

A mathematical model of
receptor-mediated apoptosis:
Fas Trimerization and FADD Recruitment

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Abstract

Apoptosis or programmed cell death is crucial to cell renewal. Understanding apoptosis can advance the field of cancer cell research because cancerous cells have lost the ability to undergo apoptosis. In order for apoptosis to occur, a cell must be directed to self-destruct. Cells are removed when malfunctions occur; whether they may be cell stress, damage, or conflicting cell division signals.

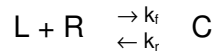
Before we can begin, it is important to first understand the receptor-ligand relationship. In order for signals to occur; a ligand must bind to a receptor. The activating signal is initiated when a protein (ligand) binds to the surface of a cell (receptor) and due to the binding, a complex forms. This formed complex sends a signal to the cell to activate a function. Depending on the receptor and ligand, a signal can only occur when a specific number of complexes form. Cell-surface death receptors must be engaged by specific ligands that transmit apoptotic signals for apoptosis to initiate.

For apoptosis to begin, it must also undergo further processes (recruitment, caspases) after the receptor-ligand binding has occurs. The objective of this study is to use mathematical modeling to describe and enhance the understanding of apoptosis. By using a mathematical model, we are able to easily observe different results by fluctuation of our constant values.

1. Background on Modeling Receptor/Ligand Binding

1.1 Single Monovalent Binding

For the understanding of receptor/ligand binding, consider a case in which a monovalent ligand L binds to a single, monovalent receptor R to form a receptor/ligand complex C. It is also important to note that because this model only includes one binding site, no additional receptors/ligands of the same type can bind to this complex.



The parameters k_f and k_r are the association/dissociation rates respectfully. The interaction described can be formulized with the following equations:

$$\frac{dC}{dt} = k_f R L - k_r C \quad (1)$$

$$R_T = R + C \quad (2)$$

Our desired result is the steady state value at which $dC/dt = 0$ represented by C_{eq} . To further simplify the model, we can assume that ligand depletion is negligible, ie. $L = L_0$. By making this simplification, we can simplify our model to estimate results.

$$C_{eq} = \frac{R_T L_0}{K_D + L_0} \quad (3)$$

For sake of more simplification, K_D is equal to k_r / k_f . However, since assuming negligible ligand depletion is unrealistic, we have also attempted this problem without such an assumption. By assuming ligand depletion is not negligible, it is convenient to convert our variables so they are dimensionless:

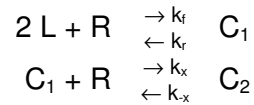
$$\bar{C} = \frac{C}{R_T} \quad (4)$$

Now using both our new dimensionless variable, and the fact that ligand depletion is not negligible: we are able to derive a new equilibrium value.

$$\bar{C}_{eq} = \frac{L}{K_D + L} \quad (5)$$

1.2 Multivalent Ligands

We now extend our model to multivalent ligands, which are, ligand with more than one binding site for a receptor. Receptors that are linked to other receptors by the means of multivalent ligands are known to be cross-linked. In this scenario, we are considering a bivalent ligand aggregating with a monovalent receptor:



This process is represented with the following equations:

$$\frac{dL}{dt} = -2k_f LR + k_r C_1 \quad (6)$$

$$\frac{dC_1}{dt} = 2k_f LR - k_r C_1 - k_x C_1 R + 2k_{-x} C_2 \quad (7)$$

$$\frac{dC_2}{dt} = k_x C_1 R - 2k_{-x} C_2 \quad (8)$$

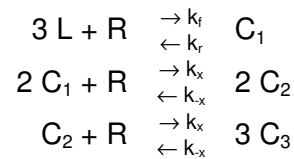
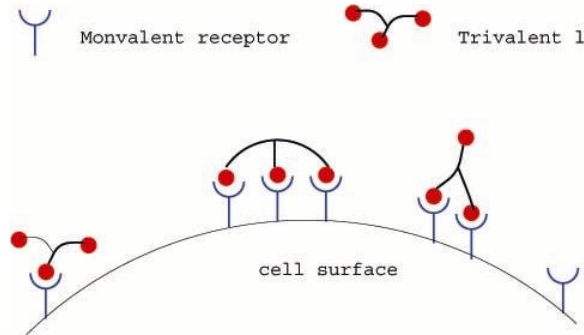
$$R_T = R + C_1 + 2C_2$$

Similar to k_f and k_r , k_x and k_{-x} describe the forward and reverse cross-linking rates. In this situation C_1 , C_2 describe a single, doubly bound complex respectfully. In order to derive a steady state, a similar process used for the monovalent binding is being used again. (Now using a definitely larger model) However, we are now interested in the total of cross-links in equilibrium C_{xTeq} , which in this case is equivalent to C_{2eq} . In order to develop our model for apoptosis, we will use a trivalent ligand/monovalent receptor system.

2. Mathematical Model Development for Fas/FasL System

Because Fas is the most commonly experimented death receptor, we are using Fas to help create our model for apoptosis. The model for the Fas/FasL engagement is based on experiments that show FasL is a trivalent ligand, which has the ability to bind up to three Fas receptors. The possible bonding sites are represented in Figure 1.

Figure 1: Trivalent Ligand bonding with a Monovalent Receptor



This process is represented with the following equations:

$$\frac{dL}{dt} = -3k_f LR + k_r C_1 \quad (9)$$

$$\frac{dC_1}{dt} = 3k_f LR - k_r C_1 - 2k_x C_1 R + 2k_{-x} C_2 \quad (10)$$

$$\frac{dC_2}{dt} = 2k_x C_1 R - 2k_{-x} C_2 - k_x C_2 R + 3k_{-x} C_3 \quad (11)$$

$$\frac{dC_3}{dt} = k_x C_2 R - 3k_{-x} C_3 \quad (12)$$

$$R_T = R + C_1 + 2C_2$$

L represents free FasL; R is the number of free receptors; and C_1 , C_2 , C_3 are the number of singly, doubly and triply bound Fas/FasL complexes respectively. The parameters k_f

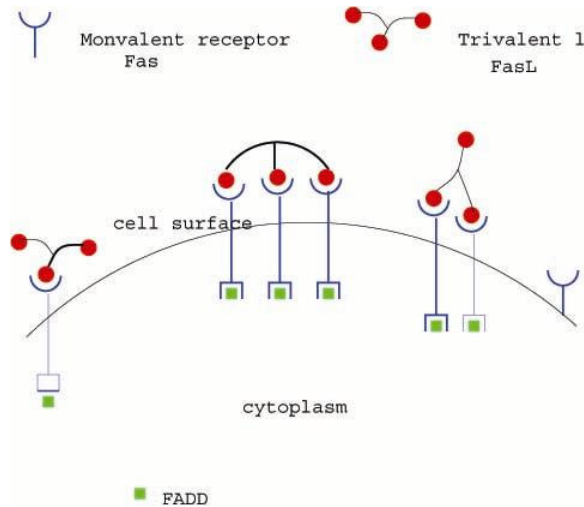
and k_r are the forward and reverse reaction rates, while k_x and k_{-x} are the forward and reverse cross-linking rates.

Again, we are interested in the cross-link equilibrium, C_{xTeq} . However, because the FasL is known to be trivalent:

$$C_{xTeq} = C_{2eq} + 2 C_{3eq}$$

As shown in Figure 2; once clustered, the Fas receptor becomes a bi or trivalent receptor for the FADD protein. It is important to note, for FADD recruitment to have an effect, the Fas/FasL must be doubly or triply bound complexes. It is then through the FADD protein and these newly formed receptors that the signal for apoptosis occurs.

Figure 2: FADD Recruitment



This process is represented by the following equations:

$$\frac{d\check{c}_2}{dt} = -2k_f f \check{c}_2 + k_r d_{12} \quad (13)$$

$$\frac{d\check{c}_3}{dt} = -3k_f f \check{c}_3 + k_r d_{13} \quad (14)$$

$$\frac{dd_{12}}{dt} = 2k_f f \check{c}_2 - k_r d_{12} - k_x f d_{12} + k_{-x} d_{22} \quad (15)$$

$$\frac{dd_{22}}{dt} = k_x f d_{12} - k_{-x} d_{22} \quad (16)$$

$$\frac{dd_{13}}{dt} = 3 \kappa_f f \check{c}_3 - \kappa_r d_{13} - 2 \kappa_x f d_{13} + 2 \kappa_{-x} d_{23} \quad (17)$$

$$\frac{dd_{23}}{dt} = 2 \kappa_x f d_{13} - 2 \kappa_{-x} d_{23} - \kappa_x f d_{23} + 3 \kappa_{-x} d_{33} \quad (18)$$

$$\frac{dd_{33}}{dt} = \kappa_x f d_{23} - 3 \kappa_{-x} d_{33} \quad (19)$$

$$f_T = f + d_{12} + 2 d_{22} + d_{13} + 2 d_{23} + 3 d_{33}$$

$$\check{c}_2^0 = \check{c}_2 + d_{12} + d_{22}$$

$$\check{c}_3^0 = \check{c}_3 + d_{13} + d_{23} + d_{33}$$

\check{c}_2 and \check{c}_3 represent the concentrations of doubly and triply clustered Fas death domains; d_{ij} are the concentrations of j clustered death domains with i FADD molecules attached. The parameters, κ_f and κ_r are the forward and reverse reaction rates associated with a single FADD molecule binding to the death domain, κ_x and κ_{-x} are the forward and reverse binding rates once at a least one FADD molecule has attached. The total intracellular FADD concentration is f_T and the initial concentrations of the doubly and triply clustered Fas death domains are denoted by \check{c}_2^0 and \check{c}_3^0 respectively.

3. Analysis

3.1 Fas Engagement and Clustering

The model given in (9), (10), (11), (12) describes the first steps in the receptor engagement pathway of programmed cell death initiation. Studying this system in isolation can provide important information regarding the dynamics of Fas/FasL binding and the sensitivity of the predicted behavior to the model parameters. In order to reduce confusion a few notes are to be made:

- We derive the equilibrium values by using algebra
- We are able to generate both the graphs and our results by using MatLab
- We are generating results for when $\kappa = .1, 1, 10$ and 100.
- When assuming non-negligible ligand depletion, $r^* = .1, 10$
- When assuming non-negligible ligand depletion, we must convert the variables so they are dimensionless

When FasL depletion is negligible (i.e. $L = L_0$), the equilibrium number of singly, doubly, and triply bound receptors is:

$$C_{1eq} = 3 \left(\frac{L_0}{K_D} \right) R_{eq} \quad (20)$$

$$C_{2eq} = 3 K_x \left(\frac{L_0}{K_D} \right) R_{eq}^2 \quad (21)$$

$$C_{3eq} = K_x^2 \left(\frac{L_0}{K_D} \right) R_{eq}^3 \quad (22)$$

$$3 \left(\frac{L_0}{K_D} \right) K_x^2 R_{eq}^3 + 6 \left(\frac{L_0}{K_D} \right) K_x R_{eq}^2 + \left(1 + 3 \left(\frac{L_0}{K_D} \right) \right) R_{eq} - R_T = 0 \quad (23)$$

From this information, the total number of crosslinks at equilibrium can be computed, $C_{xTeq} = C_{2eq} + 2 C_{3eq}$ and a cross-linking curve which plots C_{xTeq} versus the ligand concentration can be constructed. This type of diagram can determine the range of ligand concentrations for which clustering is successful and from Figure 3 it is clear that successful clustering occurs for an intermediate range of scaled ligand concentrations and as expected, increasing the nondimensional cross-linking constant $\kappa = K_x R_T$ leads to an enhanced receptor aggregation.

$$C_{xTeq} = \sum_{i=2}^3 (i-1) C_{ieq} = K_x \left(\frac{L_0}{K_D} \right) R_{eq}^2 (3 + 2 K_x R_{eq})$$

Figure 3: Equilibrium crosslinking curve plotting

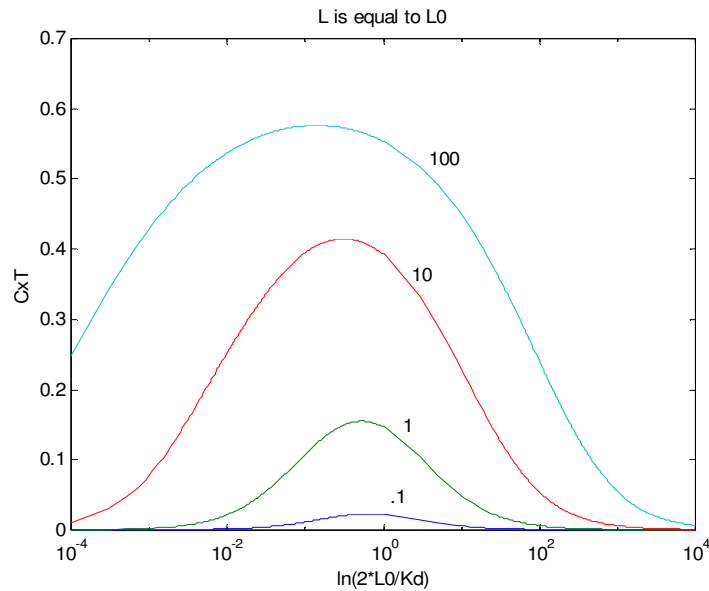


Fig 3: The scaled equilibrium number of crosslinks (C_{xTeq}) as a function of the scaled ligand concentration ($2 L_0 / K_D$) for various choices of the crosslinking equilibrium constant, κ .

Table 1: Percentage of Triply Bound Complexes

κ	max C_{xT}	$2L_0/K_D$	C_3 %
.1	0.02345487073137	0.6459	3.12750
1	0.15514620139585	0.5343	20.7096
10	0.41487253289877	0.3147	56.5687
100	0.57641190199192	0.1429	82.1874

Table 1 lists the nondimensional ligand concentration, $2 L_0 / K_D$, which results in the maximum percentage of crosslinks for $\kappa = 0.1, 1.0, 10, 100$. As κ increases the ligand concentration required for maximal crosslinking decreases. Table 1 also shows that the percentage of the total crosslinks at equilibrium which are triply bound increases as κ increases. Therefore if the death signal is to be successful at low receptor concentration (i.e. $\kappa = K_x R_T$, small) and high ligand concentration, Fas trimerization is unlikely to be the driving force in the signal propagation, as only a very small percentage of the total number of crosslinked receptors are in the triply bound form. However at high receptor and low ligand concentrations, the majority of the total number of crosslinks is made up of triply bound complexes and Fas trimerization could be important for the downstream events.

Table 2: Percentage of Doubly Bound Complexes

κ	Max C_{xTeq}	$2 L_0/K_D$
.1	0.01191151829848	1
1	0.08578643762690	1
10	0.26833752096446	1
100	0.40950124378879	1

Table 2 demonstrates the consequences that result from only dimmers forming. Note that if Fas trimerization should fail completely, that is none of the crosslinks are triply bound; $\kappa = 1.0$ would result in at most 8.5% crosslinking compared to 16% when triply bound clustered form (most of this %16 are doubly bound complexes). Further, should κ increases to 100 failure to produce triples would yield at most 41% total crosslinking compared to 58% when triply bound complexes are possible (in this case, most of the 58% are triply bound complexes). Therefore when κ is large, the fact that triply bound clusters can form results only in a moderate increase in the total number of crosslinks at equilibrium.

Significance of FasL Depletion:

The above steady state solutions are based on the assumption that ligand depletion is negligible, i.e. $L = L_0$ if this assumption is relaxed, the steady state solutions become:

$$\bar{C}_{1eq} = 3 \left(\frac{L_0}{K_D} \right) \bar{R}_{eq} \bar{L}_{eq} \quad (24)$$

$$\bar{C}_{2eq} = 3 \kappa \left(\frac{L_0}{K_D} \right) \bar{R}_{eq}^2 \bar{L}_{eq} \quad (25)$$

$$\bar{C}_{3eq} = \kappa^2 \left(\frac{L_0}{K_D} \right) \bar{R}_{eq}^3 \bar{L}_{eq} \quad (26)$$

$$\bar{L}_{eq} = (\bar{R}_{eq} r^* (3 + \kappa \bar{R}_{eq} (3 + \kappa \bar{R}_{eq})) + 1)^{-1} \quad (27)$$

$$\kappa^2 r^* \bar{R}_{eq}^4 + \left((3 - \kappa) r^* + 3 \kappa \left(\frac{L_0}{K_D} \right) \right) \kappa \bar{R}_{eq}^3 + \quad (28)$$

$$3 \left((1 - \kappa) r^* + 2 \kappa \left(\frac{L_0}{K_D} \right) \right) \bar{R}_{eq}^2 +$$

$$\left(1 + 3 \left(\left(\frac{L_0}{K_D} \right) - r^* \right) \right) \bar{R}_{eq} - 1 = 0$$

$$\bar{C}_{xTeq} = \sum_{i=2}^3 (i-1) \bar{C}_{ieq} = \kappa \left(\frac{L_0}{K_D} \right) \bar{R}_{eq}^2 (3 + 2 \kappa \bar{R}_{eq}) \quad (29)$$

The effects of ligand depletion are simulated in Figures 4 and 5. In this case, as $r^* = R_T / K_D$ increases, the range of ligand concentrations for which clustering is successful decreases and maximum crosslinking occurs for higher ligand concentrations.

When $r^* = .10$

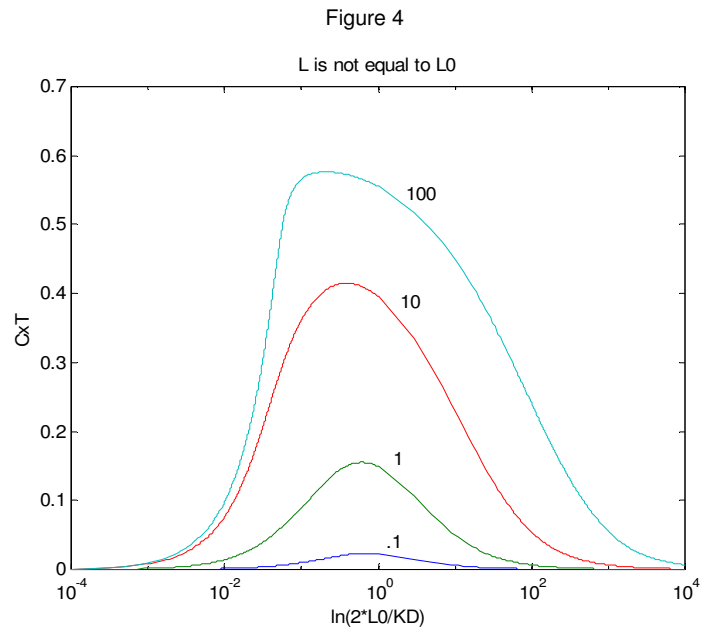


Fig 4: Equilibrium crosslinking curve plotting the scaled equilibrium number of crosslinks (C_{XTeq}) as a function of the scaled ligand concentration ($2 L_0/K_D$) for various choices of the crosslinking equilibrium constant, κ , when ligand depletion is considered. In this figure, $r^* = 0.1$.

Figure 5

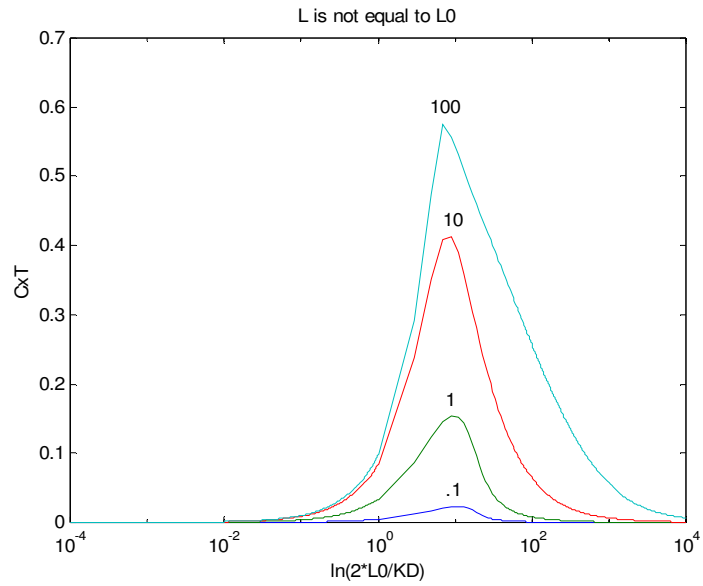


Fig 5: Equilibrium crosslinking curve plotting the scaled equilibrium number of crosslinks ($C_{xT_{eq}}$) as a function of the scaled ligand concentration ($2 L_0/K_D$) for various choices of the crosslinking equilibrium constant, κ , when ligand depletion is considered. In this figure, $r^* = 10$.

From this point on, the equilibrium number of crosslinks as determined by the Fas/FasL model (eqn (9) – (13)), described and analyzed above will be considered the signal for the initiation of the apoptotic cascade, i.e. the death signal. Most importantly, these equilibrium values will be used as initial conditions for the intracellular events of FADD recruitment and caspase-8 activation.

3.2 FADD Recruitment

The aggregation of Fas results in a conformational change in the receptors' death effector domain, which is part of its cytoplasmic tail. The intracellular adaptor protein, FADD (Fas-associated death domain), is then recruited and binds to the clustered death domains of the Fas receptors. Therefore once the death signal is received, that is, once Fas clustering is in equilibrium, FADD recruitment begins and the initial values of the clustered death domains are known. If FADD depletion is negligible, we can suggest the following steady states for FADD recruitment:

$$C_{2eq} = \frac{\check{C}_2^0}{1 + 2\alpha(1 + \beta)} \quad (30)$$

$$C_{3eq} = \frac{\check{C}_3^0}{1 + \alpha(3 + \beta(3 + \beta))} \quad (31)$$

$$d_{22eq} = 2\alpha\beta C_{2eq} \quad (32)$$

$$d_{32eq} = 3\alpha\beta C_{3eq} \quad (33)$$

$$d_{33eq} = \alpha\beta^2 C_{3eq} \quad (34)$$

$$d_{Teq} = d_{22eq} + d_{32eq} + 2d_{33eq}$$

Figure 6a

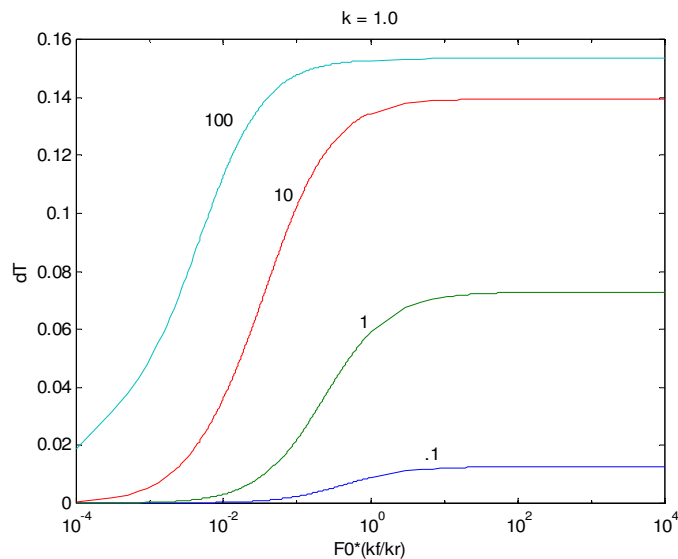
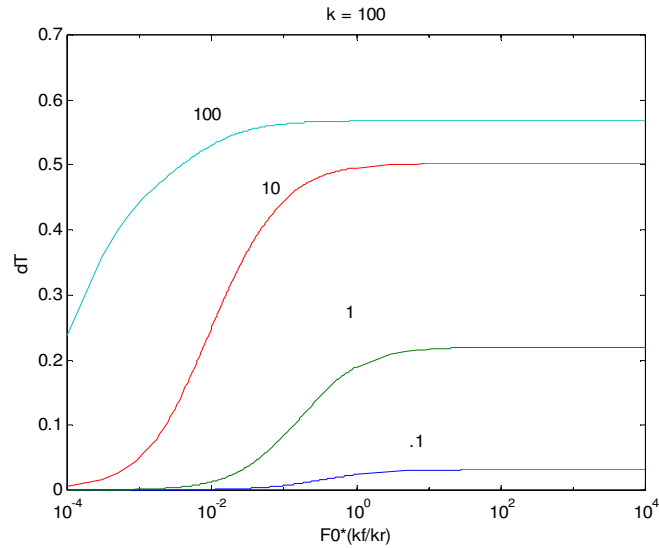


Figure 6b



Equilibrium FADD binding curve plotting: the scaled equilibrium concentration of bound FADD (d_T) a function of the scaled FADD concentration ($\alpha = f_T / K_D$) for various choices of the crosslinking equilibrium constant, $\beta = f_T / K_X$ when FADD depletion is ignored.

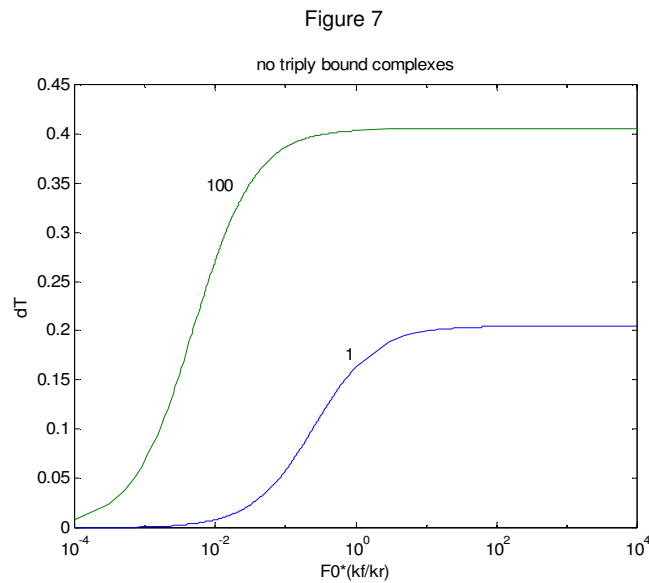
- a. $\kappa = 1.0$ and less than 20% of the Fas receptors are crosslinked; this gives $\tilde{C}_2^0 = .1222$, $\tilde{C}_3^0 = .0164$
- b. $\kappa = 100$ so that over 50% of the Fas receptors are crosslinked; this gives $\tilde{C}_2^0 = .1021$, $\tilde{C}_3^0 = .2371$

Figure 6 illustrates the steady state profiles of FADD recruitment when depletion is negligible. The initial conditions for clustered Fas death domains are taken to be the maximum levels (determined in Section 3.1, see Figure 3 and Table 1) when $\kappa = 1.0$ for Figure 6a and when $\kappa = 100$ for Figure 6b. This provides insight into two different scenarios for FADD recruitment.

It is clear that for a fixed constitutive level of FADD, f_T , not only is the dissociation constant important for successful recruitment (as determined by varying the nondimensional parameter, α), but the nondimensional crosslinking constant β is also a critical parameter. Therefore even if Fas aggregation is successful as is the case for

initial conditions, FADD recruitment can fail if its crosslinking constant is not sufficiently large.

Similar analytical solutions can be obtained for the case when FADD depletion is considered. For a given β , the overall effect of FADD depletion is to decrease the maximum concentration of bound FADD and to shift to the right the FADD concentration corresponding to the change in concavity (results not shown).



Equilibrium FADD binding curve plotting the scaled equilibrium concentration of bound FADD (d_T) as a function of the scaled FADD concentration ($\alpha = f_T / K_D$) for various choices of the crosslinking equilibrium constant, $\beta = f_T / K_X$. Here, FADD depletion is ignored, FasL behaves like a dimer in that no triply bound Fas/FasL complexes are able to form, and $\tilde{c}_2^0 = .41$, $\tilde{c}_3^0 = 0.0$.

In order to determine the role of Fas trimerization at the level of FADD recruitment, Figure 7 plots the steady state profiles of bound FADD as a function of FADD concentration when trimerization fails completely. Here, $\kappa = 100$ corresponding to $\tilde{c}_2^0 = 0.41$ and $\tilde{c}_3^0 = 0$, that is a maximum of percentage of crosslinks equal 41%. Notice in this case, even when β is large, FADD recruitment is significantly reduced and less than half as much of the protein is bound compared to the case when triply bound

complexes are able to form. Further, this difference in FADD recruitment becomes more pronounced as β decreases. Therefore, although Fas trimerization has only marginal effects on the total number of crosslinks at equilibrium when κ (the nondimensional crosslinking constant for Fas/FasL) is large; trimerization is of crucial importance for successful FADD recruitment.

4. Conclusion

4.1 Summary

I was given the task to develop and analyze a mathematical model for death receptor engagement using Fas/FasL as our model system. In order to accomplish this, I looked at a trivalent ligand – monovalent receptor system. In order to obtain numerical results, we investigated the most commonly experimented death receptor, Fas. After completion of this model, we used the results to provide initial conditions for the intracellular event, FADD recruitment.

Based on our results, we are able to understand that in the situation of low receptor concentration and high ligand concentration (κ , small); Fas trimerization is unlikely to be the driving force for signaling apoptosis. We know this is the case since only a very small percentage of the crosslinks are in a triply bound form. In the opposite regard, a high receptor concentration and low ligand concentration shows that most of the crosslinks are in a triply bound form. There are also consequences that result from only dimmers forming. If Fas trimerization completely fails, the resulting percentage of crosslinking will be far smaller. An important step for apoptosis to occur is that of FADD recruitment. However, if Fas trimerization completely fails, FADD recruitment is significantly reduced. Because of this, successful Fas trimerization is crucial for FADD recruitment, and ultimately for signaling apoptosis.

4.2 Future Work

In order to complete this model, we must consider the two mechanisms for caspase-8 activation: (i) autocatalysis and (ii) the close proximity model which assumes that two or more procaspase-8 molecules bind to FADD (multivalent as a receptor) once in place on the death domain of Fas and activate each other. The model will describe the way in which free procaspase-8 molecules bind to death complexes composed of doubly bound Fas receptors with one or two FADD proteins attached. The former results in a terminal complex from which no activation can occur; whereas, the latter forms a complex which can accept an additional procaspase-8 molecule. These two procaspase-8 molecules which have been brought into close proximity via binding now activate each other, releasing two molecules of activated caspase-8, and an unoccupied receptor. The methodology for the binding of procaspase-8 to two death complexes consisting of triply bound Fas receptors with one, two, or three FADD proteins follows in a similar manner.

Alternatively, two free molecules of procaspase-8 may activate each other producing two caspase-8 molecules; although the probability of this is very small.

This model is of interest because it is at this step that the cell is irreversibly committed to death. Our major objective will be to determine what effects the initial trimerization of the Fas receptor has on the downstream events of caspase activation.

Bibliography

Author: Fussenegger, M. and Bailey, J.E. and Varner J.
Title: A mathematical model of caspase function in apoptosis
Journal: Nature Biotech.
Volume: #18
Pages: 768
Year: 2000

Author: Thornberry, N.A. and Lazeuik, Y.
Title: Caspases: enemies within
Journal: Science
Volume: 281
Pages: 1312
Year: 1998

Author: Ashkenazi, A. and Dixit, V.M.
Title: Death receptors: signaling and modulation
Journal: Science
Volume: 281
Pages: 1305
Year: 1998

Author: Nagata, S.
Title: Apoptosis by death factor
Journal: Cell
Volume: 88
Pages: 355.
Year: 1997

Author: Budihardjo, I. and Oliver, H. and Lutter, M. and Luo, X. and Wang, X.
Title: Biochemical pathways of caspase activation during apoptosis
Journal: Annu. Rev. Cell Dev. Biol.
Volume: 15
Pages: 269
Year: 1999

Author: Edelstein-Keshet, Leah
Title: Mathematical models in biology
Edition: 1st ed.
Pages: 586
Year: 1988