | − | F | + | + | + | + | | | | | |

**Table S6. Expression of wild-type and single mutant populations contemplated for the sectors of the threshold of selection when omega equals forty.** Descriptions as in Table S4.

| | | Negative | | Positive | |
|---|---|---|---|---|---|
| | | *LacI* | *TrpR* | *MalT* | *Cbl* |
| mu (μ) | $D_{min}$ | 2, ↑ | 2, ↑ | 3, ↑ | 2, ↓ |
| | $D_{max}$ | 3, ↓ | 3, ↓ | 2, ↓ | 3, ↑ |
| pi (π) | $D_{min}$ | 1, – | 2, ↑ | N/A | N/A |
| | $D_{max}$ | 1, – | 1, – | N/A | N/A |
| upsilon (υ) | $D_{min}$ | N/A | N/A | 1, – | 1, – |
| | $D_{max}$ | N/A | N/A | 2, ↓ | 1, – |
| tau (τ) | $D_{min}$ | 1, – | 1, – | 1, – | 1, – |
| | $D_{max}$ | 1, – | 1, – | 1, – | 1, – |
| rho (ρ) | $D_{min}$ | 1, – | 1, – | 3, ↑ | 1, – |
| | $D_{max}$ | 3, ↓ | 3, ↓ | 1, – | 3, ↑ |
| omega (ω) | $D_{min}$ | 2, ↑ | 1, – | 3, ↑ | 2, ↓ |
| | $D_{max}$ | 3, ↓ | 3, ↓ | 1, – | 3, ↑ |
| epsilon (ε) | $D_{min}$ | 1, – | 1, – | 1, – | 1, – |
| | $D_{max}$ | 1, – | 1, – | 4, ↓ | 1, – |
| psi (ψ) | $D_{min}$ | 1, – | 1, – | 1, – | 1, – |
| | $D_{max}$ | 1, – | 1, – | 1, – | 1, – |
| gamma (γ) | $D_{min}$ | 1, – | 1, – | 1, – | 1, – |
| | $D_{max}$ | 1, – | 1, – | 1, – | 1, – |
| delta (δ) | $D_{min}$ | 2, ↑ | 2, ↑ | 3, ↓ | 2, ↓ |
| | $D_{max}$ | 2, ↑ | 2, ↑ | 2, ↓ | 3, ↓ |
| lambda (λ) | $D_{min}$ | 5, ↑ | 5, ↑ | 5, ↑ | 1, – |
| | $D_{max}$ | 1, – | 1, – | 1, – | 5, ↑ |
| sigma (σ) | $D_{min}$ | 1, – | 1, – | 1, – | 4, ↓ |
| | $D_{max}$ | 5, ↓ | 5, ↓ | 4, ↓ | 1, – |
| theta (θ) | $D_{min}$ | 2, ↓ | 2, ↓ | 3, ↓ | 2, ↑ |
| | $D_{max}$ | 3, ↑ | 3, ↑ | 2, ↑ | 3, ↓ |

**Table S7. Influence of parameters sensibility on minimum and maximum values for demand.** Analysis from parameters represented in Figs. 13a-m, for the minimum ($D_{min}$) and maximum ($D_{max}$) values for the demand. As in [1], the influence of the parameters are classified into five categories: 1 = no discernible influence, 2 = nearly linear variation, 3 = cube-root influence, 4 = moderate amplification (order of magnitude), 5 = extreme amplification (1000-fold). Starting from the basal value: ↑ = increase of $D_{min}$ ($D_{max}$), ↓ = decrease of $D_{min}$ ($D_{max}$), – = no discernible change of $D_{min}$ ($D_{max}$).

| | | *LacI* | *TrpR* | Comments |
|---|---|---|---|---|
| mu (μ) | → | = | = | $D_{min}$ increase for both |
| | | = | = | $D_{max}$ decrease for both |
| | ← | = | = | $D_{min}$ decrease for both |
| | | = | = | $D_{max}$ increase for both |
| pi (π) | → | A | | $D_{min}$ increase for *TrpR* narrowing their WT region |
| | | = | = | $D_{max}$ no discernable change in either |
| | ← | | A | $D_{min}$ decrease for *TrpR* increasing the WT region |
| | | = | = | $D_{max}$ no discernable change in either |
| tau (τ) | → | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| | ← | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| rho (ρ) | → | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ sharp decrease for both |
| | ← | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ sharp increase for both |
| omega (ω) | → | | A | $D_{min}$ linear increase for *LacI* narrowing their WT region. Advantage for *TrpR* |
| | | = | = | $D_{max}$ decrease for both |
| | ← | A | | $D_{min}$ linear decrease for *LacI* increasing the WT region. Advantage for *LacI* |
| | | = | = | $D_{max}$ decrease for both |
| epsilon (ε) | → | | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| | ← | | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| psi (ψ) | → | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| | ← | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |

**Table S8.** (cont.)

| | | *LacI* | *TrpR* | Comments |
|---|---|---|---|---|
| gamma (γ) | → | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| | ← | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| delta (δ) | → | = | = | $D_{min}$ increase for both narrowing the WT region |
| | | = | = | $D_{max}$ increase for both narrowing the WT region |
| | ← | = | = | $D_{min}$ decrease for both increasing the WT region |
| | | = | = | $D_{max}$ increase for both narrowing the WT region |
| lambda (λ) | → | = | = | $D_{min}$ abrupt increase for both increasing WT regions |
| | | = | = | $D_{max}$ no discernable change in either |
| | ← | = | = | $D_{min}$ abrupt decrease for both increasing WT regions |
| | | = | = | $D_{max}$ no discernable change in either |
| sigma (σ) | → | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ decrease abrupt for both |
| | ← | = | = | $D_{min}$ no discernable change in either |
| | | = | = | $D_{max}$ no discernable change in either |
| theta (θ) | → | = | = | $D_{min}$ with decrease in both WT regions |
| | | = | = | $D_{max}$ increase in the WT region for both |
| | ← | = | = | $D_{min}$ with increase in both |
| | | = | = | $D_{max}$ with decrease in both |

**Table S8. Parameter sensibility analysis for the *apo* and *holo* conformations within the negative mode of regulation.** The mutation and growth rate parameter sensitivities from Figs. 6 and S15 are analyzed for the minimum ($D_{min}$) and maximum ($D_{max}$) values of the demand around the nominal values. The analysis focuses on the effects that small increments (→) or decrements (←) have around the parameter's nominal maximum values of the demand for the wild type. The results were compared within the two TFs with negative regulation and classified as: no difference (=) between them or with advantage (A) of one over another.

| | | *MalT* | *Cbl* | Comments |
|---|---|---|---|---|
| mu (μ) | → | | A | 1-$D_{min}$ increase for *Cbl* and decrease for *MalT*. Advantage for *Cbl* with decrease in $D_{min}$ |
| | | | A | 1-$D_{max}$ increase for *MalT* and decrease for *Cbl*. Advantage for *Cbl* with an increase in the $D_{max}$ |
| | ← | A | | 1-$D_{min}$ decrease for *Cbl* and then increase in $D_{min}$ . Advantage for *MalT* |
| | | A | | 1-$D_{max}$ decrease for *MalT* and then increase in $D_{max}$. Advantage for *MalT* |
| upsilon (υ) | → | = | = | 1-$D_{min}$ no discernable change in either |
| | | | A | 1-$D_{max}$ increase for *MalT* and then decrease in $D_{max}$. Advantage for *Cbl* |
| | ← | = | = | 1-$D_{min}$ no discernable change in either |
| | | A | | 1-$D_{max}$ decrease for *MalT* and then increase the $D_{max}$. Advantage for *MalT* |
| tau (τ) | → | = | = | 1-$D_{min}$ no discernable change in either |
| | | = | = | 1-$D_{max}$ no discernable change in either |
| | ← | = | = | 1-$D_{min}$ no discernable change in either |
| | | = | = | 1-$D_{max}$ no discernable change in either |
| rho (ρ) | → | | A | 1-$D_{min}$ decrease for *MalT* which implies increase of $D_{min}$ with a decrease of the WT region. Advantage for *Cbl* |
| | | | A | 1-$D_{max}$ decrease for *Cbl* which implies increase in $D_{max}$ and advantage for *Cbl* |
| | ← | A | | 1-$D_{min}$ increase for *MalT* which implies decrease in $D_{min}$. Advantage for *MalT* |
| | | A | | 1-$D_{max}$ increase for Cbl which implies decrease of the WT region. Advantage for *MalT* |
| omega (ω) | → | | A | 1-$D_{min}$ slight decrease for *MalT* which implies increase in $D_{min}$. 1-$D_{min}$ linear increase for *Cbl* which implies decrease in $D_{min}$. Advantage for *Cbl* |
| | | | A | 1-$D_{max}$ slight decrease in *Cbl* implying the increase in $D_{max}$ . Advantage for *Cbl* |
| | ← | A | | 1-$D_{min}$ slight increase for *MalT* which implies decrease in $D_{min}$ . Advantage for *MalT* |
| | | A | | 1-$D_{max}$ slight increase for *Cbl* which implies decrease in $D_{max}$ . 1-$D_{min}$ linear decrease for *Cbl* which implies increase in $D_{min}$. Advantage for *MalT* |
| epsilon (ε) | → | = | = | 1-$D_{min}$ no discernable change in either |
| | | | A | 1-$D_{max}$ increase in *MalT* which implies a decrease in $D_{max}$. No discernible effect for *Cbl*. Advantage for *Cbl* |
| | ← | = | = | 1-$D_{min}$ no discernable change in either |
| | | A | | 1-$D_{max}$ decrease for *MalT* which implies increase in $D_{max}$ . Advantage for *MalT* |
| psi (ψ) | → | = | = | 1-$D_{min}$ no discernable change in either |
| | | = | = | 1-$D_{max}$ no discernable change in either |
| | ← | = | = | 1-$D_{min}$ no discernable change in either |
| | | = | = | 1-$D_{max}$ no discernable change in either |

**Table S9.** (cont.)

| | | *MalT* | *Cbl* | Comments |
|---|---|---|---|---|
| gamma (γ) | → | = | = | 1-$D_{min}$ no discernable change in either |
| | | = | = | 1-$D_{max}$ no discernable change in either |
| | ← | = | = | 1-$D_{min}$ no discernable change in either |
| | | = | = | 1-$D_{max}$ no discernable change in either |
| delta (δ) | → | | A | 1-$D_{min}$ more pronounced increase for *Cbl* which implies decrease in $D_{min}$. Advantage for *Cbl* |
| | | | A | 1-$D_{max}$ more pronounced increase for *MalT* which implies decrease in $D_{max}$. Advantage for *Cbl* |
| | ← | A | | 1-$D_{min}$ more pronounced decrease for *Cbl* which implies increase in $D_{min}$. Advantage for *MalT* |
| | | A | | 1-$D_{max}$ more pronounced decrease for *MalT* which implies increase of $D_{max}$. Advantage for *MalT* |
| lambda (λ) | → | | A | 1-$D_{min}$ abrupt decrease for *MalT* with no room for increase. Advantage for *Cbl* |
| | | A | | 1-$D_{max}$ abrupt decrease for *Cbl* with no room for increase. Advantage for *MalT* |
| | ← | A | = | 1-$D_{min}$ sharp increase for *MalT* which implies increase in $D_{min}$. Advantage for *MalT* |
| | | = | A | 1-$D_{max}$ sharp increase for *Cbl* which implies increase in $D_{min}$. Advantage for *Cbl* |
| sigma (σ) | → | A | | 1-$D_{min}$ abrupt increase in *Cbl* which implies decrease of $D_{min}$ but with no room for further increase of the parameter. Advantage for *MalT* |
| | | | A | 1-$D_{max}$ abrupt increase in *MalT* which implies decrease of $D_{max}$ but with no room for further increase of the parameter. Advantage for *Cbl* |
| | ← | | A | 1-$D_{min}$ decrease in Cbl which implies increase in $D_{min}$. Advantage for *Cbl* |
| | | A | | 1-$D_{max}$ decrease in *MalT* which implies increase in $D_{max}$ . Advantage for *MalT* |
| theta (θ) | → | A | | 1-$D_{min}$ decrease for *Cbl* which implies an increase in $D_{min}$ . 1-$D_{min}$ increase for *MalT* which implies a decrease in $D_{min}$ . Advantage for *MalT* |
| | | A | | 1-$D_{max}$ decrease for *MalT* which implies an increase in the $D_{max}$ . 1-$D_{max}$ increase for *Cbl* which implies a decrease in $D_{max}$. Advantage for *MalT* |
| | ← | | A | 1-$D_{min}$ increase for *Cbl* which implies decrease in $D_{min}$ . Advantage for *Cbl* |
| | | | A | 1-$D_{max}$ increase for *MalT* which implies a decrease in the $D_{max}$. 1-$D_{max}$ decrease for *Cbl* which implies an increase in $D_{max}$. Advantage for *Cbl* |

**Table S9. Parameter sensibility analysis for the *apo* and *holo* conformations within the positive mode of regulation.** Descriptions as in Table S8.

| | Negative | | Positive | |
|---|---|---|---|---|
| | *LacI* | *TrpR* | *MalT* | *Cbl* |
| Total | 2 | 2 | 16 | 16 |
| Mutation | 2 | 2 | 8 | 8 |
| Growth | 0 | 0 | 8 | 8 |
| Increase (→) | 1 | 1 | 4 | 12 |
| Decrease (←) | 1 | 1 | 12 | 4 |
| $D_{min}$ | 2 | 2 | 7 | 7 |
| $D_{max}$ | 0 | 0 | 9 | 9 |

**Table S10. Summary of the advantages from Tables S8 and S9**. Grouped according to: a) total number of advantages, b) mutation and growth parameters, c), increase and decrease around the nominal value, and d) maximum and minimum values for the demand. The parameter theta (θ) is included in the count.

## 3.1.- SI Model description

*Savageau's seminal model*

The demand theory of gene regulation (DTGR) equations from the Savageau's original model [2] include the wild type ($X_w$), mutant promoter ($X_p$), mutant modulator ($X_m$), and the double mutant ($X_d$) populations. The $X_w$ can mutate to $X_p$ or $X_m$ and, if these two mutations are produced in the same organism, then the double mutant $X_d$ is originated (Eqs. S1-S4). The growth and mutation rates are given by the symbols $g$ and $m$ with the corresponding subscripts from the set $\{w, p, m, d\}$.

$$dX_w/dt = g_w X_w - g_w m_{pw} X_w - g_w m_{mw} X_w \quad [S1]$$
$$dX_p/dt = g_p X_p + g_w m_{pw} X_w - g_p m_{dp} X_p \quad [S2]$$
$$dX_m/dt = g_m X_m + g_w m_{mw} X_w - g_m m_{dm} X_m \quad [S3]$$
$$dX_d/dt = g_d X_d + g_m m_{dm} X_m + g_p m_{dp} X_p \quad [S4]$$

A simplification of Eqs. S1-S4 can be achieved by grouping growing and mutational rates into alpha terms with the corresponding subscripts:

$$\alpha_d = g_d$$
$$\alpha_{pw} = g_w m_{pw}$$
$$\alpha_{mw} = g_w m_{mw}$$
$$\alpha_{dm} = g_m m_{dm}$$
$$\alpha_{dp} = g_p m_{dp}$$
$$\alpha_{pp} = g_p(1 - m_{dp})$$
$$\alpha_{mm} = g_m(1 - m_{dm})$$
$$\alpha_{ww} = g_w\left(1 - (m_{pw} + m_{mw})\right)$$

Representing Eqs. S1-S4 in alpha terms,

$$dX_w/dt = \alpha_w X_w \quad [S5]$$
$$dX_p/dt = \alpha_{pw} X_w + \alpha_p X_p \quad [S6]$$
$$dX_m/dt = \alpha_{mw} X_w + \alpha_m X_m \quad [S7]$$
$$dX_d/dt = \alpha_d X_d + \alpha_{dm} X_m + \alpha_{dp} X_p \quad [S8]$$

The Transcription Factor Conformation (TFC) model, represented in Fig 2, include the populations: wild type ($X_w$), mutant promoter ($X_p$), mutant modulator ($X_m$), and six double mutants ($X_{d_1} \dots X_{d_6}$),

$$dX_w/dt = g_w X_w - g_w m_{pw} X_w - g_w m_{mw} X_w - g_w m_{r_1 w} X_w - g_w m_{r_2 w} X_w \quad [S9]$$
$$dX_p/dt = g_p X_p + g_w m_{pw} X_w - g_m m_{d_1 p} X_p - g_p m_{d_3 p} X_p - g_p m_{d_4 p} X_p \quad [S10]$$
$$dX_m/dt = g_m X_m + g_w m_{mw} X_w - g_m m_{d_1 m} X_m - g_m m_{d_5 m} X_m - g_m m_{d_6 m} X_m \quad [S11]$$
$$dX_{r_1}/dt = g_{r_1} X_{r_1} + g_w m_{r_1 w} X_w - g_{r_1} m_{d_2 r_1} X_{r_1} - g_{r_1} m_{d_3 r_1} X_{r_1} - g_{r_1} m_{d_6 r_1} X_{r_1} \quad [S12]$$
$$dX_{r_2}/dt = g_{r_2} X_{r_2} + g_w m_{r_2 w} X_w - g_{r_2} m_{d_2 r_2} X_{r_2} - g_{r_2} m_{d_4 r_2} X_{r_2} - g_{r_2} m_{d_5 r_2} X_{r_2} \quad [S13]$$
$$dX_{d_1}/dt = g_{d_1} X_{d_1} + g_m m_{d_1 m} X_m + g_p m_{d_1 p} X_p \quad [S14]$$
$$dX_{d_2}/dt = g_{d_2} X_{d_2} + g_{r_1} m_{d_2 r_1} X_{r_1} + g_{r_2} m_{d_2 r_2} X_{r_2} \quad [S15]$$
$$dX_{d_3}/dt = g_{d_3} X_{d_3} + g_{r_1} m_{d_3 r_1} X_{r_1} + g_p m_{d_3 p} X_p \quad [S16]$$
$$dX_{d_4}/dt = g_{d_4} X_{d_4} + g_{r_2} m_{d_4 r_2} X_{r_2} + g_p m_{d_4 p} X_p \quad [S17]$$
$$dX_{d_5}/dt = g_{d_5} X_{d_5} + g_{r_2} m_{d_5 r_2} X_{r_2} + g_m m_{d_5 m} X_m \quad [S18]$$
$$dX_{d_6}/dt = g_{d_6} X_{d_6} + g_{r_1} m_{d_6 r_1} X_{r_1} + g_m m_{d_6 m} X_m \quad [S19]$$

Grouping Eqs. S9-S19 into alpha terms,

$$\alpha_{d_1} = g_{d_1} \qquad \alpha_{d_3 r_1} = g_{r_1} m_{d_2 r_1}$$
$$\alpha_{d_2} = g_{d_2} \qquad \alpha_{d_3 p} = g_p m_{d_3 p}$$
$$\alpha_{d_3} = g_{d_3} \qquad \alpha_{d_4 r_2} = g_{r_2} m_{d_4 r_2}$$
$$\alpha_{d_4} = g_{d_4} \qquad \alpha_{d_4 p} = g_p m_{d_4 p}$$
$$\alpha_{d_5} = g_{d_5} \qquad \alpha_{d_5 r_2} = g_{r_2} m_{d_5 r_2}$$
$$\alpha_{d_6} = g_{d_6} \qquad \alpha_{d_5 m} = g_m m_{d_5 m}$$

$\alpha_{pw} = g_w m_{pw}$ $\quad$ $\alpha_{d_6 r_1} = g_{r_1} m_{d_6 r_1}$

$\alpha_{mw} = g_w m_{mw}$ $\quad$ $\alpha_{d_6 m} = g_m m_{d_6 m}$

$\alpha_{r_1 w} = g_w m_{r_1 w}$ $\quad$ $\alpha_p = g_p[1 - (m_{d_1 p} + m_{d_3 p} + m_{d_4 p})]$

$\alpha_{r_2 w} = g_w m_{r_2 w}$ $\quad$ $\alpha_m = g_m[1 - (m_{d_1 m} + m_{d_5 m} + m_{d_6 m})]$

$\alpha_{d_1 m} = g_m m_{d_1 m}$ $\quad$ $\alpha_{r_1} = g_{r_1}[1 - (m_{d_2 r_1} + m_{d_3 r_1} + m_{d_6 r_1})]$

$\alpha_{d_1 p} = g_p m_{d_1 p}$ $\quad$ $\alpha_{r_2} = g_{r_2}[1 - (m_{d_2 r_2} + m_{d_4 r_2} + m_{d_5 r_2})]$

$\alpha_{d_2 r_1} = g_{r_1} m_{d_2 r_1}$ $\quad$ $\alpha_w = g_w[1 - (m_{pw} + m_{mw} + m_{r_1 w} + m_{r_2 w})]$

$\alpha_{d_2 r_2} = g_{r_2} m_{d_2 r_2}$

Collapsing Eqs. S9-S19 into alpha terms,

$dX_w/dt = \alpha_w X_w$ [S20]

$dX_p/dt = \alpha_{pw} X_w + \alpha_p X_p$ [S21]

$dX_m/dt = \alpha_{mw} X_w + \alpha_m X_m$ [S22]

$dX_{r_1}/dt = \alpha_{r_1 w} X_w + \alpha_{r_1} X_{r_1}$ [S23]

$dX_{r_2}/dt = \alpha_{r_2 w} X_w + \alpha_{r_2} X_{r_2}$ [S24]

$dX_{d_1}/dt = \alpha_{d_1} X_{d_1} + \alpha_{d_1 m} X_m + \alpha_{d_1 p} X_p$ [S25]

$dX_{d_2}/dt = \alpha_{d_2} X_{d_2} + \alpha_{d_2 r_1} X_{r_1} + \alpha_{d_2 r_2} X_{r_2}$ [S26]

$dX_{d_3}/dt = \alpha_{d_3} X_{d_3} + \alpha_{d_3 r_1} X_{r_1} + \alpha_{d_3 p} X_p$ [S27]

$dX_{d_4}/dt = \alpha_{d_4} X_{d_4} + \alpha_{d_4 r_2} X_{r_2} + \alpha_{d_4 p} X_p$ [S28]

$dX_{d_5}/dt = \alpha_{d_5} X_{d_5} + \alpha_{d_5 r_2} X_{r_2} + \alpha_{d_5 m} X_m$ [S29]

$dX_{d_6}/dt = \alpha_{d_6} X_{d_6} + \alpha_{d_6 r_1} X_{r_1} + \alpha_{d_6 m} X_m$ [S30]

The linear equations S20-S30 are solved and the solutions at the end of a life cycle, which started in high-demand (*H*) environment, read as:

$$X_w(t + C) = X_w(t) exp[C(\alpha_w^H D + \alpha_w^L (1 - D))],$$

[S31]

$$X_*(t+C) = X_*(t)\left(\frac{\alpha_{*w}^{L}}{\alpha_w^L - \alpha_*^L}\left\{exp\left[C\left(\alpha_w^H D + \alpha_w^L(1-D)\right)\right] - exp\left[C\left(\alpha_w^H D + \alpha_*^L(1-D)\right)\right]\right\} + \frac{\alpha_{*w}^{H}}{\alpha_w^H - \alpha_*^H}\left\{exp\left[C\left(\alpha_w^H D + \alpha_*^L(1-D)\right)\right] - exp\left[C\left(\alpha_*^H D + \alpha_*^L(1-D)\right)\right]\right\}\right) + X_*(t)exp\left[C\left(\alpha_*^H D + \alpha_*^L(1-D)\right)\right]$$

[S32]

where *C*, is the average time for a gene to complete an on/off cycle, and demand *D*, corresponds at the fraction of the cycle time that the gene is on. The asterisks represent one in the set $\{p, m, r_1, r_2\}$, which are the corresponding variables for each of the mutant populations. The superscripts represent the fraction of the cycle time spent in environment with high (*H*) and low (*L*) demand with respect to the average total cycle time. The solutions at the end of a life cycle that start in a low-demand (*L*) environment can be obtained simply by interchanging superscripts *H* and *L* and the symbols *D* and $(1 - D)$.

The steady state pattern of mutant populations vs. wild type at can be expressed as the ratio of the two.

$$\frac{X_*}{X_w} = \frac{\left(\frac{\alpha_{*w}^{L}}{\alpha_w^L - \alpha_*^L}(1 - exp[(\alpha_*^L - \alpha_w^L)(1-D)C]) + \frac{\alpha_{*w}^{H}}{\alpha_w^H - \alpha_*^H}(1 - exp[(\alpha_*^H - \alpha_w^H)DC])\right)exp[(\alpha_*^L - \alpha_w^L)(1-D)C]}{1 - exp[(\alpha_*^H - \alpha_w^H)DC + (\alpha_*^L - \alpha_w^L)(1-D)C]}$$

[S33]

$$\frac{X_*}{X_w} = \frac{\left(\frac{\alpha_{*w}^{H}}{\alpha_w^H - \alpha_*^H}(1 - exp[(\alpha_*^H - \alpha_w^H)DC]) + \frac{\alpha_{*w}^{L}}{\alpha_w^L - \alpha_*^L}(1 - exp[(\alpha_*^L - \alpha_w^L)(1-D)C])\right)exp[(\alpha_*^H - \alpha_w^H)DC]}{1 - exp[(\alpha_*^L - \alpha_w^L)(1-D)C + (\alpha_*^H - \alpha_w^H)DC]}$$

[S34]

Equations [S33] and [S34] correspond to the steady state ratios analogous to equations 10 and 11 from the original Savageau manuscript [2]. The thresholds of selection (TS) were calculated equating the Eqs. S33 and S34 to the criteria of selection ($\theta$) and then using the bisection method to calculate $C$ and $D$.

The wild-type time course enrichment dynamics of selection from Figs. S11-S14 were defined as the fraction of wild type against mutants $f_m = X_w/(X_p + X_m + X_{r_1} + X_{r_2})$ along $n$ cycles times $nC$. This was calculated for each cycle time $C$ with the abscissa as number of cycles and the ordinate as the log of a normalized ratio $10\, f_m/f_m^{steady-state}$.

Following Savageau's methodology [1, 2], the influence of the TFC constituent parameters (Tables 1 and S1) on the extreme minimum ($D_{\min}$) and maximum ($D_{\max}$) values of the demand (Figs. 6, S15, and S16) were obtained after approximation from the corresponding Eqs. S33 or S34 with the first three Taylor terms when $C$ <<1 and solving for $C = 0$ as a function of $D$. The Taylor approximations were used to obtain $D_{\min}$ and $D_{\max}$ curves after the parameters varied around its nominal value.

## 3.2- SI Results

3.2.1. *Time course of selection.* The enrichment recovery dynamics of the wild-type regulatory mechanism represent the time course of the ratio of wild-type to all the single mutant populations at different high demand intervals ($D$). It starts at time zero with equal numbers of mutants and a ratio of $X_w/(X_p + X_m + X_{r_1} + X_{r_2})$ of 10% of its steady state value.

As in the Savageau work [1], the time course of selection for the TFs with negative modes of regulation (*LacI* and *TrpR*) emerges more rapidly when the demand for gene expression ($D$) increases (Figs. S11 and S12). By contrast, the positive mode of gene control emerges more rapidly as the demand for gene expression ($D$) decreases (Figs. S13 and S14).

Within the negative mode of regulation, *TrpR* presents a faster recovery with short $D$ cycles (Fig. S12a) than *LacI* (Fig. S11a). For longer $D$ cycles, the wild-type enrichments are very similar among the negative *apo* and *holo* conformations (Figs. S11b-c vs. S12b-c).

Within the positive mode of regulation, faster recovery is observed for *MalT* over *Cbl*, especially at higher values of $D$ (Figs. S13b-c vs. S14b-c).

3.2.2. *Influence of parameters on minimum and maximum values for demand.*

*Positive mode of regulation*. Some of the parameter sensitivities display similar behavior to that published in {Savageau, 1998 #643}; this is the case of *LacI* and *MalT* for: μ (Fig. S15-a), ε (Fig. S15-g), δ (Fig. S15-j), σ (Fig. S15-l), θ (Fig. S15-m), and ρ for *MalT* (Fig. S15-e).

Some of the thresholds of selection not delimiting the wild-type regions (Figs. S16a-m) manifest sensitivities similar to those described in [1]. This is the case for *LacI*: μ (Fig. S16-a), π (Fig. S16-b), τ (Fig. S16-d), ε (Fig. S16-g), δ (Fig. S16-j), λ (Fig. S16-k), and θ (Fig. S16-m).

In some cases only one of the thresholds matches in shape with [1]. These are: *MalT* in μ for the modulator (Fig. S16-a), υ for $r_1$ (Fig. S16-c), τ for the modulator (Fig. S16-d), ε for $r_1$ (Fig. S16-g), δ for $r_1$ (Fig. S16-j), σ for $r_1$ (Fig. S16-l), θ for $r_1$ (Fig. S16-m).

3.3.3.- *Enrichment dynamics*. The dynamics for the negative and positive modes of regulation follow the pattern reported by Savageau [3], *i.e*. a more rapid emergence of the negative mode of regulation with an increase in the demand, and the opposite pattern for the positive mode of regulation.

Within the negative modes of regulation, the *holo* conformation has a sharper increase for the wild type than the *apo* conformation (Figs. S11-S12). In the positive mode of control, the opposite trend was observed, with a faster recovery for the *apo* conformation as compared with the *holo* conformation (Figs. S13-S14).

## 4.- SI References


1. M. A. Savageau, *Genetics*, 1998, **149**, 1677-1691.
2. M. A. Savageau, *Genetics*, 1998, **149**, 1665-1676.
3. M. A. Savageau, *J Theor Biol*, 1969, **25**, 370-379.