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Linear Control Analysis of Eissing's Apoptosis Model

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***Abstract*—Apoptosis is a key regulator for replacing unused, old and damaged cells. Here we analyse a popular model of Apoptosis. We deconstruct this model by linearising it about the life steady state and applying methods from linear control theory. This control viewpoint uncovers a decentralised control scheme with a clear separation of plant and controller. The small gain theorem is used to analyse stability and to give insight into how the inhibitors naturally modulate cell death and highlighting the role played by positive and negative feedback. Finally we use simulations to examine the extent to which reversal of apoptosis is possible once initiated.**

***Index Terms*—// Apoptosis, Caspase Activation, Biochemical Pathways, Feedback control, Dementia.**

I. Introduction

Apoptosis or programmed cell death is essential for maintaining homoeostasis in a living organism and controls physiological tissue growth by conterbalancing proliferation in a delicate manner [21]. It is a key regulator for replacing unused, old and damaged cells. Misregulation of the apoptosis pathway may lead to excessive cell proliferation as in cancer or viral infections and excessive cell death as in neurodegenerative diseases [17]. Apoptosis can be induced by intrinsic or extrinsic signalling pathways. The intrinsic pathway is triggered in response to defective cell cycle, organelle or DNA damage, detachment from the extracellular matrix or other severe cell stress [13]. The activation of specific pro-apoptotic receptors by their ligands, i.e. cytokines from the tumor necrosis factors (TNF)-family such as TRAIL (TNF-related apoptosis inducing ligand), FasL and TNF-α activate the extrinsic pathway. [26]. The core of the apoptotic pathway consists of a mutual activation of proteins, called caspases (cysteine-dependent aspartate-directed proteases). The first step towards apoptosis is the activation of initiator caspases, such as Caspase-8, by the cleavage of their corresponding pro-caspases. The final and irreversible step is the cleaving of a large number of effector caspases, e.g. Caspase-3. In all cases initiator caspases directly activate effector caspases. In the so called Type I cells this is sufficient to induced apoptosis, while Type II cells require the amplification of the triggering signal. This is obtained by releasing pro-apoptotic proteins like Cytochrome C from the mitochondria. The onset of apoptosis is controlled by the caspase inhibitors XIAP (X-linked inhibitor of apoptosis protein) and BAR (Bifunctional apoptosis inhibitor).

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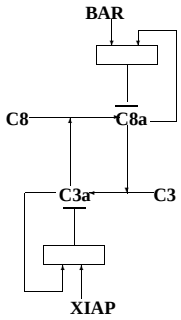

Fig. 1. Motif illustrating caspase activation controlled by decentralised inhibitors.

Why study the regulation of apoptotic signalling? In cell death arising from dementia and related diseases apoptosis is triggered prematurely and here apoptosis should be restored to the normal decision time for a particular cell type. In cancer cells apoptosis is disabled and here we would like to trigger apoptosis. Caspase inhibitors regulate this process by acting as controllers in a negative feedback loop.

Numerous mathematical models of apoptosis have been published [Huber H, Bullinger E, Rehm M: Systems Biology Approaches to the Study of Apoptosis. In Essentials of Apoptosis, 2nd Edition. Edited by Yin X-M, Dong Z: Humana press; 2009: 283-297]. Common to many of them is a description of the form,

$$\dot{x}(t) = f(x(t), x(0), p) \tag{1}$$

where $x(t) \in R_+^n$ is a state vector of molecule concentrations and $p \in R_+^k$ is the vector of kinetic constants of the described reaction rates. In this study, we analyse a popular model of apoptosis. This model has two steady-states in the non-negative orthant. Without a trigger signal, the model remains in a steady state, the so-called life steady state. The linear control viewpoint uncovers a decentralised control scheme with a clear separation of plant and controller. The small gain theorem is used to analyse stability which gives insights into how the inhibitors naturally modulate cell death and highlighting the role played by positive and negative feedback.

The rest of this paper is organized as follows. In section II we describe Eissing's model as a feedback system. In section III develop linear input/output models. In section IV we use the small gain theorem to analyse stability. Section V considers control of apoptosis. Conclusions are given in section VI.

II. A Feedback Model of Eissing's Dynamics

In this section we briefly describe Eissing's model of the direct or intrinsic apoptosis dynamics [14]. The full state

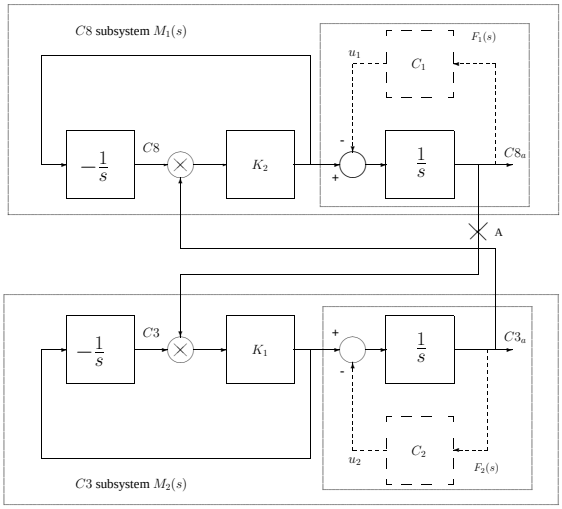

Fig. 2. Simplified feedback representation of Eissing's model as the interconnection of subsystems M_1 and M_2 and also showing the feedback controllers C_1 and C_2 for each subsystem.

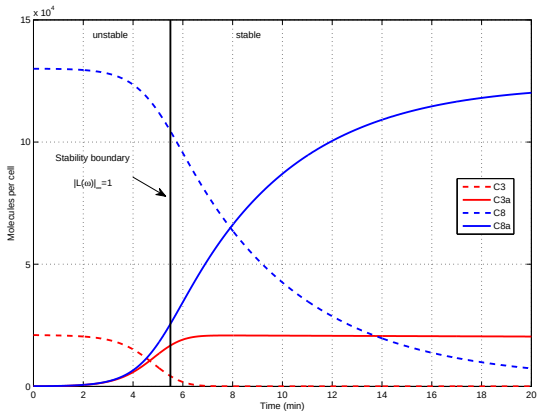

Fig. 3. Plant Transient Response $C8a(0) = 100, u_1 = u_2 = 0$. (p16, [11]). Initially the loop-gain $||M_1 M_2||_\infty >> 1$. Also shown is the time at which the loop gain decreases to unity.

model is given in the appendix along with the initial conditions used. Only the initial concentration of C8a(0) is varied, all other initial conditions remain fixed. Concentrations are in molecules per cell (mpc) and time is measured in minutes (m). Rates are $(mpc\, m^{-1})$ and constants are consistent with this convention. In the model there is an initial pool of the inactive caspase proteins C3 and C8 and an initial pool of the protein inhibitors XIAP and BAR. The activated caspase proteins are C3a and C8a. The initial condition $C8a(0)$ (mpc) can be thought of as an impulse input to the system. When $C8a(0) = 0$ the initial concentration of inhibitors acts to stabilise the autocatalytic reaction resulting in a stable fixed point. For the model provided $C8a(0) \approx< 75\ mpc$ the model returns to this stable fixed point. Otherwise the two caspase proteins are activated in an autocatalytic reaction. (It is interesting to note that when linearised about the life steady state the stability radius of the Jacobian is significantly smaller than for the full nonlinear model.) When apoptosis is triggered the initial concentration of the protein inhibitors XIAP and BAR slows down the activation of C3 and C8. However inhibitors are consumed because XIAP binds to C3a to form the complex C3aXIAP and BAR binds to C8a to form the complex C8aBAR. Once XIAP and BAR have been almost completely consumed then rapid activation of C3 to C3a occurs. This signals the cell to proceed with apoptosis.
Eissing's and similar nonlinear models have been analysed extensively in the literature from different viewpoints [30], [10], [4], [5]. Next we show that Eissing's model naturally fits into the framework of a feedback system consisting of a plant and decentralised feedback controller. The plant P represents the autocatalytic reaction activating the caspase protiens C3 and C8 and modelled by the reactions

$$\begin{aligned} C8a &+ C3 \rightarrow C8a + C3a \\ C3a &+ C8 \rightarrow C8a + C3a. \end{aligned} \tag{2}$$

The feedback controller C arises from the inhibitor reactions, for example for $C3$ we have

$$\begin{aligned} C3a &+ XIAP \leftrightarrow C3aXIAP \\ C3a &+ XIAP \rightarrow C3a. \end{aligned} \tag{3}$$

and a similar set of reactions for BAR. Note that forming the complex C3aXIAP (C8aBAR) is a reversible reaction. Apoptosis can now be viewed as the following MIMO feedback system with plant dynamics

$$\begin{aligned} \frac{dC8}{dt} &= -k_9\, C_8 - k_2\, C8 \cdot C3a + k_{d9} \\ \frac{dC8a}{dt} &= -k_5\, C8a + k_2\, C8 \cdot C3a + u_1 \\ \frac{dC3}{dt} &= -k_{10}\, C3 - k_1\, C3 \cdot C8a + k_{d10} \\ \frac{dC3a}{dt} &= -k_6\, C3a + k_1\, C3 \cdot C8a + u_2. \end{aligned}$$

where the outputs are C8a and C3a and two inputs u_1 and u_2. The controller dynamics are given by,

$$\begin{aligned} \frac{dXIAP}{dt} &= -k_8\, XIAP - k_4\, C3a \cdot XIAP + k_{d8} + u_2 \\ \frac{dC3aXIAP}{dt} &= -k_7\, C3aXIAP - u_2 \\ \frac{dBAR}{dt} &= -k_{12}\, BAR + k_{d12} + u_1 \\ \frac{dC8aBAR}{dt} &= -k_{13}\, C8aBAR - u_1 \end{aligned}$$

where

$$\begin{aligned} u_1 &= -k_{11}\, C8a \cdot BAR + k_{_{d11}}\, C8aBAR \\ u_2 &= -k_3\, C3a \cdot XIAP + k_{d3}\, C3aXIAP. \end{aligned}$$

The controller outputs are u_1 and u_2 and the inputs are $C8a$ and $C3a$. The controller is decentralised because there is no cross coupling between the XIAP and BAR dynamics. Each

control signal contains two terms. The first term inhibits protein activation while the second term promotes protein activation. Switching to the stable death state depends on the initial concentration of C8a(0) and a fine balance between the two control terms and. As noted in [13] the plant without inhibitors (open-loop $u_1 = 0$ and $u_2 = 0$) is unstable at the life steady state where $[C8,\ C8a,\ C3,\ C3a] = [13.10^4,\ 0,\ 21.10^3,\ 0]$, (i.e. $C8a(0) = 0$). A simplified block diagram of the core dynamics with decentralised controllers is shown in Figure 2. If we set $u_1 = 0$ and $u_2 = 0$ the dynamics show in Figure 3 are similar to p16 in [11]. To analyse this system we use the linearised dynamics similar to the approach taken with the glycolytic pathway [6].

III. Linear Analysis

Simulations indicate that about the life steady state Eissing's model behaves in a reasonably linear manner [8]. Computing the Jacobian about the life steady state x_e,

$$A_e = \frac{\partial df}{\partial x}|_{x=x_e} \tag{4}$$

gives the linearised system

$$\dot{x} = A_e x. \tag{5}$$

The Jacobian is given in the appendix where the states are labelled as $x_1, x_2, \cdots, x_8$ and $x_1 ... etc$ also refers to the protein concentration at the life steady state unless otherwise stated.

The Jacobian may be partitioned in two ways. First as a MIMO **negative** feedback system with linear plant $P(s)$ and controller $C(s)$. Identification at the life steady state reveals the plant to be strictly proper and the controller to be decentralised but proper. Using this information we can assume the following state space models for the linearised plant and controller.

$$P(s) = \left[\begin{array}{c|c} A & B \\ \hline C & 0 \end{array}\right], \tag{6}$$

$$C(s) = \left[\begin{array}{c|c} A_c & B_c \\ \hline C_c & D_c \end{array}\right]. \tag{7}$$

The feedback system (P, C) can be assembled into the following matrix

$$A_e = \begin{bmatrix} A + BD_cC & BC_c \\ B_cC & A_c \end{bmatrix}$$

and compared to the Jacobian of (4) to obtain the plant and controller state matrices. Note that the death steady state is less interesting because presumably by that time apoptosis is irreversible .

A. Linear Plant

The linearised plant has four states however for small $t << 1\ m$, $C3(t)$ and $C8(t)$ can be treated as constants giving the following model valid when $C8a(0) <\approx 75\text{mpc}$,

$$\begin{pmatrix} \dot{C8a} \\ \dot{C3a} \end{pmatrix} = \begin{pmatrix} -k_5 & k_2\ C8(0) \\ k_1\ C3(0) & -k_5 \end{pmatrix} \begin{pmatrix} C8a \\ C3a \end{pmatrix} + \begin{pmatrix} u_1 \\ u_2 \end{pmatrix}.$$

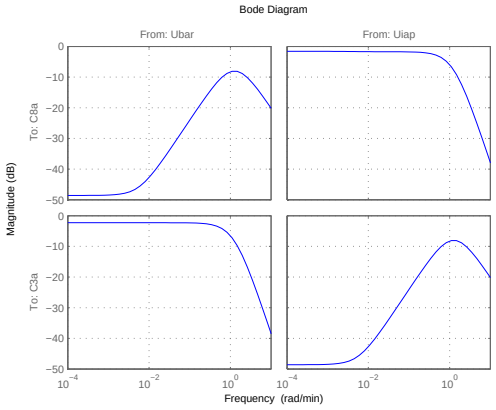

Fig. 4. Linearized Plant Frequency Response at $t = 0, C8a(0) \approx 10$

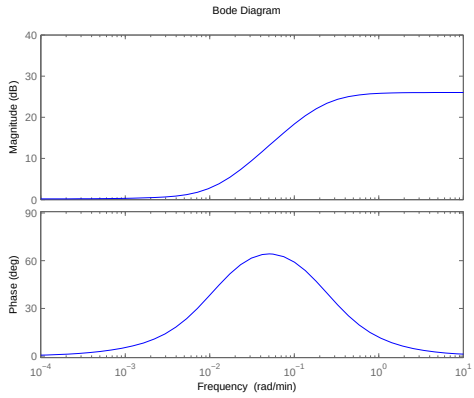

Fig. 5. C_1 (BAR) and C_2 (XIAP) Frequency Response: at $t = 0$

The plant transfer function

$$P(s) = C(sI - A)^{-1}\ B \tag{8}$$

(with $\mathrm{C} = \mathrm{I}_2$), can be further simplified using Eissing's initial conditions where $k_5k_6 << k_1\ k_2\ C3(0)\ C8(0)$ resulting in the reduced two state model

$$P(s) = \begin{pmatrix} \dfrac{s}{(s^2 - \alpha)} & \dfrac{k_2\ C8(0)}{(s^2 - \alpha)} \\ \dfrac{k_1\ C3(0)}{(s^2 - \alpha)} & \dfrac{s}{(s^2 - \alpha)} \end{pmatrix}. \tag{9}$$

This unstable system has poles at $s = \pm\sqrt{\alpha}$ where $\alpha = k_1\ k_2\ C3(0)\ C8(0) \approx 1.6$. A Bode magnitude plot of four state linearised plant (at the unstable life steady state) is shown in Figure.4. The bandwidth of the skew diagonal entries is approximately $1\ rad/m$. Tight coupling between the $C8a$ and $C3a$ subsystems due to the auto-catalytic reaction causes the plant to be skew diagonal at low frequencies. From a traditional control design viewpoint this suggests controlling $C3a$ by BAR and $C8a$ controlled by $XIAP$. Here the function of the biochemical controller is to maintain stability within a small radius of the fixed point.

B. Linear Controller

The controller linearised about the life steady state is decentralised and has has four states. The details are given

in the Appendix.

$$\begin{pmatrix} u_1 \\ u_2 \end{pmatrix} = -\begin{pmatrix} C_1 & 0 \\ 0 & C_2 \end{pmatrix}\begin{pmatrix} C8a \\ C3a \end{pmatrix} \tag{10}$$

It is possible to obtain the more illuminating reduced approximations

$$\begin{aligned} C_1(s) &\approx K_1\,\frac{s+k_{13}}{s+k_{d11}} \\ C_2(s) &\approx K_2\,\frac{s+k_7}{s+k_{d3}} \end{aligned} \tag{11}$$

where $K_1 = k_{11}\ BAR(\cdot)$, $K_2 = k_3\ XIAP(\cdot)$. Both compensator's have a zero break frequency less than the pole break frequency, $k_{13} < k_{d11}$ and $k_7 < k_{d3}$ and therefore providing lead compensation. The maximum phase lead is approximately 1 rad or around 60 deg at approximately $5\ 10^{-2}\ rad/m$. A Bode plot of C_1 is shown in Figure 5, with C_2 being very similar. The compensator break frequencies and phase angle do not vary significantly as the system evolves. In the approximate model they are constant. The gain term of the simplified compensator's is proportional to the inhibitor concentration. When apoptosis is activated these gains slowly decrease as the inhibitors are consumed. The effect of inhibitor compensation is to add feedback around the integrators as show in Figure 2. This results in the transfer functions

$$F_1(s) = \frac{s+kd_{11}}{(s+K_1)(s+k_{13})} \tag{12}$$

$$F_2(s) = \frac{s+kd_3}{(s+K_2)(s+k_7)}. \tag{13}$$

where $K_1 = k_{11}\ BAR(\cdot)$ and $K_2 = k_3\ XIAP(\cdot)$. The poles at $s = -K_1$ and $s = -K_2$ are approximately the two stable fast eigenvalues in the Jacobian at the life steady state. It is easily checked that the magnitude of these fast poles is proportional to the inhibitor concentration. Lead compensation introduces a fast initial control action which drives the state vector onto a slow manifold [30]. If the reduced compensator is used to stabilise the core plant at the life steady state then the fast control signals generated are indistinguishable from the control signals in the full nonlinear model. In fact the initial fast controller signals are well approximated by simple linear transfer functions with time constants equal to the fast poles. Provided $C8a(0) < 75$ then the nonlinear system returns to the life steady state where $C3a(0) \approx 0$ and $C8a(0) \approx 0$ and so the control signals are close to zero.

It is not immediately obvious that in this case the inhibitor reaction equations should behave like a lead compensator. However the linearised dynamics are not unlike the dynamics of the inverted pendulum which is stabilised by lead type compensation. To complete the analogy, when the pendulum is in the up position it is at an unstable fixed point, c.f. the life steady state of the uncompensated plant. In the down position the pendulum is stable, c.f. the death steady state. The decision point is close to the horizontal position of the pendulum.

IV. Stability analysis and the small gain theorem

Here we analyse the system as the SISO interconnection of two stable linear systems $M_1(s), M_2(s)$ in a **positive** feedback loop. The caspase subsystems C8 and C3 are coupled in a positive feedback loop as shown in Figure 6 and also Figure 2. Both $M_1(s), M_2(s) \in \mathbb{R}H_\infty$ are two fourth order stable strictly proper transfer functions each with relative degree one. The loop gain is defined by

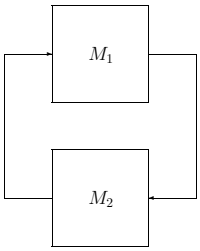

Fig. 6. Interconnection of caspase subsystems M_1(C8) and M_2(C3)

$$L(s) = M_1 M_2 \tag{14}$$

where

$$\begin{aligned} M_1(s) &= C_1(sI - A_1)^{-1}B_1 \\ M_2(s) &= C_2(sI - A_2)^{-1}B_2. \end{aligned}$$

By applying a suitable permutation matrix to the Jacobian is put in the form

$$A_{12} = \begin{bmatrix} A_1 & B_1C_2 \\ B_2C_1 & A_2 \end{bmatrix}$$

and the state matrices are eaisly recovered. See appendix for details. The small gain theorem states that if the loop gain is stable then a sufficient condition for closed-loop stability is that $||L(i\omega)||_\infty < 1$, [2]. This is true regardless of the sign of the feedback and so the transfer function $L(s)/\left(1 \pm L(s)\right) \in \mathbb{R}H_\infty$. Importantly the maximum gain of each of these low pass subsystems is the DC gain. This allow us to conclude that when $||L(i\omega)||_\infty > 1$ the closed-loop system is unstable [39]. In other words in this example of positive feedback the small gain theorem is sharp. As $C8a(0)$ is increased the loop gain increases until $||L(i\omega)||_\infty > 1$ and the closed-loop system becomes unstable. The loop gain as function of $C8a(0)$ reaches a maximum (with the initial conditions) when $C8a(0) = 500$ and decreases to less than unity when $C8a(0) \geq 5000$ where the eigenvalues of the Jacobian are all stable. Of course this initial condition is so far from the stable fixed point of the nonlinear system that apoptosis is still triggered. As the system evolves we can compute $||L(i\omega)||_\infty$ for the nonlinear system by sampling the state vector. This creates a family of linear time invariant plants [35]. When apoptosis is triggered then initially $||L(i\omega)||_\infty > 1$. As the system evolves and the loop gain decreases until $||L(i\omega)||_\infty < 1$. Then by small gain theorem the closed-loop system will be stable as the Nyquist plot of $||L(i\omega)||_\infty$ is inside the unit circle [2]. In the death steady state the $C3a >> C3$ and $C8a >> C8$ and so the gain $0 < L(0) << 1$. In Figure 7 we repeat the simulation from [11], p48, and show how the control signals vary. The stability transition boundary where $||L(i\omega)||_\infty = 1$ is shown in the figure. At the life steady state the linearised system is stable for significantly smaller concentrations of

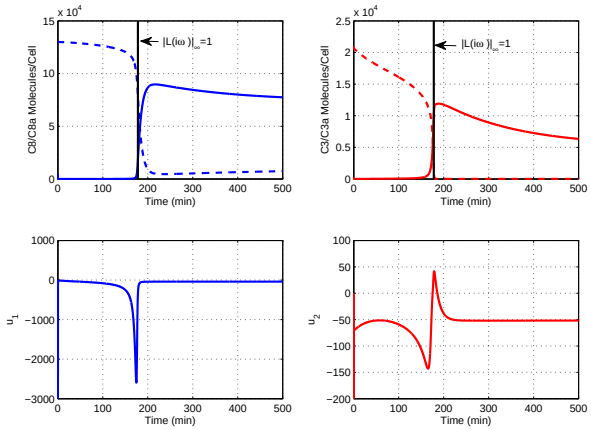

Fig. 7. Plant response and Control Signals Response for $C8a(0) = 5000$, p48, [11]. Initially the loop-gain $||M_1 M_2||_\infty > 1$. Also shown is the time at which the loop gain decreases to unity.

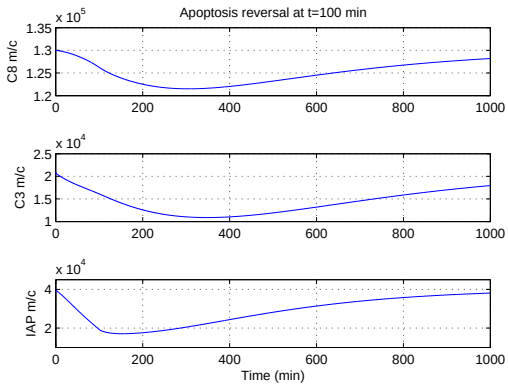

Fig. 8. Reversing apoptosis at t=100 min, $C8a(0) = 5000$

$C8a(0) < 2$. Interestingly the nonlinear system can tolerate a larger $C8a(0)$ before triggering apoptosis where the loop gain of the linearised system is slightly greater than unity. If the initial concentration of XIAP and BAR is zero then the compensator gains are zero and the system is open-loop. Then the loop gain can then be approximated by the double integrator

$$L(s) \approx \frac{k_1\, k_2\, x_1(0)\, x_3(0)}{s^2}. \tag{15}$$

The closed-loop poles (positive feedback) are at at $s = \pm\sqrt{K}$ where $K = k_1\, k_2\, x_1(0)\, x_3(0) \approx 1.6$. (Of course we know a priori that the uncompensated system at the life steady state is unstable). The stability boundary when $||L(i\omega)||_\infty = 1$ is shown in Figure 3.

The sensitivity of the closed-loop system is given by

$$S(s) = (I + PC)^{-1} \tag{16}$$

and for the linearised intrinsic model we find that $S(0) >> 1$ indicating that the closed-loop system is very sensitive to uncertainty. In fact this mirrors what we already know about this dynamical system in that small changes in $C8a(0)$ will drive the system unstable. Clearly unstable poles limit closed-loop performance [23], [28] but it is not clear how to interpret ”performance” other than to limit close-loop bandwidth. Closed-loop bandwidth will limit a cells ability to reverse apoptosis by limiting the speed at which biochemical changes can take place.

V. Reversing Apoptosis

Preliminary evidence suggests that apoptosis is potentially reversible with the possibility of return to the stable life steady state [36]. For example for small initial $C8a(0) \approx 100$ the lag phase is long and the state vector moves slowly away from the life steady state. Then there is ample time for a cell to recover and return to the life steady state.

In Eissing’s model reversal of apoptosis is achieved by reducing the loop gain so that $||L||_\infty < 1$. The loop gain is determined by the control law which we repeat here

$$\begin{aligned} u_1 &= -k_{11}\, C8a \cdot BAR + k_{d11}\, C8aBAR \\ u_2 &= -k_3\, C3a \cdot XIAP + k_{d3}\, C3aXIAP. \end{aligned}$$

Reducing the degradation constants k_{d3} or k_{d11} by as little as 10% reduces the loop gain sufficiently to stabilise the system. For example the simulation shown in Figure 8 illustrates reversal of apoptosis when $C8a(0) = 5000$. In Eissing’s model this triggers apoptosis at $t \approx 200$ min. At $t = 100min$ the loop gain is smoothly reduced by 10% and the system returns to the steady state. Apoptosis is not triggered. Eventually the concentration of XIAP returns to close to the initial value as forming a complex with C3a is a reversible process. The caspases C3 and C8 return close to their initial concentrations. Clearly there is a limitation on the speed at which the loop gain may be altered and if the loop gain is reduced too slowly then apoptosis is not reversed. Alternatively if the objective is to promote apoptosis for example in a cancer cell then the loop gain can be increased so that $||L||_\infty > 1$. This can be accomplished by reducing the constants k_3 or k_{11} by a sufficiently small amount and suggests how apoptosis may be manipulated using open-loop control. Manipulating these constants biochemically has been sugested [38].

Controlling apoptosis by regulating XIAP has been proposed as a way to induce apoptosis [22], [27]. Closed-loop control of biochemical pathways considered in [32] can be applied to Essing’s apoptosis model. An external controller $C_w(s)$ arguments the existing control law as shown in Figure 9. A simple choice of control law is to use decentralised lead controllers similar to the original biochemical reactions. These can be used to stabilise the dynamics at some time after the triggering of apoptosis. A more formal approach might be to consider $C_w(s)$ as a washout controller. As we have seen the core model is unstable at the life steady state and the inhibitors act to stabilise the unstable fixed point. At the stable life steady state the control signals return close to zero because the controller input depends on the concentration of activated caspase proteins which is small. In effect the inhibitors are functioning as a washout controller. A washout controller is used to stabilise nonlinear systems about an unstable possibly

uncertain fixed point. (The life fixed point is not known explicitly). This allows us to steer the state vector back to the life steady state once apoptosis has been triggered. A key point being that the location of the unstable equilibrium point is unchanged by the controller and at the stabilised fixed point the control action is zero. Intuitively the closer the system is to the switching state the more difficult it is to return to the life steady state. When the switching state is reached recovery is impossible, cell death is inevitable. The apoptosis dynamics are in the form

$$\dot{x} = f(x, u) \tag{17}$$

and in a neighbourhood of the life steady steady x_0 can be rewritten [31] as

$$\dot{x} = Ax + Bu + h(x, u) \tag{18}$$

This system is stabilized using a washout controller [31] with the following dynamics

$$\begin{aligned} \dot{z} &= P(x - z) \\ u &= K(x - z) \end{aligned} \tag{19}$$

The matrix K is the state feedback gain chosen using for example the LQR criteria. The matrix $P = \epsilon A^{-1}(A + BK)$ is nonsingular where $\epsilon > 0$ is sufficiently small. In this set-up we can think of ϵ as a design parameter to be chosen along with the LQR criteria. The above centralised controller assumes the full state is available for measurement however an observer can be used to estimate the state vector. The nominal plant dynamics are used to obtain a controller $C_w(s)$. Note that because the plant A matrix has one unstable eigenvalue the washout filter will itself be unstable [31]. However the objective here is to gain understanding as to the extent that recovery is possible using Eissing's model. Unstable controllers incur performance limitations for closed-loop control systems [23].

A. Experiment Techniques

If it is possible to identify the dynamics of a biochemical pathway then in principle the pathway can be controlled. Dynamic measurements of intracellular pathways have recently been adressed in [33] where input/output methods are used to identify, in vitro, the frequency response of a yeast G-protein signalling cascade. This opens up the possibility of intervening with feedback control to stabilise apoptosis [32]. Similar experimental techniques described in [33] may be used to identify the apoptosis biochemical pathway in yeast [37]. The identified model is then used to verify the fidelity of existing models and to test the whether or not such biochemical controllers are always decentralised and stable.

To implement the controller caspase inhibitors have to be modulated and concentrations of C3a and C8a measured [34]. Generating the control signal requires active delivery of the inhibitor protein at a cellular level to the target cells. The actuator proteins then have to cross the cell boundary and be delivered to the correct biochemical pathway taking into account body metabolism in the controller design. Dynamical models of intracellular biochemical pathways are subject to uncertainty in the kinetic constants, sparse measurement data and interaction with other pathways. Cell to cell variation can be modelled as an uncertainty [12]. Biochemical pathways in the cellular environment do no act in isolation and interact with other pathways [5] and other cells. There is also evidence of crosstalk between the biochemical pathways of apoptosis and autophagy [9]. Simply put, parts of a pathway are poorly understood.

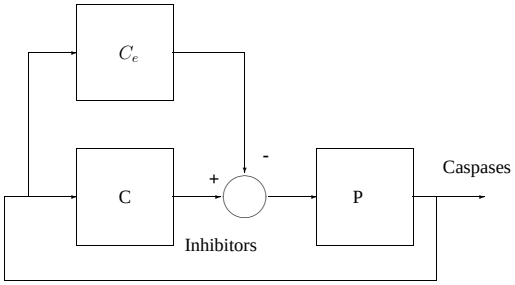

Fig. 9. Control of Apoptosis

VI. Conclusion

Linear control theory gives new insights into Eissing's intrinsic model of apoptosis. Linearising the dynamics about the life steady state clearly illustrates the function of plant and controller complementing and adding to existing analysis. Simplified models show how the inhibitors act as a stable decentralised lead control law where only the gain decreases as the system evolves towards cell death. The role played by positive and negative feedback loops and their interaction is highlighted. Our contribution posits the idea of shaping the loop-gain as possible technique to modulate apoptosis. This approach to closed-loop control is preliminary, however in a fast evolving field closed-loop control of intracellular biochemical pathways in vitro may soon be possible. Finally the methods used here may also be applied to the other models of the apoptosis pathway [1] [26], [40].

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APPENDIX

Eissing's state equations $\dot{x}(t) = f(x(t))$, from [14] on p38.

$$
\begin{aligned}
\frac{dC8}{dt} &= -k_9\, C_8 - k_2\, C8 \cdot C3a + k_{d9} \\
\frac{dC8a}{dt} &= -k_5\, C8a + k_2\, C8 \cdot C3a + u_1 \\
\frac{dC3}{dt} &= -k_{10}\, C3 - k_1\, C3 \cdot C8a + k_{d10} \\
\frac{dC3a}{dt} &= -k_6\, C3a + k_1\, C3 \cdot C8a + u_2 \\
\frac{dXIAP}{dt} &= -k_8\, XIAP - k_4\, C3a \cdot XIAP + k_{d8} + u_2 \\
\frac{dC3aXIAP}{dt} &= -k_7\, C3aXIAP - u_2 \\
\frac{dBAR}{dt} &= -k_{12}\, BAR + k_{d12} + u_1 \\
\frac{dC8aBAR}{dt} &= -k_{13}\, C8aBAR - u_1
\end{aligned}
$$

where the control is defined by

$$
\begin{aligned}
u_1 &= -k_{11}\, C8a \cdot BAR + k_{_{d11}}\, C8aBAR \\
u_2 &= -k_3\, C3a \cdot XIAP + k_{d3}\, C3aXIAP.
\end{aligned}
$$

The initial conditions in molecules per cell are

$$
\begin{aligned}
C8 &= 130\,10^3 \\
C8a &= C8a(0) \\
C3 &= 21\,10^3 \\
C3a &= 0 \\
XIAP &= 40\,10^3 \\
C3aXIAP &= 0 \\
BAR &= 40\,10^3 \\
C8aBAR &= 0.
\end{aligned}
$$

and constants are given in [14].

Here we represent the linearised system as a feedback system.
Defining the state vector as

$$[x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8] = [C8, C8a, C3, C3a, XIAP, C3aXIAP, BAR, C8aBAR], \quad (1)$$

then the linearised model is

$$\dot{x} = A_e x \quad (2)$$

where A_e is the Jacobian,

$$A_e = \begin{bmatrix} -\mathrm{k_9}-\mathrm{k_2\ x_4} & 0 & 0 & -\mathrm{k_2\ x_1} & 0 & 0 & 0 & 0 \\ \mathrm{k_2\ x_4} & -\mathrm{k_5}-\mathrm{k_{11}x_7} & 0 & \mathrm{k_2x_1} & 0 & 0 & -\mathrm{k_{11}x_2} & \mathrm{k_{d11}} \\ 0 & -\mathrm{k_1x_3} & -\mathrm{k_{10}}-\mathrm{k_1x_2} & 0 & 0 & 0 & 0 & 0 \\ 0 & \mathrm{k_1x_3} & \mathrm{k_1x_2} & -\mathrm{k_6}-\mathrm{k_3x_5} & -\mathrm{k_3x_4} & \mathrm{k_{d3}} & 0 & 0 \\ 0 & 0 & 0 & -\mathrm{k_3x_5}-\mathrm{k_4x_5} & -\mathrm{k_8}-\mathrm{k_3x_4}-\mathrm{k_4x_4} & \mathrm{k_{d3}} & 0 & 0 \\ 0 & 0 & 0 & \mathrm{k_3x_5} & \mathrm{k_3x_4} & -\mathrm{k_{d3}}-\mathrm{k_7} & 0 & 0 \\ 0 & -\mathrm{k_{11}x_7} & 0 & 0 & 0 & 0 & -\mathrm{k_{12}}-\mathrm{k_{11}x_2} & \mathrm{k_{d11}} \\ 0 & \mathrm{k_{11}x_7} & 0 & 0 & 0 & 0 & \mathrm{k_{11}x_2} & -\mathrm{k_{d11}}-\mathrm{k_{13}} \end{bmatrix}$$

and with a slight abuse of notation protein concentrations at the initial conditions or some other time are also referred to by x_i.
The linearised model is written as a feedback system with linear plant and controller by partitioning Jacobian. Linear plant $[A, B, C]$

$$A = \begin{bmatrix} -\mathrm{k_9}-\mathrm{k_2\ x_4} & 0 & 0 & -\mathrm{k_2\ x_1} \\ \mathrm{k_2\ x_4} & -\mathrm{k_5} & 0 & \mathrm{k_2x_1} \\ 0 & -\mathrm{k_1x_3} & -\mathrm{k_{10}}-\mathrm{k_1x_2} & 0 \\ 0 & \mathrm{k_1x_3} & \mathrm{k_1x_2} & -\mathrm{k_6} \end{bmatrix}$$

$$B = \begin{bmatrix} 0 & 0 \\ 1 & 0 \\ 0 & 0 \\ 0 & 1 \end{bmatrix}, \quad C = B^T, \quad D = 0_{2\times 2}$$

Linear decentralised controller $[A_c, B_c, C_c, D_c]$

$$A_c = \begin{bmatrix} -\mathrm{k_8}-(\mathrm{k_3}+\mathrm{k_4})\mathrm{x_4} & \mathrm{k_{d3}} & 0 & 0 \\ \mathrm{k_3x_4} & -\mathrm{k_{d3}}-\mathrm{k_7} & 0 & 0 \\ 0 & 0 & -\mathrm{k_{12}}-\mathrm{k_{11}x_2} & \mathrm{k_{d11}} \\ 0 & 0 & \mathrm{k_{11}x_2} & -\mathrm{k_{d11}}-\mathrm{k_{13}} \end{bmatrix}$$

$$B_c = \begin{bmatrix} 0 & -(k_3+k_4)x_5 \\ 0 & k_3x_5 \\ -k_{11}x_7 & 0 \\ k_{11}x_7 & 0 \end{bmatrix}' \quad C_c = \begin{bmatrix} 0 & 0 & -k_{11}x_2 & -k_{d11} \\ -k_3x_4 & k_{d3} & 0 & 0 \end{bmatrix}, D_c = \begin{bmatrix} -k_{11}x_7 & 0 \\ 0 & -k_3x_5 \end{bmatrix}$$

The linearised and strictly proper subsystems $M_1,\ M_2$ are obtained by applying a coordinate transformation (permutation matrix) to the Jacobian. This reorders the state vector so the state matrices can then be read out from the Jacobian. The $M_1(s)$ or C8 subsystem dynamics are given by.

$$\mathrm{A}_1 = \begin{bmatrix} -k_9 - k_2 x_4 & 0 & 0 & 0 \\ k_2 x_4 & -k_5 - k_{11} x_7 & -k_{11} x_2 & k_{d11} \\ 0 & -k_{11} x_7 & -k_{12} - k_{11} x_2 & k_{d11} \\ 0 & k_{11} x_7 & k_{11} x_2 & -k_{d11} - k_{13} \end{bmatrix}$$

$$B_1 = \begin{bmatrix} \text{- } \mathrm{k}_2\ \mathrm{x}_1 & \mathrm{k}_2\ \mathrm{x}_1 & 0 & 0 \end{bmatrix}^T$$

$$C_1 = \begin{bmatrix} 0 & 1 & 0 & 0 \end{bmatrix}$$

$$D_1 = 0$$

The $M_2(s)$ or C3 subsystem dynamics are given by.

$$\mathrm{A}_2 = \begin{bmatrix} -k_{10} - k_1 x_2 & 0 & 0 & 0 \\ k_1 x_2 & -k_6 - k_3 x_5 & -k_3 x_4 & k_{d3} \\ 0 & -(k_3 + k_4) x_5 & -k_8 - (k_3 - k_4) x_4 & k_{d3} \\ 0 & k_3 x_5 & k_3 x_4 & -k_{d3} - k_7 \end{bmatrix}$$

$$B_2 = \begin{bmatrix} \text{- } \mathrm{k}_1\ \mathrm{x}_3 & \mathrm{k}_1\ \mathrm{x}_3 & 0 & 0 \end{bmatrix}^T$$

$$C_2 = \begin{bmatrix} 0 & 1 & 0 & 0 \end{bmatrix}$$

$$D_2 = 0$$

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