Parameter Sensitivity and Bistability in a Caspase Activation Model of Apoptosis

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Biological Background

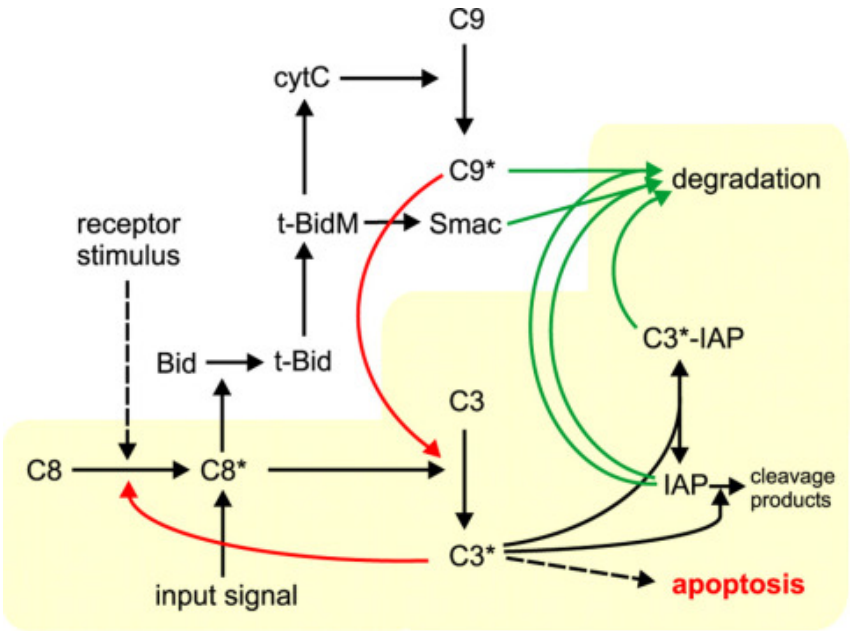

Figure 1: Caspase model depicting feedback regulation of apoptosis. [2].

- **Apoptosis:** Programmed cell death removes damaged cells and shapes tissue during development.

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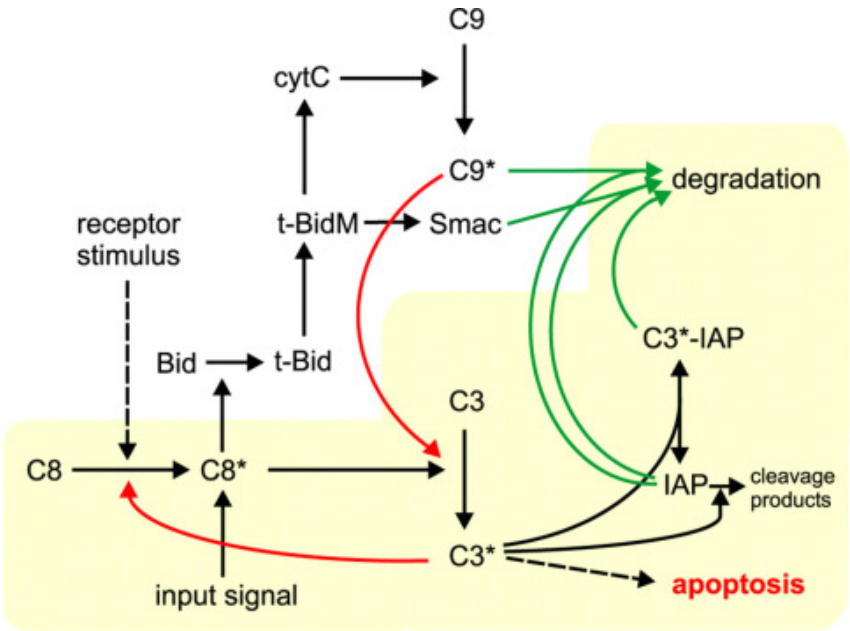

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- **Caspases:** Enzymes that initiate in a cascade. Caspase-3 is the executioner caspase triggering irreversible cell death.

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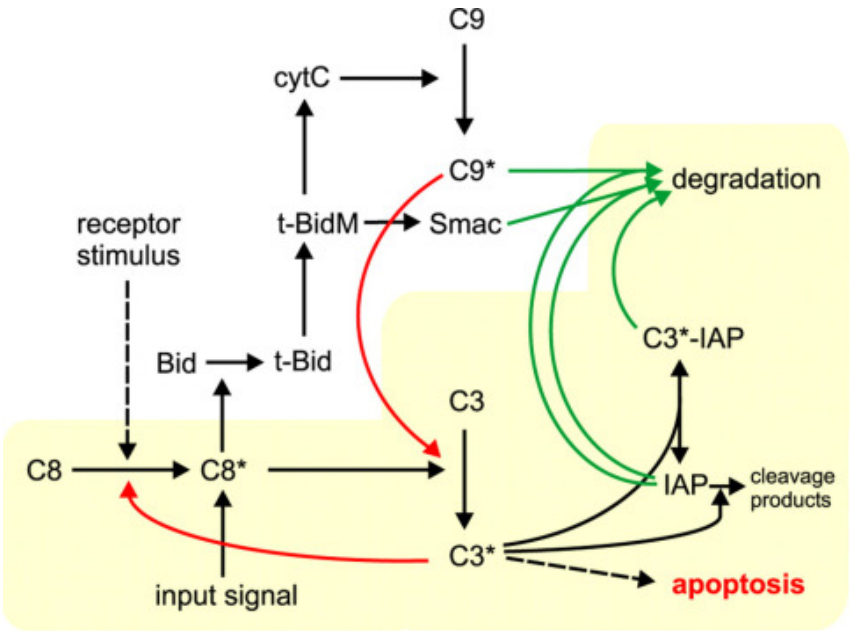

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- **Apoptosis:** Programmed cell death removes damaged cells and shapes tissue during development.
- **Caspases:** Enzymes that initiate in a cascade. Caspase-3 is the executioner caspase triggering irreversible cell death.
- **Bistability:** Biological switches that allow all-or-nothing decisions. Key to understanding how cells commit to death.

Biochemical Reaction Networks

What is a Biochemical Reaction Network?

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Example Reaction:

$$A + B \underset{\ell_1}{\overset{k_1}{\rightleftharpoons}} C$$

- Law of Mass Action leads to the following system:

$$\begin{aligned}
\frac{d[A]}{dt} &= -k_1[A][B] + \ell_1[C] \\
\frac{d[B]}{dt} &= -k_1[A][B] + \ell_1[C] \\
\frac{d[C]}{dt} &= k_1[A][B] - \ell_1[C]
\end{aligned}$$

Caspase Reaction Network

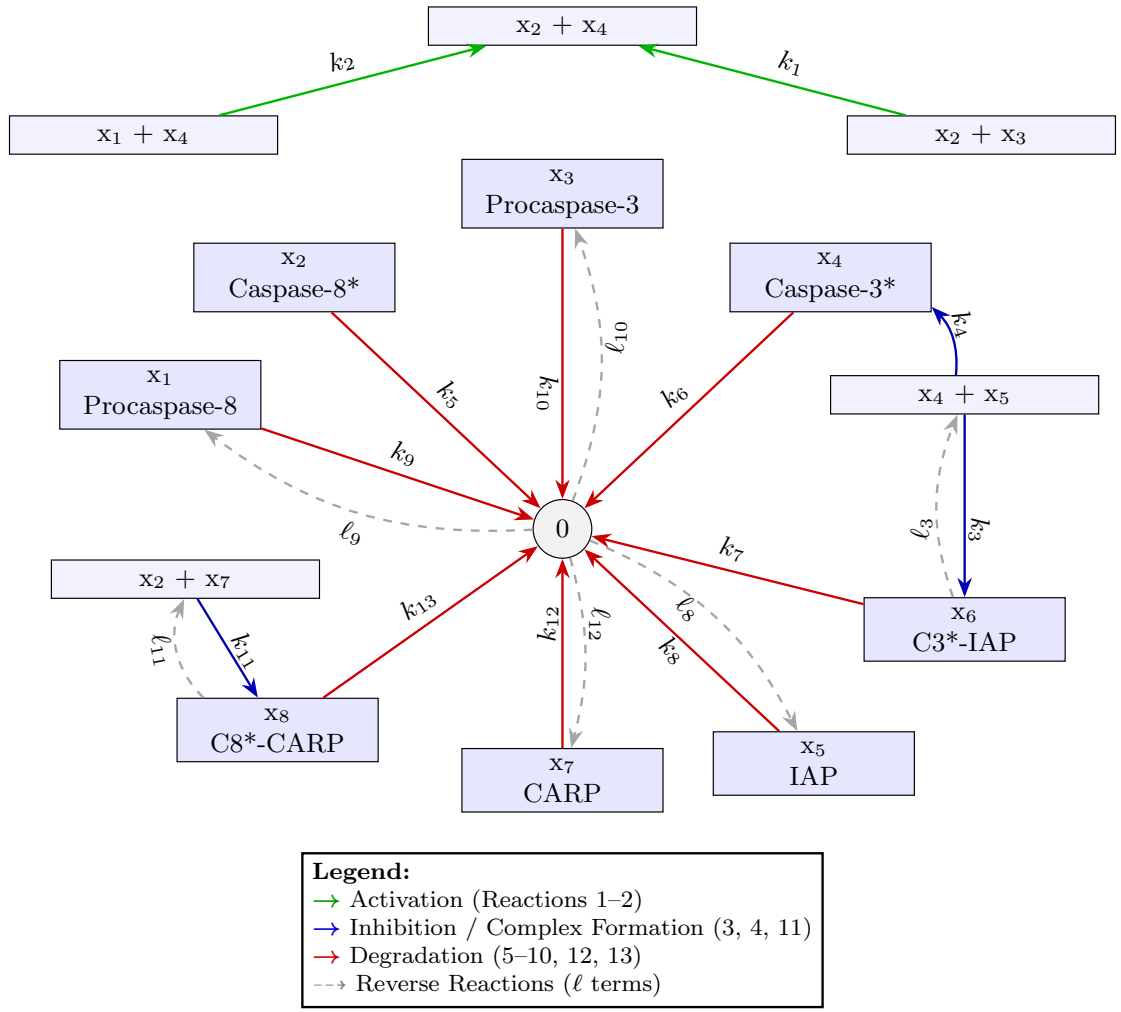

Figure 2: Reaction network of the caspase model.

ODE System and Species Key

ODEs from Model:

$$\frac{dx_1}{dt} = -k_2 x_1 x_4 - k_9 x_1 + \ell_9$$
$$\frac{dx_2}{dt} = k_2 x_1 x_4 - k_5 x_2 - k_{11} x_2 x_7 + \ell_{11} x_8$$
$$\frac{dx_3}{dt} = -k_1 x_2 x_3 - k_{10} x_3 + \ell_{10}$$
$$\frac{dx_4}{dt} = k_1 x_2 x_3 - k_3 x_4 x_5 - k_6 x_4 + \ell_3 x_6$$
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$$\frac{dx_6}{dt} = k_3 x_4 x_5 - k_7 x_6 - \ell_3 x_6$$
$$\frac{dx_7}{dt} = -k_{11} x_2 x_7 - k_{12} x_7 + \ell_{11} x_8 + \ell_{12}$$
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ODE system from [2].

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ODE system from [2].

Species Key:

x_1: Procaspase-8 (C8)

x_2: Active Caspase-8 (C8*)

x_3: Procaspase-3 (C3)

x_4: Active Caspase-3 (C3*)

x_5: Inhibitor of Apoptosis Protein (IAP)

x_6: Caspase-3*–IAP complex

x_7: CARP (Caspase-activated Regulation Protein)

x_8: C8*-CARP complex

Mathematical Concepts: Steady States

Definition

A **steady state** occurs when all time derivatives $\dot{x}_i = 0$, meaning the concentrations of species remain constant over time. These can be:

- Stable: System returns to the steady state after small changes
- Unstable: Small changes drive the system away

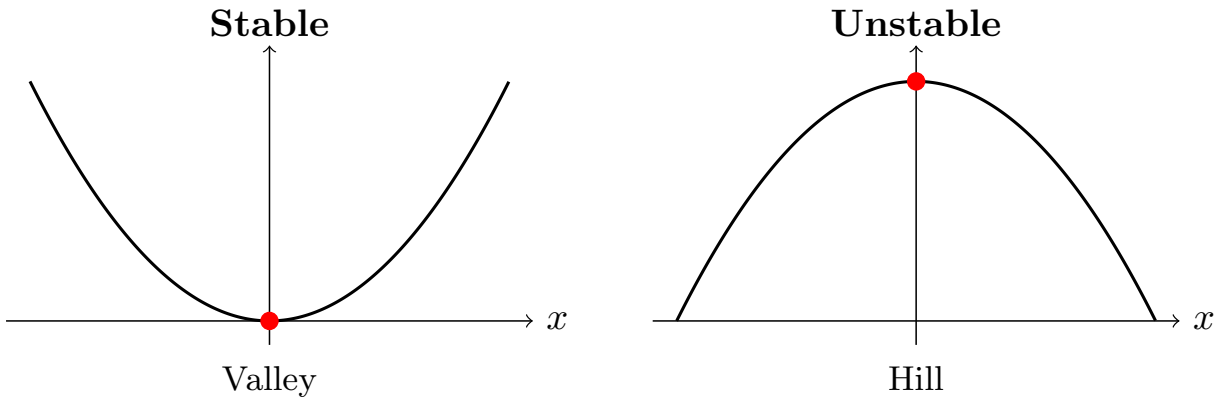

Figure 3: Energy landscape illustrating steady-state stability

Mathematical Concepts: Bistability

Bistability

A system is **bistable** if it admits **two or more stable steady states** under the same parameter conditions. This enables *switch-like behavior* (see [4]) — the system can rest in one state or the other depending on perturbations or initial conditions.

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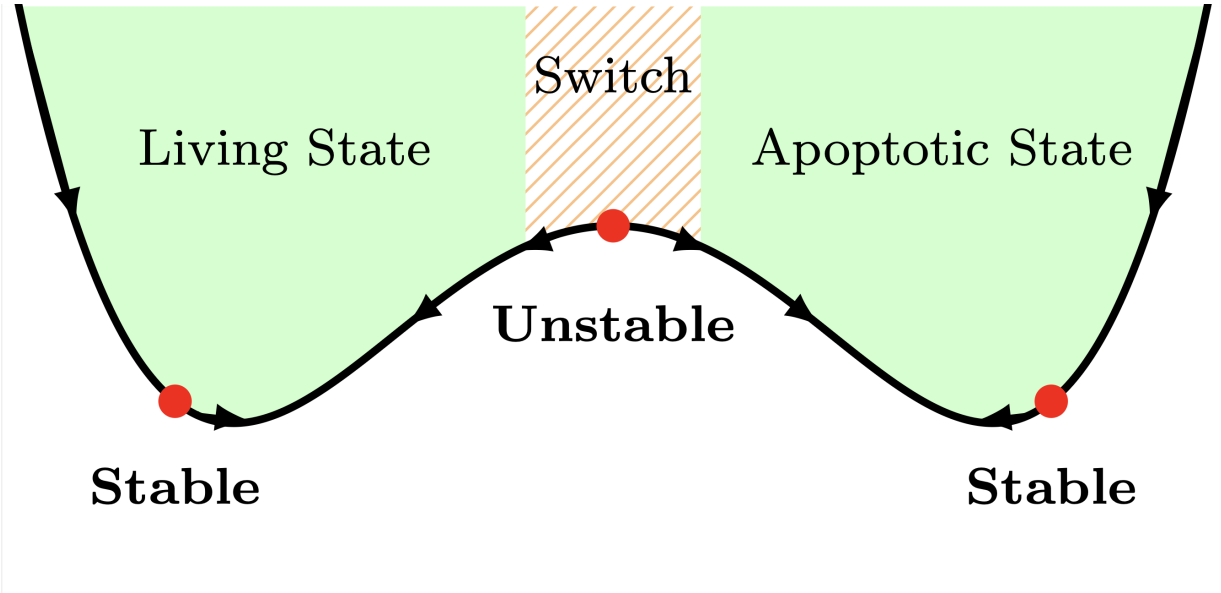

Figure 4: Illustration of bistability

Waldherr et al. (2007): Sensitivity-Based Reduction

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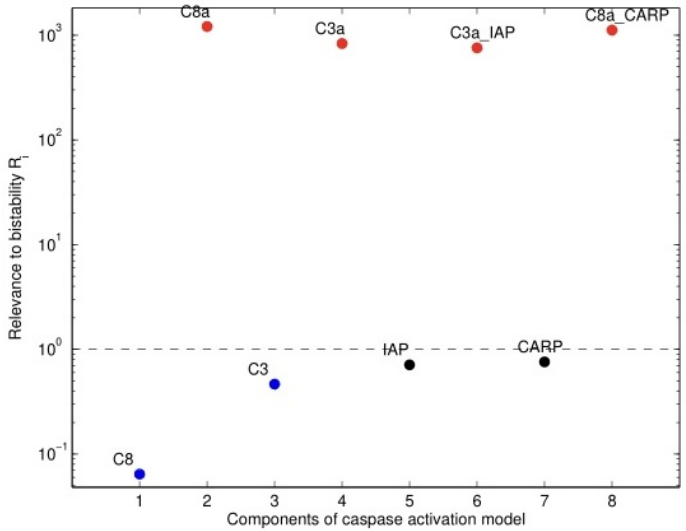

Figure 5: Sensitivity analysis results identifying species with strongest influence on system dynamics. [5]

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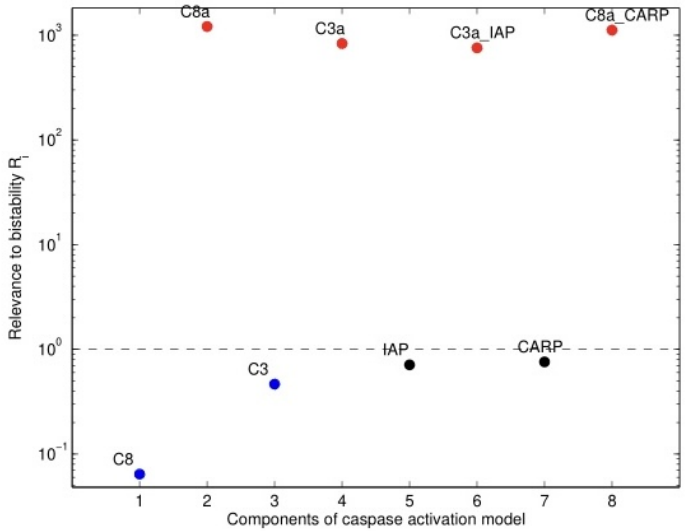


Figure 5: Sensitivity analysis results identifying species with strongest influence on system dynamics. [5]

Reduced ODEs (4-variable system):

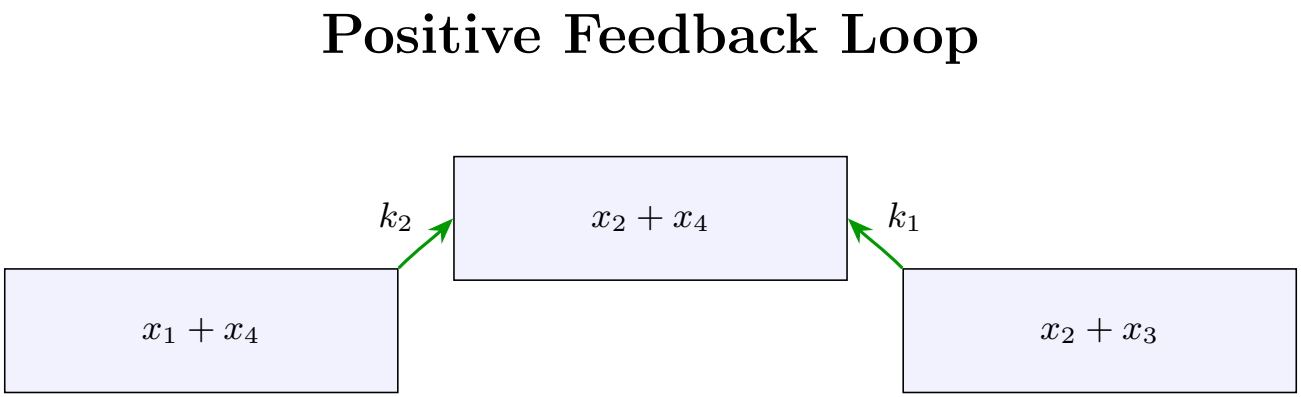
$$\dot{x}_2 = \frac{k_9 k_2 x_4}{k_9 + k_2 x_4} - k_5 x_2 - \frac{(k_{12} + k_{11} x_8) k_{11} x_2}{k_{12} + k_{11} x_2} + \ell_{11} x_8$$

$$\dot{x}_4 = \frac{k_{10} k_1 x_2}{k_{10} + k_1 x_2} - \frac{(k_8 + k_3 x_6) k_3 x_4}{(k_3 + k_4) x_4 + k_8} + k_3 x_6 - k_6 x_4$$

$$\dot{x}_6 = \frac{(k_8 + k_3 x_6) k_3 x_4}{(k_3 + k_4) x_4 + k_8} - k_3 x_6 - k_7 x_6$$

$$\dot{x}_8 = \frac{(k_{12} + k_{11} x_8) k_{11} x_2}{k_{12} + k_{11} x_2} - k_{11} x_8 - k_{13} x_8$$

Focusing on the Feedback Loop



Mechanism:

- Caspase-8* (x_2) activates Procaspase-3 (x_3) to form Caspase-3* (x_4) via k_1.
- Caspase-3* (x_4) enhances activation of Procaspase-8 (x_1) into Caspase-8* (x_2) via k_2.

This forms a reinforcing positive feedback loop.

Figure 6: Diagram of the core feedback loop: Caspase-3 enhances Caspase-8 activation, reinforcing bistable switching.

Model Consolidation: Two-Species Reduction

- We reduce to Caspase-8 (x_2) and Caspase-3 (x_4), the initiator and executioner of apoptosis.

Reduced ODEs (Symbolically Derived):

$$\dot{x}_2 = \frac{-k_{11}k_{13}l_{12}x_2\,(k_2x_4 + k_9) + k_2l_9x_4\,(k_{11}k_{13}x_2 + k_{12}k_{13} + k_{12}l_{11}) - k_5x_2\,(k_2x_4 + k_9)\,(k_{11}k_{13}x_2 + k_{12}k_{13} + k_{12}l_{11})}{(k_2x_4 + k_9)\,(k_{11}k_{13}x_2 + k_{12}k_{13} + k_{12}l_{11})}$$

$$\dot{x}_4 = \frac{k_1l_{10}x_2\,(k_3k_7x_4 + k_4k_7x_4 + k_4l_3x_4 + k_7k_8 + k_8l_3) - k_3k_7l_8x_4\,(k_1x_2 + k_{10}) - k_6x_4\,(k_1x_2 + k_{10})\,(k_3k_7x_4 + k_4k_7x_4 + k_4l_3x_4 + k_7k_8 + k_8l_3)}{(k_1x_2 + k_{10})\,(k_3k_7x_4 + k_4k_7x_4 + k_4l_3x_4 + k_7k_8 + k_8l_3)}$$

Simulation Framework & Parameter Setup

We simulate the reduced system using symbolic and numerical tools in Python:

- `SymPy` for symbolic expressions
- `NumPy` for numerical evaluation
- `Matplotlib` for plotting

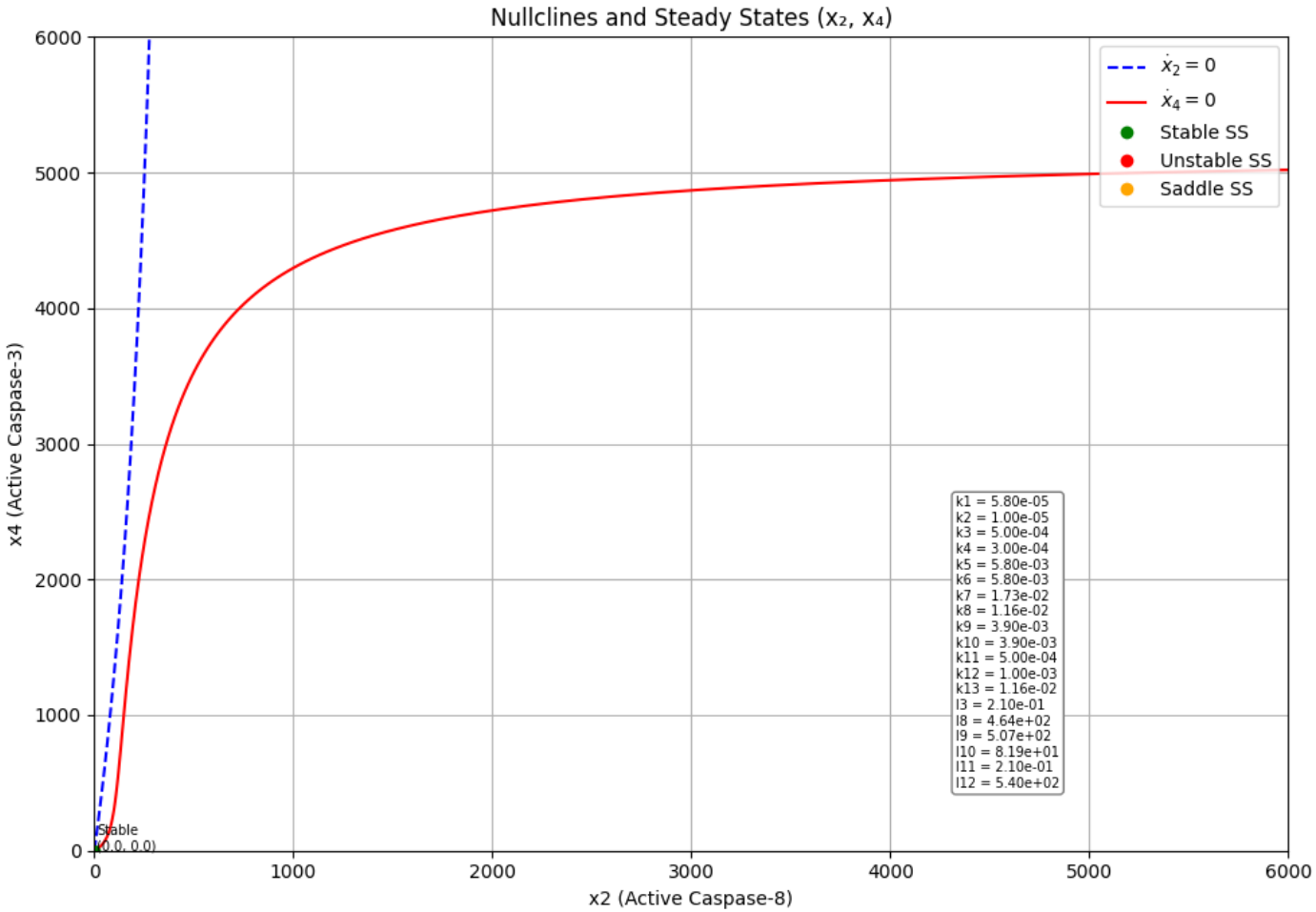
Table 1: Waldherr (2007) parameter values

Parameter	Waldherr (2007)
k_1	$5.8 \cdot 10^{-5}$
k_2	$1.0 \cdot 10^{-5}$
k_3	$5.0 \cdot 10^{-4}$
k_4	$3.0 \cdot 10^{-4}$
k_5	$5.8 \cdot 10^{-3}$
k_6	$5.8 \cdot 10^{-3}$
k_7	$1.73 \cdot 10^{-2}$
k_8	$1.16 \cdot 10^{-2}$
k_9	$3.9 \cdot 10^{-3}$
k_{10}	$3.9 \cdot 10^{-3}$
k_{11}	$5.0 \cdot 10^{-4}$
k_{12}	$1.0 \cdot 10^{-3}$
k_{13}	$1.16 \cdot 10^{-2}$
ℓ_3	0.21
ℓ_8	464
ℓ_9	507
ℓ_{10}	81.9
ℓ_{11}	0.21
ℓ_{12}	540

[5]

Nullclines of Reduced System: Monostable Case

- Plotted nullclines of $\dot{x}_2 = 0$ and $\dot{x}_4 = 0$ from reduced model
- Intersections illustrate steady states; single intersection indicate **monostability**



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Modifications to Induce Bistability:

- Increase k_1: enhances Caspase-3 activation
- Decrease ℓ_{12}: slows apoptosis inhibition

Table 2: Modified Parameter Set

Parameter	Waldherr (2007)	Modified Set
k_1	$5.8 \cdot 10^{-5}$	$1.42 \cdot 10^{-5}$
k_2	$1.0 \cdot 10^{-5}$	$1.0 \cdot 10^{-5}$
k_3	$5.0 \cdot 10^{-4}$	$5.0 \cdot 10^{-4}$
k_4	$3.0 \cdot 10^{-4}$	$3.0 \cdot 10^{-4}$
k_5	$5.8 \cdot 10^{-3}$	$5.8 \cdot 10^{-3}$
k_6	$5.8 \cdot 10^{-3}$	$5.8 \cdot 10^{-3}$
k_7	$1.73 \cdot 10^{-2}$	$1.73 \cdot 10^{-2}$
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k_9	$3.9 \cdot 10^{-3}$	$3.9 \cdot 10^{-3}$
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ℓ_3	0.21	0.21
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ℓ_9	507	507
ℓ_{10}	81.9	81.9
ℓ_{11}	0.21	0.21
ℓ_{12}	540	440

Nullclines of Reduced System: Bistable Case

- Intersections illustrate steady states; stable points act as "resting states"; the unstable one is the threshold

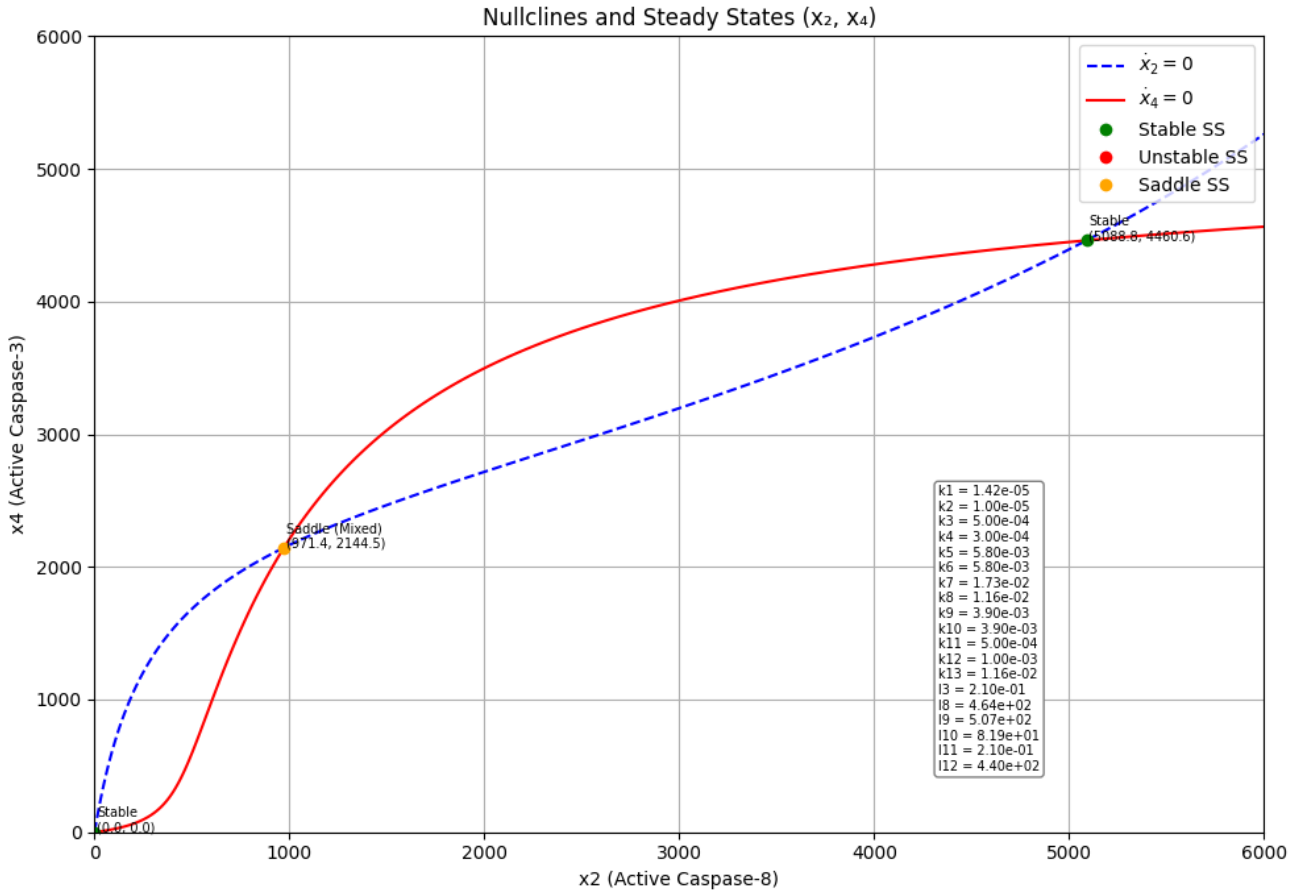

Figure 7: Nullcline plot showing bistability in the reduced system

Steady State Classification: Method

Each steady state occurs at a nullcline intersection where both $\dot{x}_2 = 0$ and $\dot{x}_4 = 0$. To classify these points, we use the Jacobian matrix of the 2D reduced system:

$$J(x_2, x_4) = \begin{bmatrix} \frac{\partial \dot{x}_2}{\partial x_2} & \frac{\partial \dot{x}_2}{\partial x_4} \\ \frac{\partial \dot{x}_4}{\partial x_2} & \frac{\partial \dot{x}_4}{\partial x_4} \end{bmatrix}$$

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We compute the eigenvalues λ_1, λ_2 of J at each steady state and assess stability by evaluating the real parts $\mathrm{Re}(\lambda_i)$:

$\mathrm{Re}(\lambda_1), \mathrm{Re}(\lambda_2) < 0$:	Stable
Mixed signs:	Saddle (unstable)
$\mathrm{Re}(\lambda_1), \mathrm{Re}(\lambda_2) > 0$:	Unstable

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We repeat this classification for the full 8D system to verify that the reduced model preserves stability structure. Stability via eigenvalue real parts follows dynamical systems theory [3].

Stability Classification Results (2D vs 8D)

Steady State	2D Eigenvalues	8D Real Parts	Classification
Low (0, 0)	$[-11.56, -1.49]$	All negative	**Stable**
Middle (971.4, 2144.5)	$[-0.037, +0.008]$	Mixed (1 positive)	**Saddle**
High (5088.8, 4460.6)	$[-0.0087, -0.0036]$	All negative	**Stable**

Table 3: Eigenvalue-based classification of steady states from both reduced and full systems.

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- All classifications match between the reduced 2D and full 8D model.

If the classifications did not match, it would indicate that the symbolic 2D model fails to preserve the core dynamics of the full biochemical system, and conclusions drawn from the reduced model would not be valid.

Model Consolidation: One-Species Reduction

Reduced ODE for x_4

$$\dot{x}_4 = \frac{\text{large polynomial in } x_4 \text{ and } k_i, \ell_i \text{ including } \sqrt{(\cdots)}}{\text{rational denominator in } x_4} + \cdots$$

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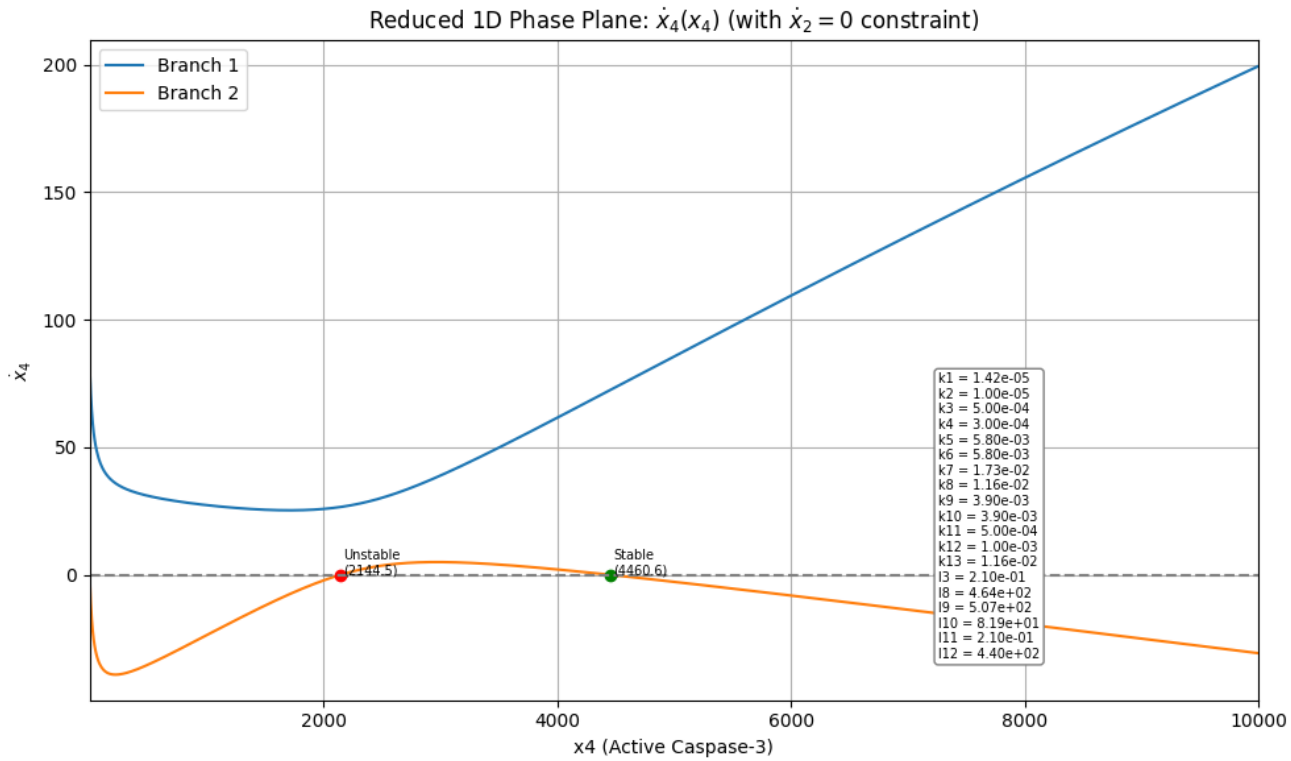

Figure 8: Single-variable nullcline projection of the caspase model

Single-Variable Nullcline: Biological Plausibility

Reducing to a single ODE for x_4 by solving $\dot{x}_2 = 0$ may produce biologically implausible results.

- Back-substituting can yield negative or nonphysical concentrations for x_2 at certain x_4.

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Conclusion: The 1D reduction is not reliable. For biologically valid results, we must analyze the full 2D (or higher) system.

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- Identify which parameters are **critical** for maintaining bistability and biological switching.

Goal: Determine which reaction rates control the cell's ability to make all-or-nothing life-or-death decisions.

Bifurcation Diagram: Varying k_1 (Caspase-3 Activation)

- Sweep k_1: controls how efficiently Caspase-8* activates Caspase-3.

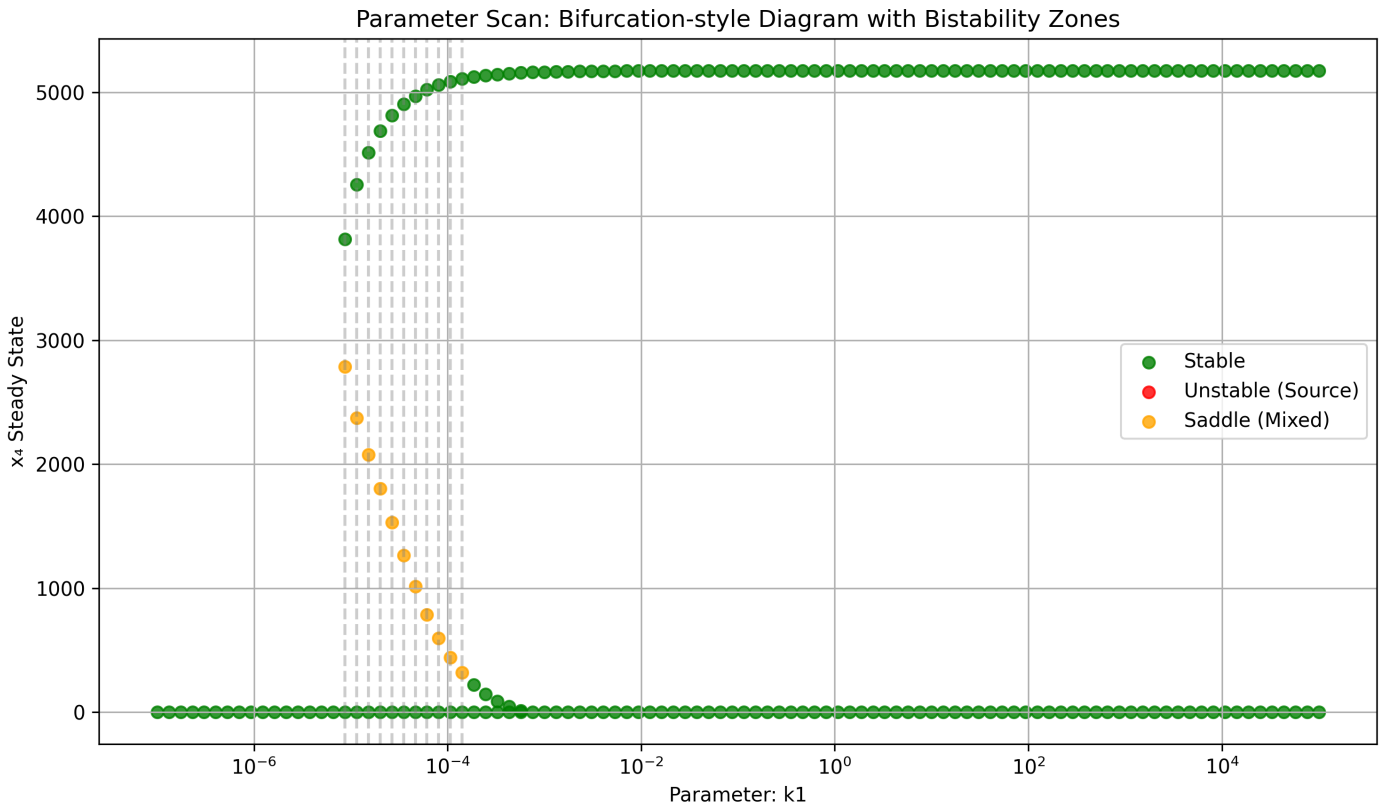
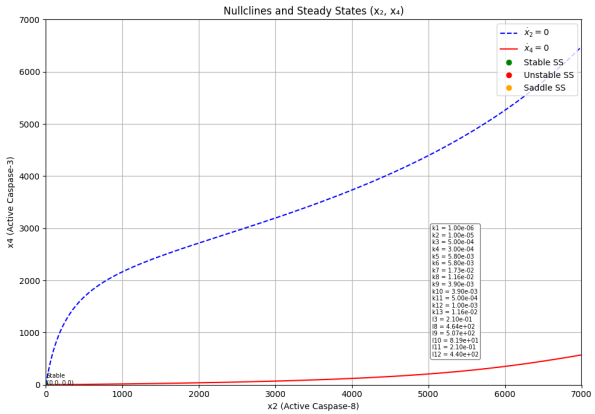
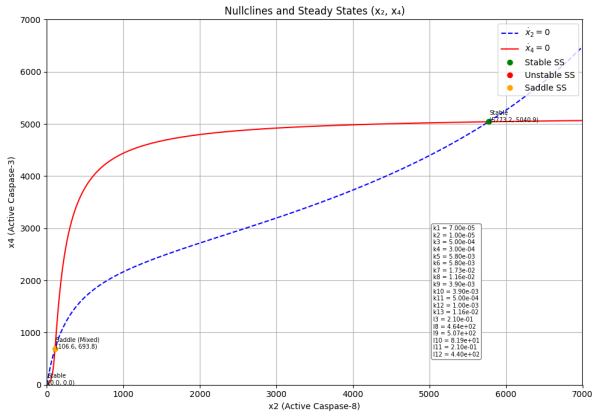


Figure 9: Bifurcation diagram showing steady state x_4 values as a function of k_1

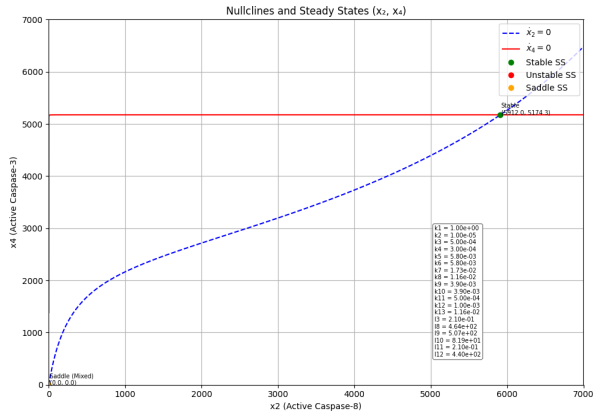
Nullclines at Key Parameter Values: k_1 Zones



Zone 1: Low k_1 (Living Only)



Zone 2: Middle k_1 (Bistable)



Zone 3: High k_1 (Apoptosis Only)

Figure 10: Nullcline plots showing steady-state changes across low, middle, and high k_1 regimes

Key Parameters Controlling Bistable Behavior

Parameter	Lower Bound	Upper Bound	Log-Width	% of Scan
l9	4.98×10^{2}	8.70×10^{2}	0.24	2.0%
k3	8.11×10^{-5}	4.33×10^{-4}	0.73	6.1%
l12	7.05×10^{1}	3.76×10^{2}	0.73	6.1%
l10	9.33×10^{1}	4.98×10^{2}	0.73	6.1%
k4	3.27×10^{-4}	2.31×10^{-3}	0.85	7.1%
l3	2.01×10^{-1}	1.42	0.85	7.1%
k7	2.31×10^{-3}	1.63×10^{-2}	0.85	7.1%
l8	7.05×10^{1}	4.98×10^{2}	0.85	7.1%
k1	8.70×10^{-6}	1.42×10^{-4}	1.21	10.1%
k9	2.48×10^{-4}	4.04×10^{-3}	1.22	10.2%
k10	3.27×10^{-4}	5.34×10^{-3}	1.22	10.2%
k2	8.70×10^{-6}	1.87×10^{-4}	1.33	11.1%
k6	3.27×10^{-4}	7.05×10^{-3}	1.34	11.2%
k5	3.27×10^{-4}	1.23×10^{-2}	1.58	13.2%
k12	1.00×10^{-7}	2.85×10^{-2}	5.45	45.4%
k8	1.00×10^{-7}	4.64×10^{-1}	6.67	55.6%
l11	1.00×10^{-7}	5.72	7.76	64.7%
k13	4.33×10^{-4}	1.00×10^{5}	8.36	69.7%
k11	2.01×10^{-5}	1.00×10^{5}	9.70	80.8%

Table 4: Parameters with narrow bistable regions (e.g., **l9**, **k3**) act as key switches. Wide ranges (e.g., **k11**) indicate robust tolerance.

Identifying Essential Parameters: Nullcline Zeroing Method

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- **Insight:** This approach provides a direct, visual way to assess the functional role of each reaction in cell fate decisions.

Parameter Zeroing: Example Nullcline Behaviors

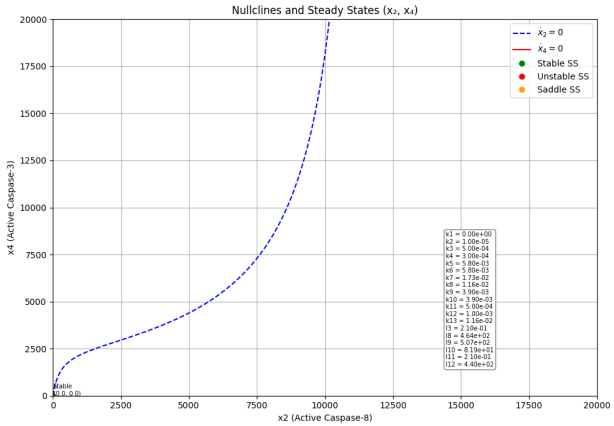

Figure 11: $k_1 = 0$: Only low stable state remains — cell lives.

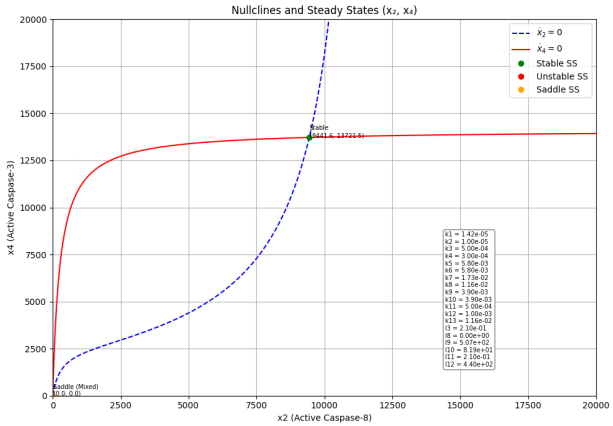

Figure 12: $I_8 = 0$: Only high stable state remains — cell death is the attractor.

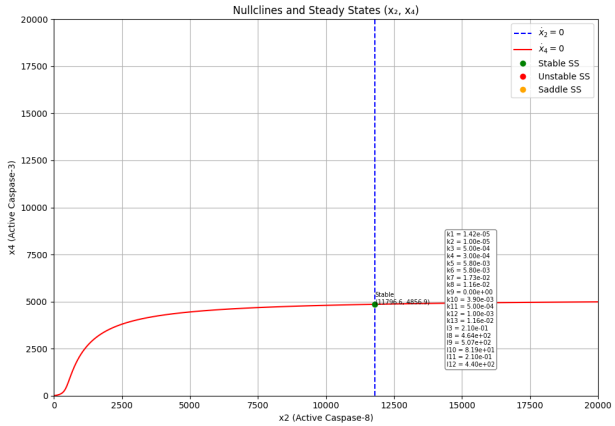

Figure 13: $k_9 = 0$: High state dominates — irreversible apoptosis.

ℓ_{11}: Unique Dispensability for the Switch

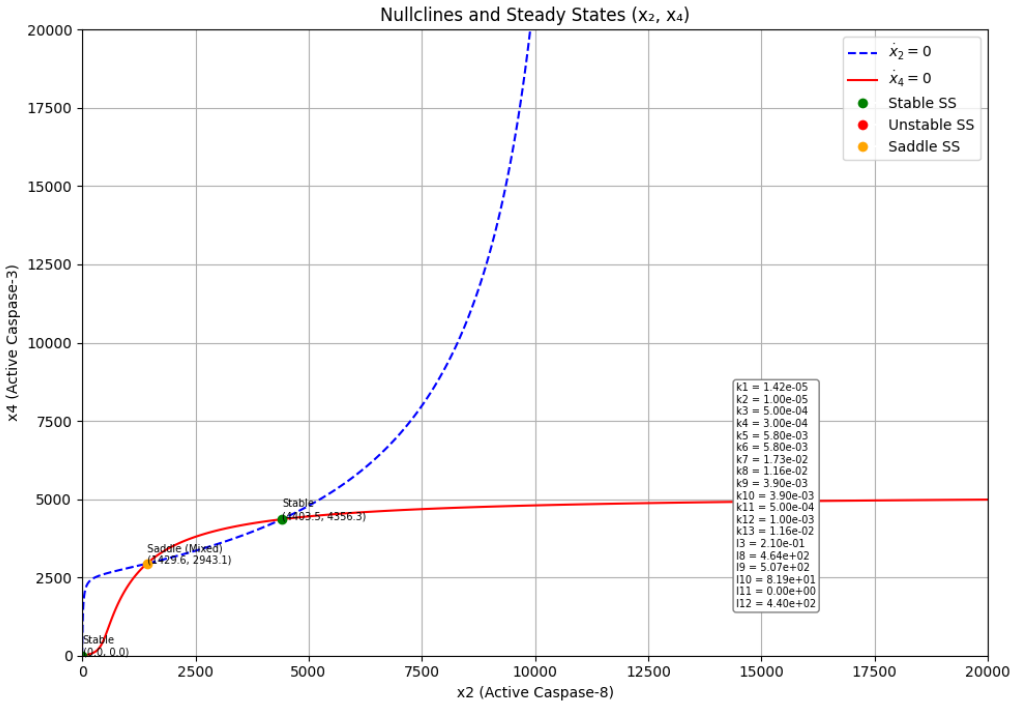

Figure 14: Nullcline plot with $\ell_{11} = 0$: Bistability is preserved, indicating that the reaction associated with ℓ_{11} is not required for the switch mechanism.

ℓ_{11}: Unique Dispensability for the Switch

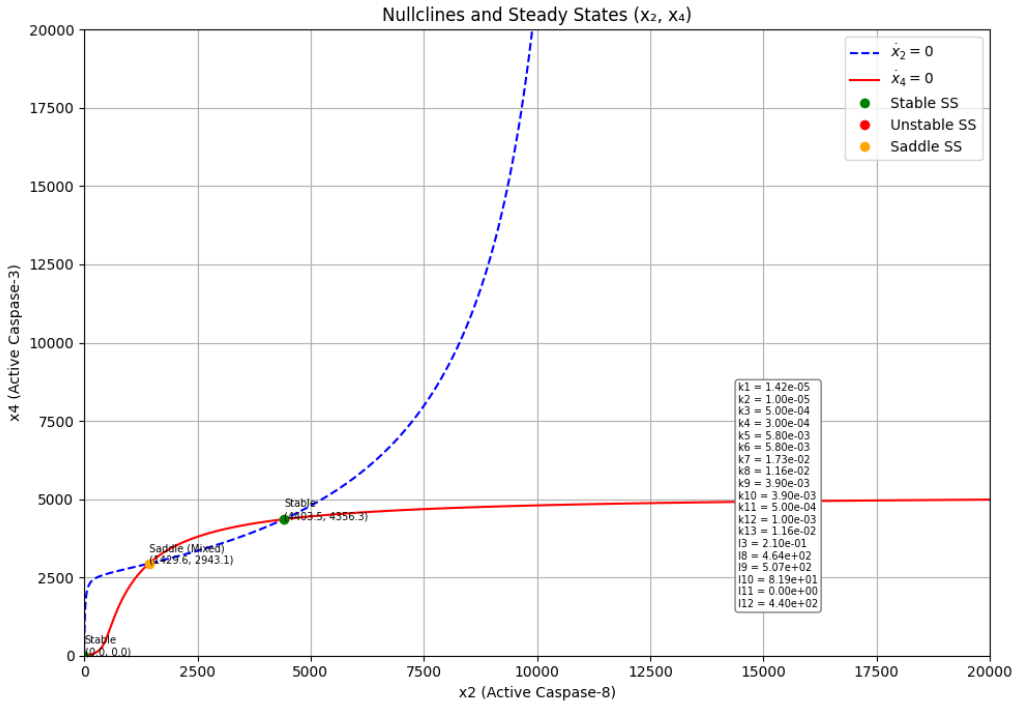

Figure 14: Nullcline plot with $\ell_{11} = 0$: Bistability is preserved, indicating that the reaction associated with ℓ_{11} is not required for the switch mechanism.

- ℓ_{11} is the only parameter whose removal ($\ell_{11} = 0$) preserves bistability.
- **Interpretation:** Bistability persists without ℓ_{11}, revealing that this reaction is not critical to the apoptotic decision-making mechanism.

Parameter Removal Reveals Distinct Stability Outcomes

Table 5: **Effect of Parameter Removal on Nullcline Structure and Bistability**

Outcome Type	**Parameters Zeroed**	**Resulting Nullcline Behavior**
Low stable only	k_1, k_2, k_4, ℓ_3, ℓ_9, ℓ_{10}	Cell is trapped in survival; apoptosis cannot occur
Low stable then saddle	k_5, k_6	Cell is locked in survival; saddle is non-functional
Low saddle then high stable	k_3, k_7, ℓ_8	Survival basin lost; unstable low region leads to apoptosis
Mid saddle then high stable	k_8, k_{12}	No survival state; flow from unstable middle leads to apoptosis
High stable only	k_9, k_{10}	Cell starts and ends in apoptosis; no other dynamics exist
Low saddle only	k_{11}, k_{13}, ℓ_{13}	No stable state; biologically implausible
Bistable	ℓ_{11}	Switch retained; bistability preserved

Interpretation: Most single parameter removals disrupt switch-like behavior: either locking cells in survival, forcing apoptosis, or eliminating stability altogether.

Conclusions

Main Research Question:
How do specific reactions and parameters in the caspase network influence bistability?

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- The **two-variable model** (Caspase-8 and -3) successfully retained full system bistable dynamics, but the **one-variable model** (Caspase-3) does not.
- **Bifurcation diagrams** and bistability windows identified which parameters most strongly shape the switch, revealing critical thresholds and sensitivities.

Future Directions

Experimental Validation

Compare simulation results with experimental data, such as measurements of cell fate or protein activity levels, to test whether the predicted bistable behavior actually occurs in biological systems. This helps confirm that the model accurately captures real-world dynamics.

Parameter Pair Interactions

Investigate how pairs of parameters interact by running two-dimensional simulations and generating heatmaps, which can highlight combinations that either promote or suppress bistability. This can reveal more complex control mechanisms not visible in one-parameter scans.

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