# Apoptosis

programmed cell death –cellular suicide– is called apoptosis (from the Greek for “a falling off”)

apoptosis is a necessary part of the development of many multicellular organisms

some cells play only transient role during development; when no longer needed, they receive signals that induce apoptosis

(e.g. tail of a tadpole, which is not needed by the adult frog)

compared with death caused by stress or injury, apoptosis tidy:

rather than spill their contents into the environment, apoptotic cells quietly implode, thus ensuring no detrimental effects on the surrounding tissue

# Caspases

apoptosis is invoked by caspase proteins, which are always present in the cell, but lie dormant until activated

the family of caspase proteins is split into two categories:

- *initiator caspases* respond to apoptosis-inducing stimuli

are triggered externally (via transmembrane receptors)

or internally, by stress signals from the mitochondria (e.g., starvation signals)

- *executioner caspases* activated by initiator caspases

they carry out the task of cellular destruction by cleaving a number of key proteins and activating DNases that degrade the cell's DNA

# Model

*Eissing et al, 2004*
caspase-8, an initiator, and caspase-3, an executioner

Caspase-8 is triggered by external stimuli
When active, it activates caspase-3

Caspase proteins activated by removal of a masking domain, revealing a catalytic site

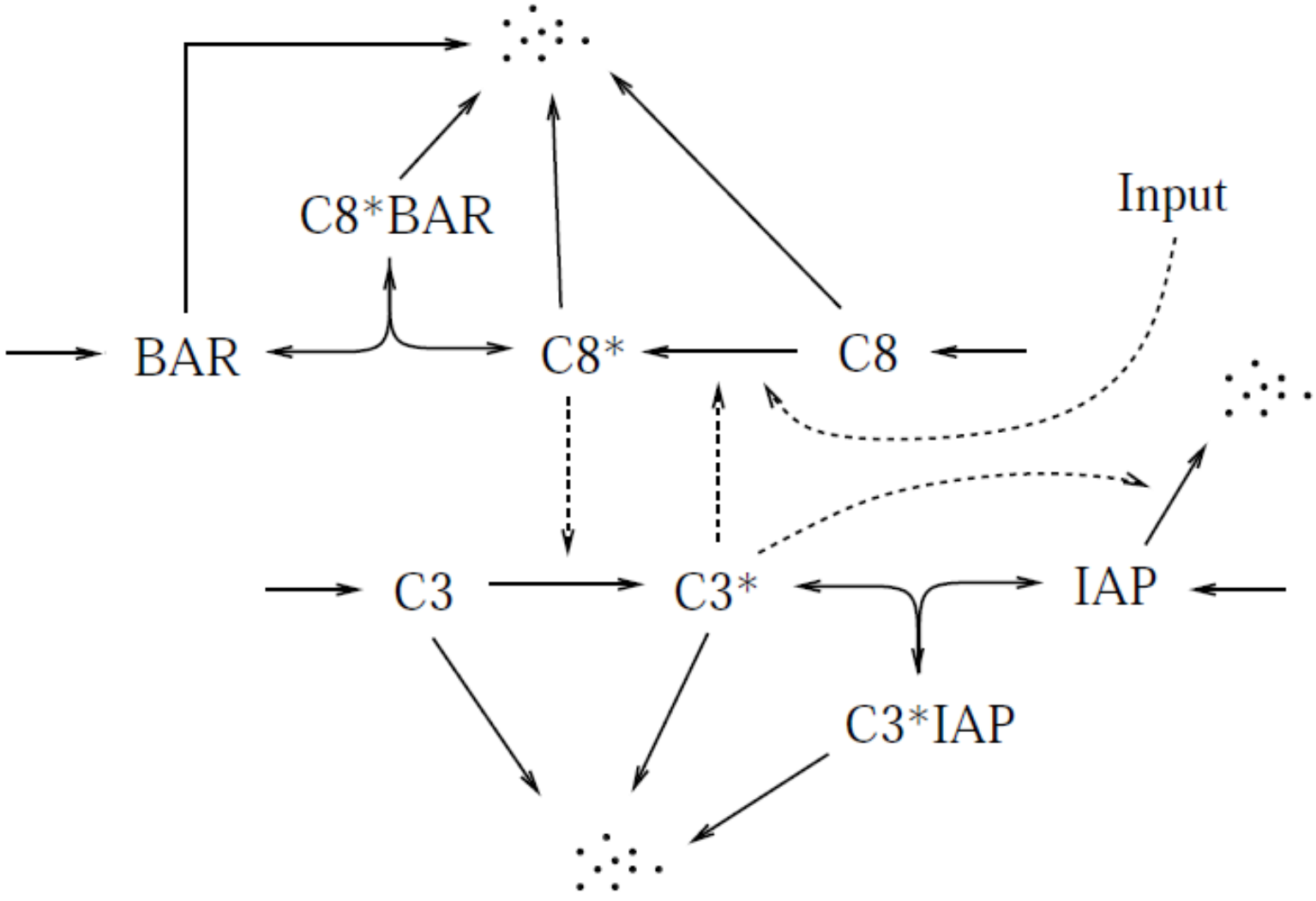
This cleavage is irreversible: protein only can be degraded
Also production processes for each protein

to guarantee decision to undergo apoptosis is irreversible, feedback mechanism: active caspase-3 activates caspase-8

positive feedback ensures caspase activity is self-perpetuating

in addition to caspases, model incorporates two proteins, IAP and BAR, which inhibit apoptosis by binding active caspases, forming inert complexes

# Model, ctd




*Eising apoptosis model (shown not as a chemical reaction network, but using a different graphical representation)*

# Players in model

An extracellular signal triggers activation of caspase-8 (C8*) from its inactive form (C8)

Once active, caspase-8 activates caspase-3 (C3 to C3*)

Active caspase-3 activates caspase-8, forming a positive feedback loop

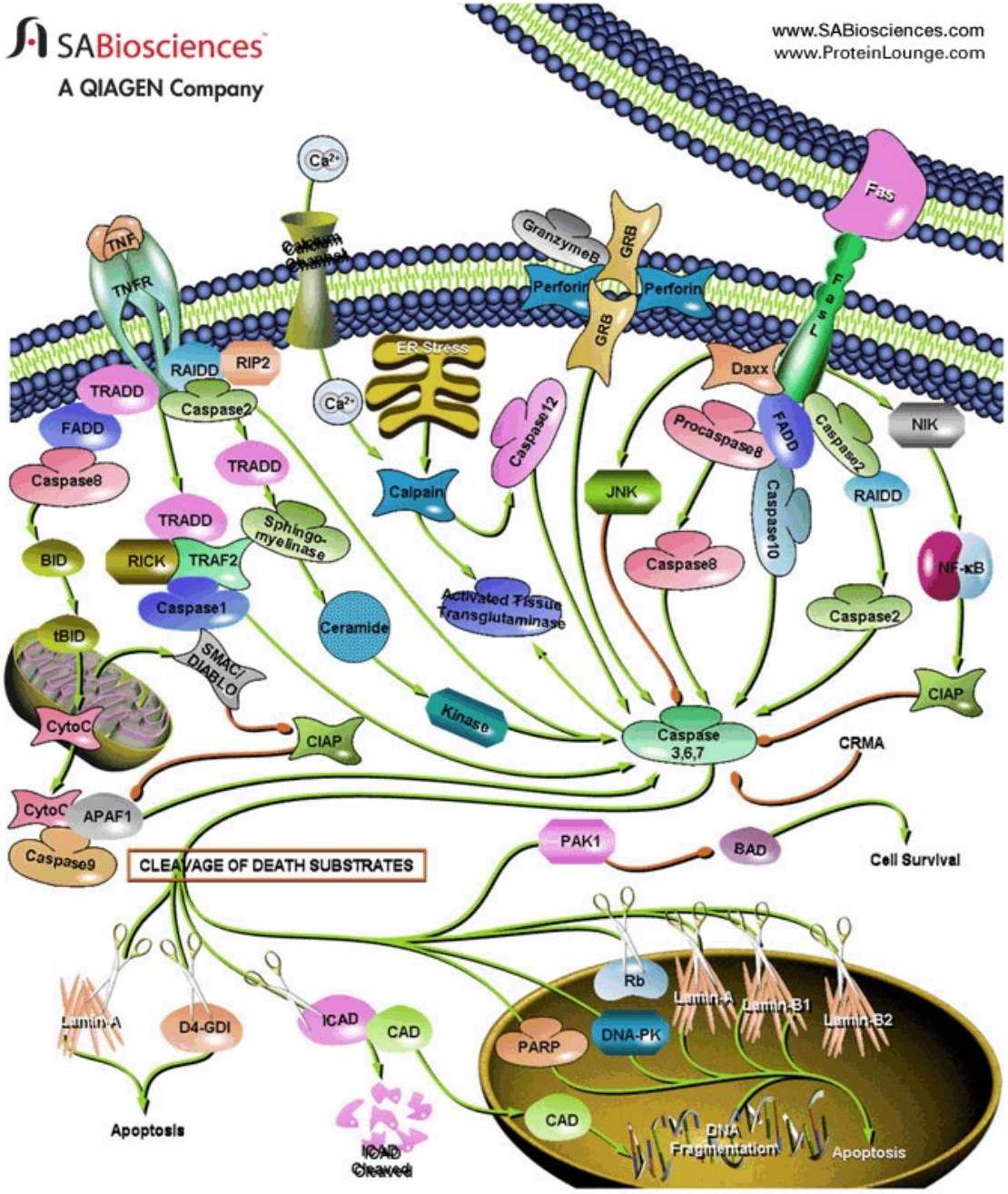
Two apoptotic inhibitors are included: BAR and IAP

These proteins bind active caspase-8 and -3, respectively, thus inhibiting the progression to apoptosis

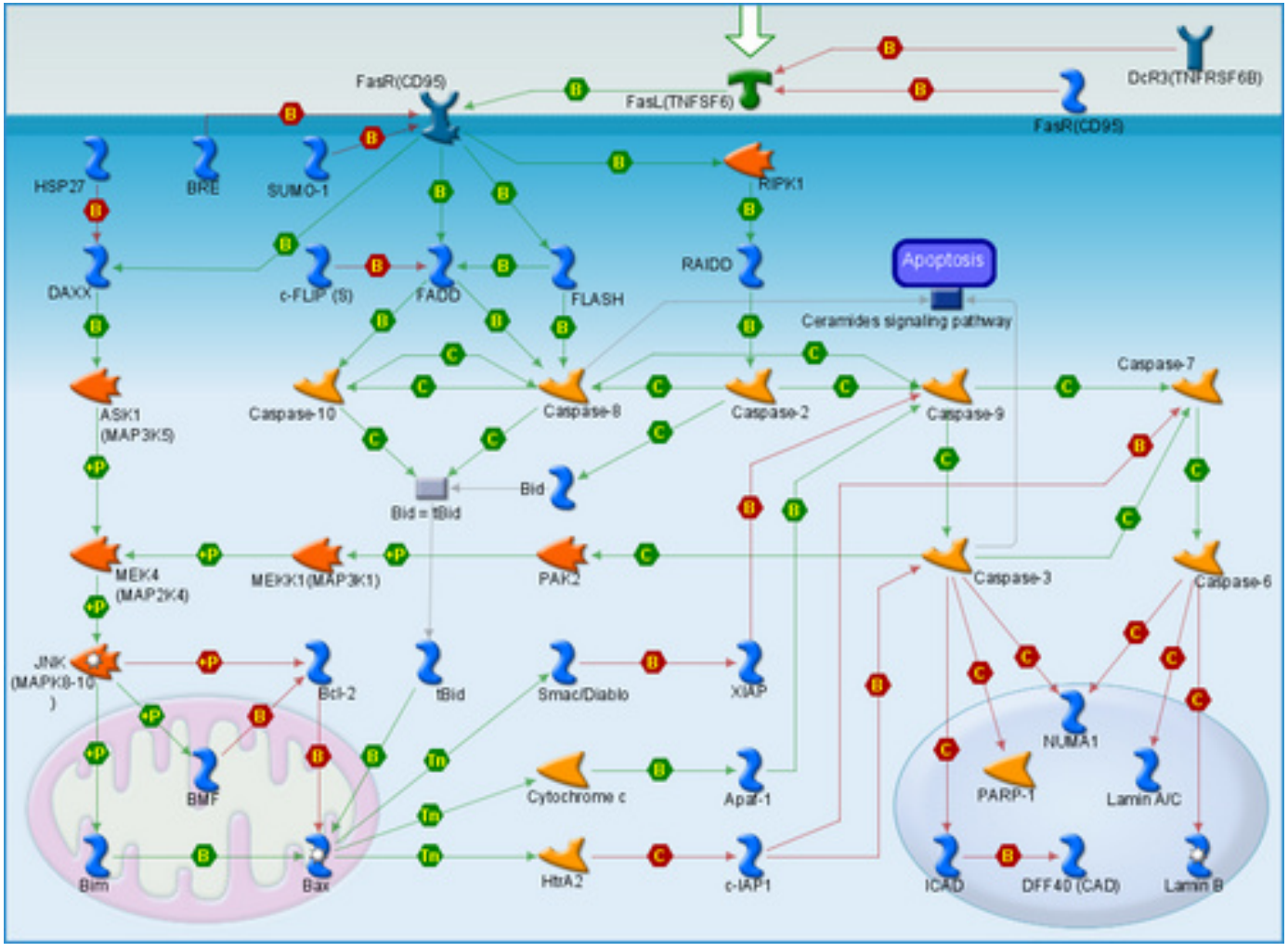
(Dots indicate degraded proteins)

(Dashed lines represent enzymatic reactions like $C3^* + C8 \rightarrow C3^* + C8^*$ which do not consume the enzyme. The authors implicitly assume these occur fast enough that we ignore intermediate complexes)

# Some figures showing fuller pathway complexity



# Some figures showing fuller pathway complexity



# Equations

Treating all enzyme-catalyzed reactions as first-order:

$$
\begin{aligned}
d/dt[\mathrm{C8}] &= k_1 - k_2[\mathrm{C8}] - k_3([\mathrm{C3}^*] + [\mathrm{Input}]) \cdot [\mathrm{C8}] \\
d/dt[\mathrm{C8}^*] &= k_3([\mathrm{C3}^*] + [\mathrm{Input}]) \cdot [\mathrm{C8}] - k_4[\mathrm{C8}^*] \\
&\quad -k_5[\mathrm{C8}^*] \cdot [\mathrm{BAR}] + k_6[\mathrm{C8}^*\mathrm{BAR}] \\
d/dt[\mathrm{C3}] &= k_7 - k_8[\mathrm{C3}] - k_9[\mathrm{C8}^*] \cdot [\mathrm{C3}] \\
d/dt[\mathrm{C3}^*] &= k_9[\mathrm{C8}^*] \cdot [\mathrm{C3}] - k_{10}[\mathrm{C3}^*] \\
&\quad -k_{11}[\mathrm{C3}^*] \cdot [\mathrm{IAP}] + k_{12}[\mathrm{C3}^* IAP] \\
d/dt[\mathrm{BAR}] &= k_{13} - k_5[\mathrm{C8}^*] \cdot [\mathrm{BAR}] + k_6[\mathrm{C8}^*\mathrm{BAR}] - k_{14}[\mathrm{BAR}] \\
d/dt[\mathrm{IAP}] &= k_{15} - k_{11}[\mathrm{C3}^*] \cdot [\mathrm{IAP}] + k_{12}[\mathrm{C3}^*\mathrm{IAP}] \\
&\quad -(k_{16} + k_{17}[\mathrm{C3}^*]) \cdot [\mathrm{IAP}] \\
d/dt[\mathrm{C8}^*\mathrm{BAR}] &= k_5[\mathrm{C8}^*] \cdot [\mathrm{BAR}] - k_6[\mathrm{C8}^*\mathrm{BAR}] - k_{18}[\mathrm{C8}^*\mathrm{BAR}] \\
d/dt[\mathrm{C3}^*\mathrm{IAP}] &= k_{11}[\mathrm{C3}^*] \cdot [\mathrm{IAP}] - k_{12}[\mathrm{C3}^*\mathrm{IAP}] - k_{19}[\mathrm{C3}^*\mathrm{IAP}]
\end{aligned}
$$

# Behavior of model

at low levels of activated caspase, at rest in a “life” state

when caspase activity rises above a threshold,

positive feedback commits the system to reaching steady state

with high levels of active caspase, a “death” state (of course, this state is transient - the cell is being dismantled – only “quasi” steady state, on the timescale of signaling)

simulation shows the response of the system to an input signal: begins at rest with zero input and low caspase activity

input $\Rightarrow$ slow increase in the activity level of caspase-8

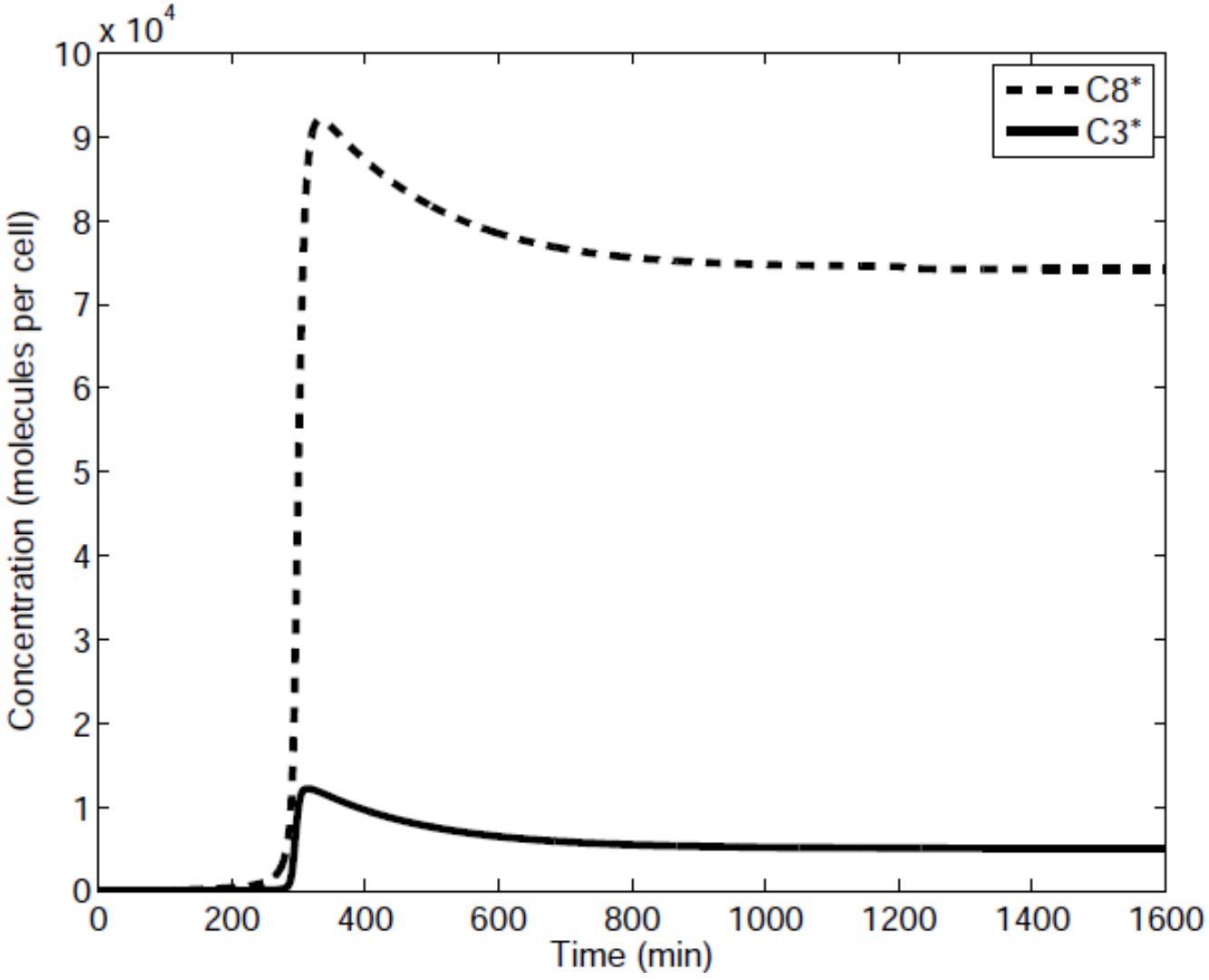
eventually leads to a rapid rise in caspase activity,

and system settles into “death” state with high caspase activity

input signal removed: this self-perpetuating state persists (bistablility) since input signal does not cause a return to the initial state,

transition is irreversible

# Plots


x 10^4
10
9
8
7
6
5
4
3
2
1
0
Concentration (molecules per cell)
C8*
C3*
0
200
400
600
800
1000
1200
1400
1600
Time (min)

# Plots

simulation begins in the low caspase activity “life” state

at time t =100 minutes, an input signal (Input=200) is introduced, causing an increase in caspase-8 activity

this triggers a positive feedback loop that results in a rapid increase in activated caspase-8 and caspase-3 abundance at about t = 300

system then settles to the high caspase-activity “death” state

input stimulus is removed at time t =1200 minutes, but there is no effect: the apoptotic switch is irreversible.

# Problems

(1) Write the chemical reaction network (e.g., $BAR + C8^* \leftrightarrow C8^*BAR$, etc.) that gives rise to the equations that were given.

(2) For this CRN, write the vector of species, the stoichiometric matrix, and the vector of reaction rates, and verify that, multiplying out, one gets the equations that were given.

(2) (Duration of triggering signal.) The simulation shown in the figure is done using the MATLAB program *apoptosis_eissing_model.m*. The input signal was maintained for 1100 minutes (until after the system had settled to the caspase-active "death" state.)

Re-run this simulation many times, with different durations, until you determine what is the shortest input pulse can trigger the irreversible life-to-death transition. Use the same input size as in the figure (Input=200). Your answer should be an integer, and you should print out a plot showing the behavior with this duration and a plot showing the behavior with 1 minute less.

# Problems, ctd

(4) Explain what one means by this statement: “In the model, active caspase-3 promotes degradation of IAP. This interaction can be seen as an additional positive feedback of $C3^*$ on itself.”

Your answer should be a couple of sentences explaining why the degradation of IAP implies that $C3^*$ gets to stay around longer than if IAP was not degraded.

Therefore, this degradation helps self-sustained caspase activity, hence also helping the positive feedback that makes caspase activation irreversible.

(5) Which $k_i$ should be set to zero to get rid of the effect of caspase-3 promoting degradation of IAP?

(6) Simulate (with an input of length 1100 and magnitude 200) what happens when the $k_i$ from the previous problem is set to zero. Plot the result and print it.