

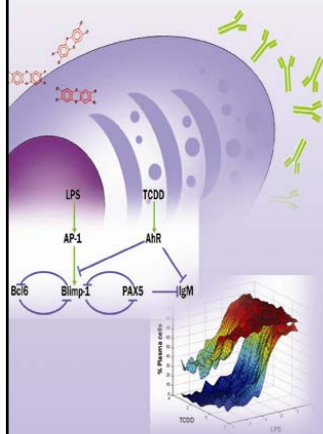
Thursday Afternoon Exercise:

Negative feedback and homeostasis (Part I)

September 25, 2008

Courtney G. Woods

Division of Computational Biology
The Hamner Institutes for Health Sciences

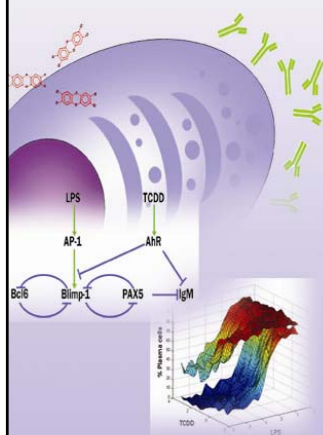


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Goals of Exercise

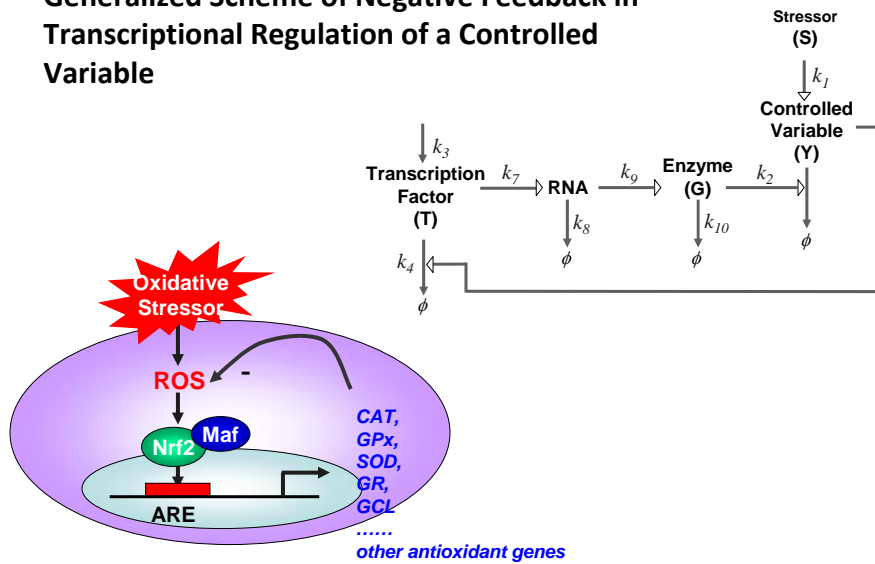
Study a negative feedback circuit which underlies homeostatic biological responses

- Incorporate the motifs that were introduced on Monday into an integrated model
- Assess how changing the local gains (thus the loop gain) can alter dose response
- Leave you a bit more on your own toward the end of the day to explore possibilities in model structure



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Generalized Scheme of Negative Feedback in Transcriptional Regulation of a Controlled Variable



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1. Open Exercises/Thursday/Neg_feedback1.mmd

```

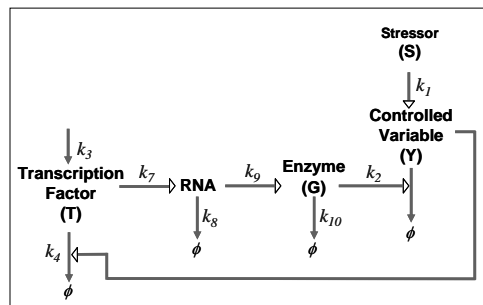
;Differential equations
d/dt (Y)  =(k1*S) - (k2*G*Y)
d/dt (T)  =(k3) - (k4*T/Y)
d/dt (RNA)=(k7*T) - (k8*RNA)
d/dt (G)  =(k9*RNA) - (k10*G)
    
```

```

; Initial conditions
init Y    =100      ;Controlled variable
init T    =1        ;Transcription factor
init RNA  =1        ;mRNA
init G    =10       ;Enzyme
    
```

```

; Parameters
S      = 10         ;mass
k1     = 10000     ;1/time
k2     = 10        ;1/mass/time
k3     = 100       ;mass/time
k4     = 10000    ;mass/time
k7     = 1         ;1/time
k8     = 1         ;1/time
k9     = 20       ;1/time
k10    = 2        ;1/time
    
```

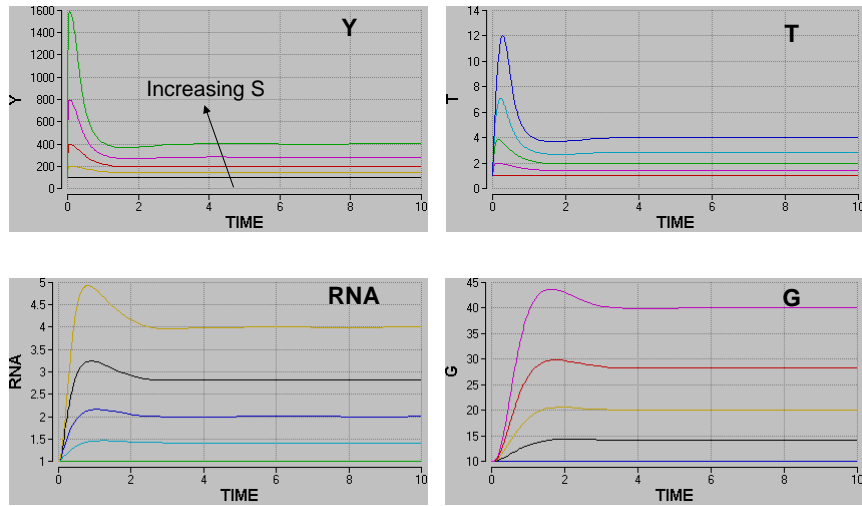


Stressor
Stressor-induced production rate constant for Y
G-catalyzed elimination rate constant for Y
Constitutive (basal) synthesis rate of T
Y-regulated degradation rate constant for T
T-activated transcription rate constant for RNA
RNA degradation rate constant
RNA-activated translation rate constant for G
G degradation rate constant

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2. Do a Batch Run by varying $S = 1, 2, 4, 8,$ and $16.$

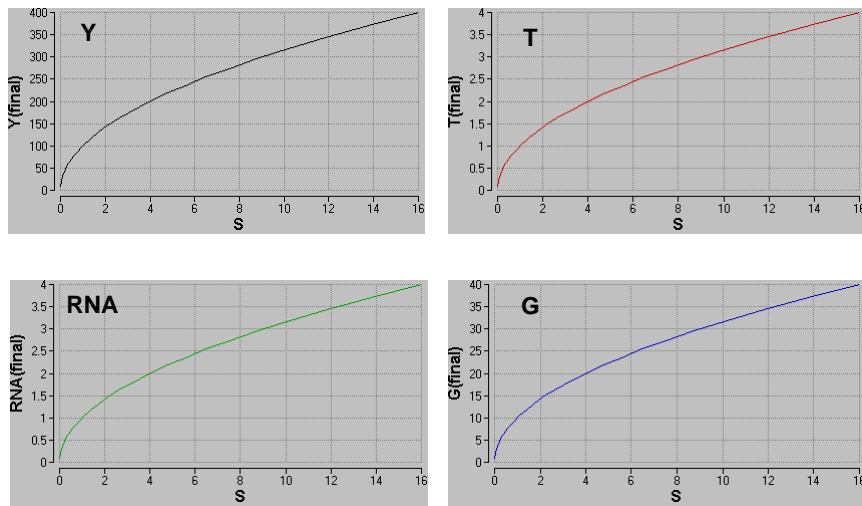
Why does Y increase initially then come back down to some lower steady states? What do you call this phenomenon? What about changes in G ?



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3. Do a Parameter Plot to get steady-state dose responses.

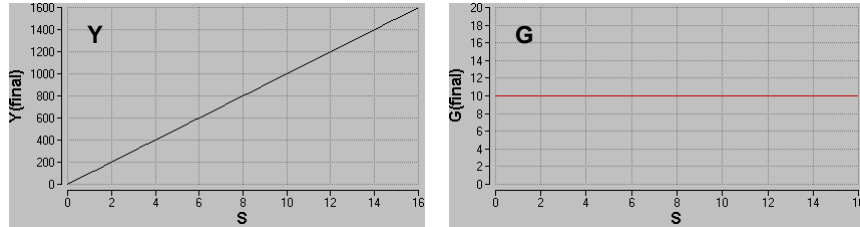
Does Y increase linearly with S ? If not, why? How about T vs. S , and G vs. S ?



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4. How will the dose responses look w/o (-) feedback?

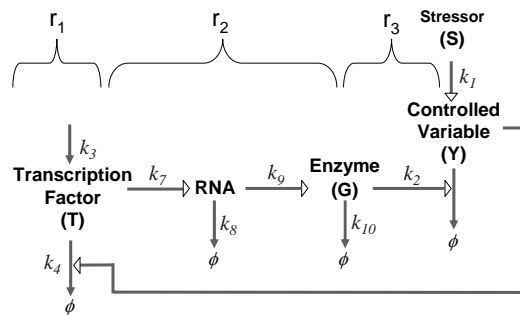
- In the equation, change $d/d(T)$ to $d/d(T)=(k_3) - (k_4*T/100)$. This decouples the degradation of transcription factor T from being regulated by controlled variable Y.
- Is Y vs. S linear now? If yes, compare it with the response in step 3 where the feedback is still intact. At the same dose of S, which has higher Y?



- What happens to G vs. S and T vs. S?
- After you are done, set $d/d(T)$ back to $d/d(T)=(k_3) - (k_4*T/Y)$.

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Recall the systems-level response coefficient for Y vs. S



Response
Coefficient

$$R_S^Y = \frac{1}{1 + |r_1 r_2 r_3|}$$

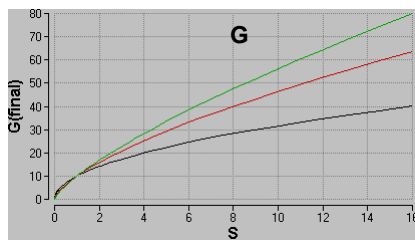
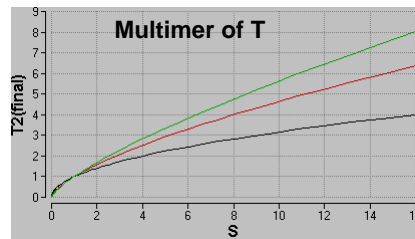
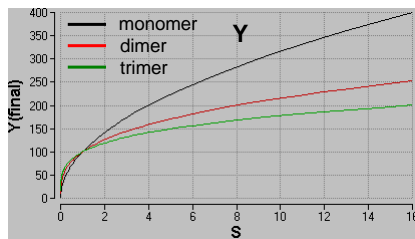
Using ultrasensitive motifs which increase local gains will reduce R_S^Y , making Y less sensitive to change of S.

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2. Do a Batch Run by varying $S = 1, 2, 4, 8,$ and 16 to see how Y, T, T_2, G evolve over time until reaching steady states.
3. Do a Parameter Plot to get steady-state dose responses. Compare the responses with previous results without dimerization. Does Y become less sensitive to changes of S now? How about the G vs. S dose response?
4. Can you figure out how to simulate trimerization of transcription factor T ?
5. Consider making changes to the model so that you can do overlay of dose responses for cases of no multimerization, dimerization, and trimerization. Why one has better homeostatic performance?

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6. The results below show overlay of steady-state dose responses for multimerization of transcription factor T



Take a break.....

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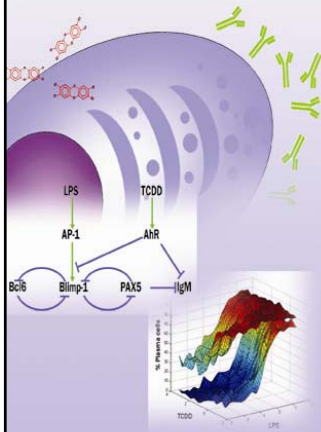
Thursday Afternoon Exercise:

Negative feedback and homeostasis (Part II)

September 25, 2008

Courtney G. Woods

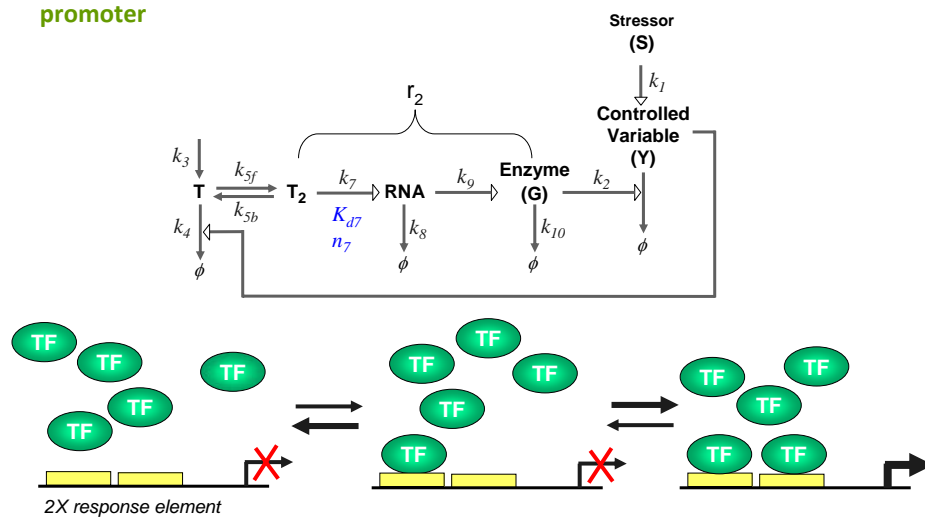
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Increasing local gain r_2 and introducing saturable promoter activation:

Cooperative binding of transcription factor dimer T_2 to gene promoter



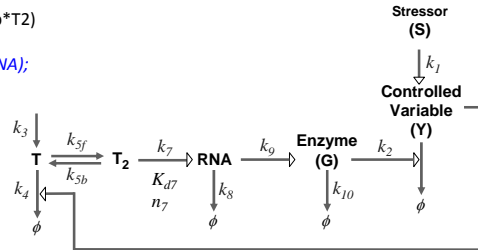
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1. Open Exercises/Thursday/Neg_feedback3.mmd.

Study how cooperative binding of T_2 to gene promoter is implemented (code in italics)

```
;Differential Equations
d/dt (Y)      =(k1*S) - (k2*G*Y)
d/dt(T)      =(k3) - (k4*T/Y) - (2*k5f*T*T) + (2*k5b*T2)
d/dt(T2)     =(k5f*T*T) - (k5b*T2)
d/dt(RNA)   =(k7*T2^n7)/(Kd7^n7+T2^n7) - (k8*RNA);
d/dt(G)      =(k9*RNA) - (k10*G)
```

```
; Initial conditions
init Y      =100      ;Controlled variable
init T      =1        ;Transcription factor
init T2     =1        ;T dimer
init RNA    =1        ;mRNA
init G      =10       ;Enzyme
```

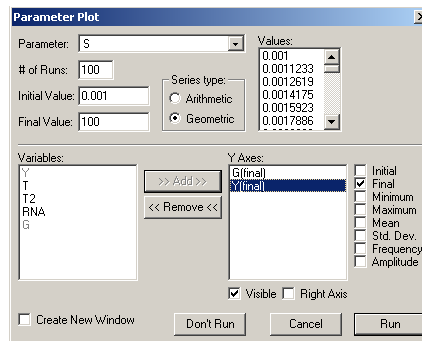


A Hill function is used to describe cooperative binding

```
; Parameters
S          = 10      ;mass      Stressor
k1         = 10000   ;1/time   Stressor-induced production rate constant for Y
k2         = 10      ;1/mass/time G-catalyzed elimination rate constant for Y
k3         = 100     ;mass/time Constitutive (basal) synthesis rate of T
k4         = 10000   ;mass/time Y-regulated degradation rate constant for T
k5f        = 100     ;1/mass/time T association (dimerization) rate constant
k5b        = 100     ;1/time   T2 dissociation rate constant
Kd7       = 3       ;mass     Dissociation constant (inverse of affinity) for T2 binding to promoter
n7        = 2       ;         Hill coefficient for cooperative binding of T2 to promoter
k7        = 10     ;mass/time T2-activated maximal transcription rate
(See Thurs3.mmd for the rest of the code)
```

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2. Do a Parameter Plot to get steady-state dose responses by using the setting below:

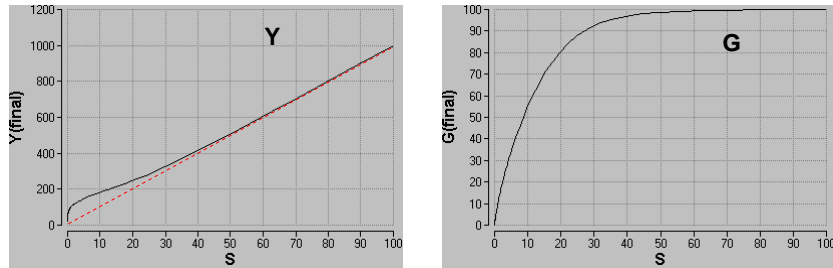


3. Observe the Y vs. S and G vs. S steady-state dose response curves (results shown in the next slide). Answer the following questions:

a) Is the Y vs. S dose response curve superlinear at low doses?

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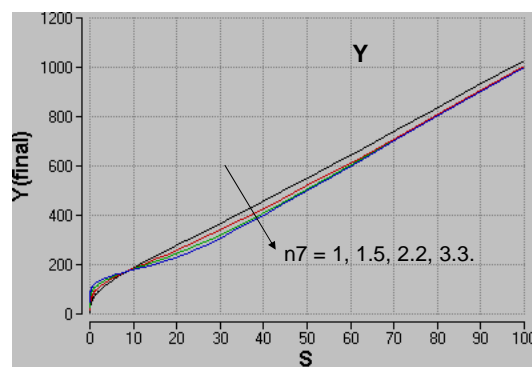
Steady-state dose response obtained from step 2 above



- b) Does it start to bend up (sublinear) as the dose increases?
- c) Does it eventually become linear? Can the linear part be perfectly extrapolated back to origin (0, 0)? Hint: use a ruler or the edge of a piece of paper to extrapolate on the computer screen. Run to higher doses if needed.
- d) Explain all the curvature changes you observed in a) – b).
- e) How does the gene expression (G vs. S) response curve look? Why does it plateau at higher doses?

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4. Use Parameter Window to change (n_7) the degree of cooperativity of transcription factor binding to promoter. Overlay the dose response result for $n_7 = 1, 1.5, 2.2, 3.3$.



Questions: With higher cooperativity, does Y vs. S response at low doses look more flat? Why? Do these curves converge at higher doses? Why?

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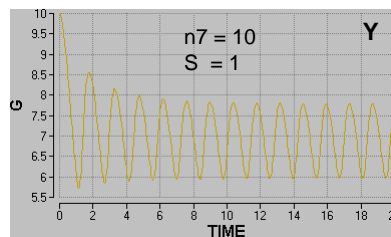
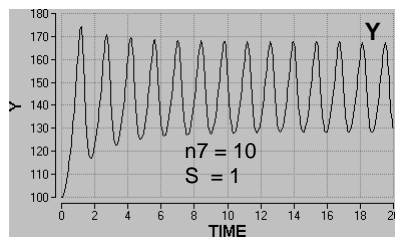
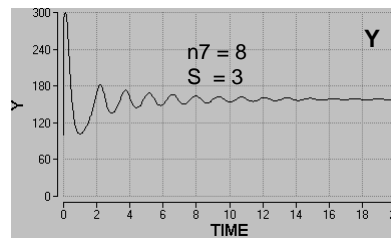
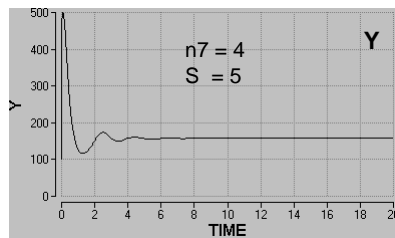
5. So it seems that the more ultrasensitive the feedback loop is (high loop gain), the better steady-state homeostatic control for Y. Is it always true? Now in Parameter Window, set n7 even higher, to 4, set stress level S to 5, hit run for time course simulation (results shown on next slide).

- What happens to Y, and G? They seem to oscillate a couple of cycles before settling to steady states. Haven you seen similar phenomenon experimentally with stress response before?
- Now increase the cooperativity even higher by setting n7 to 8, and set stress level S to 3. Hit Run. Does it look more like oscillating now but dampening out at the end?
- Let's stretch it further more. Set n7 to 10, and S to 1, do you see persistent oscillation? Don't believe it? Run for longer time then.

What's going on here? Remember this is a (-) feedback system. With a high loop gain and some time delay (gene expression is relatively slow), a (-) feedback system may oscillate. This is bad for homeostasis. Therefore high loop gain is not always desirable, because it increases the instability of the system by making it oscillate. This is one limitation of using (-) feedback control alone for homeostasis.

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Simulation results for step 5



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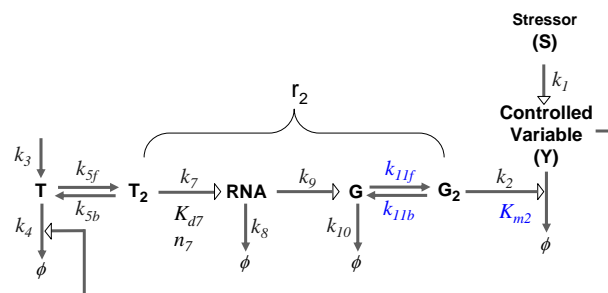
What else haven't we considered in our description of negative feedback?

- Mechanisms of activation of the transcription factor feedback loop
- Saturation of enzymatic elimination of the controlled variable Y
- Multimerization of enzymes, e.g., G in this model, that enhances homeostatic control (by increasing r_2)
- And, probably several other possibilities, including phosphorylation, MAPK-cascades, and protein-protein interactions in transcription factor activation
- So, let's.....

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Further increasing local gain r_2 and introducing saturable enzymatic elimination of Y:

(1) Gene product G dimerizes to form functional enzyme G_2 ; and (2) the reaction catalyzed by G_2 to eliminate Y can be saturated when Y is high.



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1. Open Exercises/Thursday/Neg_feedback4.mmd.

Add/modify model equations in places with ??? to incorporate enzyme saturation for elimination of Y and dimerization of enzyme G.

;Differential Equations

$$d/dt(Y) = (k1*S) - ???$$

$$d/dt(T) = (k3) - (k4*T/Y) - (2*k5f*T*T) + (2*k5b*T2)$$

$$d/dt(T2) = (k5f*T*T) - (k5b*T2)$$

$$d/dt(RNA) = (k7*T2^n7)/(Kd7^n7+T2^n7) + 2 - (k8*RNA);$$

$$d/dt(G) = (k9*RNA) - (k10*G) - ???$$

$$d/dt(G2) = ???$$

; Initial conditions

$$\text{init } Y = 100$$

$$\text{init } T = 1$$

$$\text{init } T2 = 1$$

$$\text{init } RNA = 3$$

$$\text{init } G = 30$$

$$\text{init } G2 = 90$$

;Controlled variable

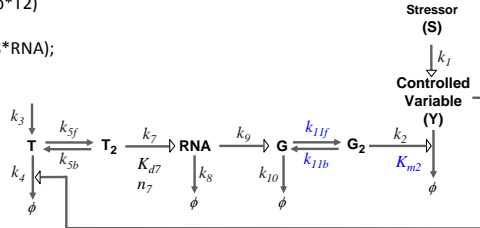
;Transcription factor

;T dimer

;mRNA

;Enzyme monomer (inactive)

;Enzyme dimer (active)



Dimerization of G and saturable elimination of Y

; Parameters

$$S = 10$$

$$k1 = 10000$$

$$k2 = 2330$$

$$Km2 = 2000$$

;mass Stressor

;1/time Stressor-induced production rate constant for Y

;1/time G2-catalyzed elimination rate constant for Y

;mass Michaelis-Menten constant of enzyme G2 elimination of Y

(Code continues on the next slide)

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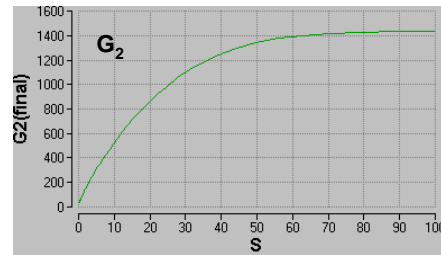
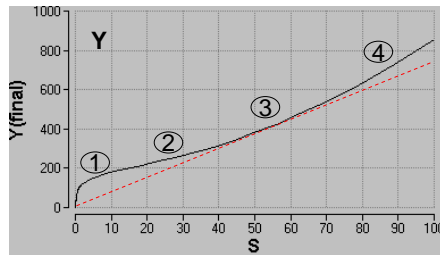
k3	=100	;mass/time	Constitutive (basal) synthesis rate of T
k4	=10000	;mass/time	Y-regulated degradation rate constant for T
k5f	=100	;1/mass/time	T association (dimerization) rate constant
k5b	=100	;1/time	T2 dissociation rate constant
Kd7	=3	;mass	Dissociation constant (inverse of affinity) for T2 binding to promoter
n7	=2	;	Hill coefficient for cooperative binding of T2 to promoter
k7	=10	;mass/time	T2-activated maximal transcription rate
k8	=1	;1/time	RNA degradation rate constant
k9	=20	;1/time	RNA-activated translation rate constant for G
k10	=2	;1/time	G degradation rate constant
k11f	=10	;1/mass/time	G association (dimerization) rate constant
k11b	=100	;1/time	G2 dissociation rate constant

2. Do a Parameter Plot to get steady-state dose responses.

- How do the Y vs. S and G_2 vs. S dose response curve look like (results shown in the next slide)? Active enzyme G_2 , which is responsible for eliminating Y, seems to increase “hyperbolically” with S without curvature changes, then why Y response has multiple phases.
- How many potential phases are there for Y vs. S dose response in terms of curvature changes. Explain what happens to the negative feedback control gene circuit as stress level S increases, which results in such dose response transition.

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3. Since this model incorporates several processes (dimerization, cooperative binding, saturable kinetics, etc), consider change parameters in these processes to see how they affect the steady-state dose response.



- ① Superlinear controlled
- ② Sublinear less controlled
- ③ Linear uncontrolled
- ④ Sublinear catastrophic

These are the equations for those with ??? marks.

$$\begin{aligned} d/dt(Y) &= (k1*S) - (k2*G2*Y/(Km2+Y)) \\ d/dt(G) &= (k9*RNA) - (k10*G) - (2*k11f*G*G) + (2*k11b*G2) \\ d/dt(G2) &= (k11f*G*G) - (k11b*G2) \end{aligned}$$

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With cellular stress response, it is quite common that a small dose of stress applied earlier appears to lessen perturbations caused by a subsequent larger dose, thus protecting the cell from damage. Let's see whether this simple negative feedback control model can do that.

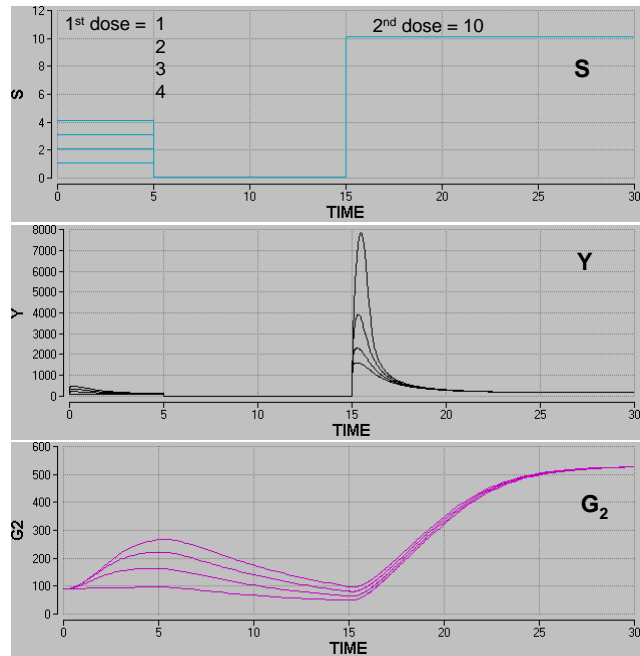
1. Comment out $S = 10$, then copy the following code in italics to the model. The code generates a pulse of first dose $S1$, followed by a second dose $S2$.

```
; Parameters
;S = 10 ;Stressor
S1 = 1 ;1st dose
S2 = 10 ;2nd dose
S1_onset = 0 ;onset of 1st dose
S1_duration = 5 ;duration of 1st dose
S2_onset = 15 ;onset of 2nd dose
S2_duration = 100 ;duration of 2nd dose
S = S1*SQUAREPULSE(S1_onset, S1_duration) + S2*SQUAREPULSE(S2_onset, S2_duration) + 0.01
```

2. Run a time course simulation, adjust x-axis to 0-30. Increase the first dose $S1$ to 2, 3, and 4, and run again (to compare, use Overlay). Observe whether the transient response of Y to the second dose $S2$ is attenuated as the dose of $S1$ increases. Explain why. Try different pulse duration and interval between the two doses.

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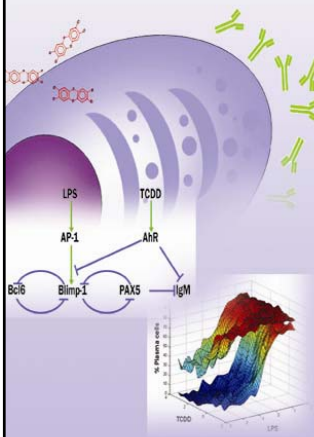
This is what you should see after step 2.



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Feedback Modeling - Summary

- Negative feedback reduces the sensitivity of the controlled variable (Y) to the imposed stressor (S)
- Enhancing the local gains in the (-) feedback circuit through dimerization, co-operative binding etc. can further decrease the responsiveness of the controlled variable to a stressor
- The negative feedback circuits contain many potential sites for ultrasensitive motifs
- Most cellular stress responses with homeostatic trajectories will have these negative feedback networks. The steady-state dose response from negative feedback control is highly nonlinear.



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Questions?

Go ahead and play some more!

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