A Bayesian approach to explore signal execution mechanisms in programmed cell death.

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Abstract

Mathematical models of biochemical reaction networks are central to the exploration of network dynamics and bridge the gap between experimental measurements and mechanistic interpretations. However, model parameters are often not available and sparse experimental data leads to challenges in model calibration and parameter estimation. This can in turn lead to inaccurate mechanistic interpretations of experimental data and generate poorly conceived hypotheses for experimental validation. To address this challenge, we evaluate whether a Bayesian probability-based approach can be used to qualitatively explore biochemical network execution mechanisms. To test this approach, we explore the parameter space of extrinsic apoptosis to identify the preferred mode of signal execution. Apoptosis signal processing can take place either through a mitochondria independent (Type I) mode or a mitochondria dependent (Type II) mode. We first show that model subnetworks successfully identify the most likely execution mode for a specific concentration of key molecular regulators. We then show that changes in molecular regulator concentrations alter the overall reaction flux through the network shifting modulating signal flow primarily through either a caspase-only pathway or through the mitochondria. Our work thus demonstrates that Bayesian probability approaches can be used to explore the dynamic behavior of model biochemical systems even with missing or sparse data.

Introduction

Emergent cellular behaviors from complex biochemical network processes are difficult to characterize due to the complex interplay between system components and their interactions [1, 2, 3]. Mathematical models of biochemical processes have evolved alongside experimental measurements to explain observed cellular behaviors and guide hypothesis construction for further testing [4, 5]. Models of biochemical reaction networks are typically based on the Mass-Action kinetics formalism and built to represent known pathway mechanics with knowledge garnered from years or even decades of experimentation [6, 7]. Although these models have yielded important predictions and insights about biochemical network processes, they also depend on kinetic rate parameters and protein concentrations that are often poorly characterized or simply unavailable. A typical work-around is to employ model calibration methods to estimate suitable parameters values via optimization to protein concentration time-course data, which is often scarce [8, 9, 10]. It has also been shown that vector-based methods can find multiple parameter sets whereby models agree with experimental data, based on goodness-of-fit metrics, but the dynamics of the network can exhibit significantly different execution modes [7, 9]. This poses a challenge for the study of dynamic network processes as the mode of signal execution can be highly dependent on a specific parameter set and could in turn lead to inadequate model-based interpretation. Therefore, an approach that can enable researchers to explore the execution mechanisms of a biochemical process from a probabilistic perspective, constrained only by available data, would facilitate a rigorous exploration of network dynamics and accelerate the generation of testable mechanistic hypotheses [11].

In this work, we explore whether a Bayesian probability approach can identify network execution modes in extrinsic apoptosis restricted only by experimental observations. Two execution modes have been identified for extrinsic apoptosis signaling: a mitochondria independent (Type I) mode, whereby initiator caspases directly activate effector caspases and induce cell death, and a mitochondria dependent (Type II) mode whereby initiator caspases engage the Bcl-2 family of proteins, which eventually lead to effector caspase activation (see Box 1 for biology details). Most mammalian cells execute apoptosis via the Type II mechanism, yet the Type I mechanism plays a central role in specific cell types, particularly certain types of lymphocytes [12]. A significant body of experimental and modeling work has identified key regulators for Type I vs Type II execution (see Box 1). However, it is still unclear how network structure and the interplay among multiple regulators can modulate signal execution for either Type I or Type II modes. A more traditional approach would prescribe intricate and detailed experimental measurements of cellular response to yield the desired data and improve our understanding of signal execution. However, the time and cost associated with such experiments makes it unlikely and at times unfeasible to obtain said data. It is here that we see Bayesian inference approaches as a complementary alternative to experimentation that can provide qualitative insights about signal execution mechanisms, by exploring broad parameter space ranges, subject only to available computer time.

Here we show that a Bayesian parameter exploration approach, constrained by network structure or molecular concentrations, can identify the dominant signal execution modes in a reaction network. Specifically, we demonstrate the dependence of Type I or a Type II cellular apoptosis execution on network structure and chemical-species concentrations. We use Bayesian model evidence as a metric for comparisons of signal flow through different pathways of the network and subnetworks to identify how regulators affect execution modes. We introduce two complementary approaches that can be used in tandem to explore signal execution modulation. We first define a *multimodel exploration method* to explore multiple hypothesis about apoptosis execution by deconstructing an established apoptosis network model into functional subnetworks. We also define a *pathway flux method* to characterize the signal flux through specific network pathways within the complete network. Combined, these two approaches enable us to qualitatively identify key network components and molecular regulator combinations that shed mechanistic insights about apoptosis execution. Our approach is generalizable to other mass-action kinetics-based networks where signal execution modes play important roles in cellular outcomes. The work leverages Nested Sampling algorithm methods to efficiently calculate Bayesian evidence on high performance computing (HPC) platforms, both of which are seldom used in biological applications. In this manner we are able to carry out the necessary calculations to explore parameter space and estimate the model evidence within the timespan of days.

Methods

Apoptosis model and simulations

The base model used in this work is a modified version of the Extrinsic Apoptosis Reaction Model (EARM) from Lopez et al. (EARM v2.1) [7] . The original EARM was simplified to reduce complexity in the number of parameters, but still retains the key features of the network for apoptosis execution. Specifically, we reduced the molecular complexity of mitochondrial outer membrane permeabilization (MOMP) down to a representative set of Bcl-2 proteins that capture the behavior of activators, inhibitors, effectors, and sensitizers. We also eliminated intermediate states for Cytochrome-c and SMAC to streamline effector caspase activation, and w added an explicit FADD molecule, a part of the death-inducing signaling complex (DISC) to achieve a more realistic representation of signal initiation. Overall, EARM v2.1 comprised 16 chemical species at non-zero initial concentrations, 50 total chemical species, 62 reactions, and 62 kinetic parameters. The modified model was recalibrated to recapitulate the time-dependent concentration trajectories of truncated Bid, Smac release from the mitochondria, and cleaved PARP analogous to the approach reported previously [42] (Figure S1). The modified EARM was partitioned into six sub-models, encoded in PySB, and are summarized in Figure 2. All simulations were run using the mass-action kinetics formalism as a system of Ordinary Differential Equations (ODEs) using the VODE integrator in SciPy, within the PySB modeling framework. All data results, representative models, and software are distributed with open-source licensing and can be found in the GitHub repository https://github.com/LoLab-VU/BIND.

Bayesian evidence estimation

Bayesian evidence is the normalizing term in a Bayesian calculation and provides a measure for model comparison with regard to their fit to experimental data. It is expressed as:

$$P(D|M) = \int L(D|\theta, M)\, P(\theta|M)\, d\theta \tag{1}$$

Where M is the model under consideration, D is the experimental data, θ is a specific set of parameter values, $L(D|\theta, M)$ is the likelihood function describing the fit of the data to the model under those parameter values, and $P(\theta|M)$ is the prior distribution of parameters. All evidence estimates were made using the nested sampling method, introduced by Skilling [43]. This method simplifies the evidence calculation by introducing a prior mass element $dX = P(\theta|M)\, d\theta$ that is estimated by $(X_{i-i} - X_i)$ where $X_i = e^{-i/N}$, i is the current iteration of the algorithm, and N is the total number of live points. The evidence is then written as

$$Z = \int_0^1 L\, dX \approx \sum_{i=1} L_i (X_{i-1} - X_i) \tag{2}$$

Initialization of the algorithm is carried out by randomly selecting an initial population of parameter sets (points in parameter space) from the prior distribution, scoring each one with the likelihood function, and ranking them from L_{high} to L_{low}. At each iteration of the algorithm a new set of parameter values is selected and scored. If that score is higher than L_{low}, then it is added to the population, at the appropriate rank, and L_{low} is removed from the population and added to the evidence sum (2).

Nested sampling software

All evidence estimates in this work are calculated with MultiNest, a nested sampling-based algorithm designed for efficient evidence calculation on highly multimodel posterior distributions [44, 45]. MultiNest works by clustering the live points (population of parameter sets) and enclosing them in ellipsoids at each iteration. The enclosed space then constitutes a reduced space of admissible parameter sets. This lowers the probability of sampling from low likelihood areas and evaluating points that will only be discarded. The evidence estimate is accompanied by an estimate of the evidence error. The algorithm terminates when the presumed contribution of the highest likelihood member of the current set of live points, $L_{high}X_i$ is below a threshold. Here, we use a threshold of 0.0001 and a population size and 16,000 unless otherwise noted. See [44, 45], for more details on the MultiNest algorithm. We use MultiNest with the Python wrapper PyMultiNest [46], which facilitates the integration with PySB into the parameter sampling pipeline.

Multimodel exploration analysis.

To explore multiple hypotheses for signal execution under different biochemical network structures, we carried out an analysis inspired in the multi-model inference approach [47, 48]. We broke down the EARM network into six subnetworks and tested each across increasing concentrations of the regulator XIAP for efficacy in achieving apoptosis. We define the proportion of cleaved PARP, relative to total PARP, as a metric for effective apoptosis execution. That is, if all PARP has been cleaved, then apoptosis has been completely achieved. We therefore define an objective function that estimates the amount of cleaved PARP as:

$$Obj_{multimodel} = \frac{cParp}{tParp} \quad (3)$$

where $cParp$ is the amount of PARP that has been cleaved and $tParp$ is the total amount of PARP in the system.

When this objective function is substituted into equation (1) in place of the likelihood function, the evidence calculation produces an expected value, an average over the chosen prior parameter range for the proportion of PARP that has been cleaved. In this work we compare the expected values for different subnetworks, pathways, and regulatory conditions only in qualitative terms and as a relative measure of fit to an outcome represented by the objective function.

Pathway flux analysis.

We also explored the effect of molecular regulators of Type I vs Type II execution relative to the apoptosis signal flux through the network as we have done in previous work [49]. Briefly, signal flux is defined as the chemical reaction flux in units of molecules per unit time, that traverses through a given pathway. In the apoptosis network there are two potential pathways that can lead to Caspase-3 activation and subsequently PARP cleavage. In the direct caspase pathway initiator caspases represented here as "Caspase-8" directly cleave and activate the effector caspases represented here as "Caspase-3". By contrast, in the mitochondrial pathway, effector caspases are activated via the apoptosome, and are dependent on mitochondrial outer membrane permeabilization (MOMP). Therefore, the dominant pathway responsible for Caspase-3 activation defines the route of the signal. To estimate the flux through the Type I vs Type II pathway, we define an objective function as:

$$Obj_{pathway} = \sum_{t=0}^{T} \frac{\sum_0^t C3_{pathway}}{\sum_0^t C3_{total}} \times (cParp_t - cParp_{t-1}) \quad (4)$$

where t represents time in seconds, $\sum_0^t C3_{pathway}$ is the amount of Caspase-3 activated via the target pathway up to time t, $\sum_0^t C3_{total}$ is the total Caspase-3 activated up to time t, and $\sum_0^t C3_{caspase} / \sum_0^t C3_{total}$ is the proportion of activated Caspase-3 that was produced via the target pathway up to time t. $(cParp_t - cParp_{t-1})$ is the total PARP that has been cleaved, and activated, by Caspase-3 from time $t-1$ to time t.Thus, at any given time t we can estimate the amount of Caspase-3 that has been activated through a specific pathway. Multiplication of these two terms returns an estimate for the amount of PARP cleaved via the specific pathway at time t. Summing over T then returns an estimate for the total apoptosis signal flowing through the target pathway. Like the PARP cleavage objective function, the signal flux objective substituted into equation (1) produces an estimate of the average flux over a defined prior distribution. We estimated this quantity over increasing concentrations of the of the molecular regulator XIAP, but also at high and low levels of the DISC components FADD and Caspase-8. The total signal flux was estimated by summing the evidence estimates for the flux through either the direct caspase or mitochondrial pathway.

Parameter ranges and initial conditions.

The prior distribution takes the form of a set of parameter ranges, one for each reaction rate parameter. The chosen ranges span four orders of magnitude around generic reaction rates

deemed plausible [4] and are specific to the type of reaction taking place. The ranges of reaction rate parameters, in Log_{10} space, are 1st order forward: [-4.0, 0.0], 2nd order forward: [-8.0, -4.0], 1st order reverse: [-4.0, 0.0], catalysis: [-1.0, 3.0]. These ranges were also used in calibration of the base model. Initial conditions were either gleaned from the literature [50, 51] or taken from a previous model of extrinsic apoptosis [7]. Because the baseline model was designed to concur with Type II apoptotic data (see above), literature derived initial conditions were based on Type II Jurkat or Hela cell lines (Table S1).

Bayes factors.

Evidence estimates are often used to select between two competing models by calculating the Bayes factor (i.e. the ratio of their evidence values). This provides a measure of confidence for choosing one model over another. We can likewise use trends in evidence values to produce trends in Bayes factors that provide additional insights into the dynamical relationship between pathways. To facilitate construction of Bayes factor trends with a continuous and symmetric range, the we calculated Bayes factors as:

$$Bf = \begin{cases} -\frac{Z_2}{Z_1} + 1 \ \ if \ Z_1 < Z_2 \\ \frac{Z_1}{Z_2} - 1 \ \ if \ Z_1 > Z_2 \end{cases}$$

where Z_1 and Z_2 are the evidence estimates for two pathways under comparison.

Computational resources

Because of the high computational workload necessary for this analysis, a wide range of computational resources were used. The bulk of the work was done on the ACCRE cluster at Vanderbilt University which has more than 600 compute nodes running Intel Xeon processors and a Linux OS. As many as 300 evidence estimates were run in parallel on this system. Additional resources included two local servers, also running Intel processors and a Linux OS, as well as a small local four node cluster running Linux and AMD Ryzen 1700 processors. A detailed breakdown of CPU time can be found in the results section. In all evidence estimates for 14 different networks/initial conditions were made across the range of XIAP concentrations. We estimate all 14 runs would take ~9 days each on a typical university server with 32 cores/64 threads.

Results

Overview: A Bayesian evidence approach to explore mechanistic hypotheses.

Our overarching goal is to understand the mechanisms and dynamics of biochemical networks responsible for cellular commitment to fate, given incomplete or unavailable data. We take a Bayesian probability approach to study model subnetworks and specific model pathways as summarized schematically in Figure 1 to estimate the evidence for signaling execution mechanisms given a set of experimental data and existing priors.

In the *Multimodel Exploration Analysis* (Figure 1, left path), the network model is deconstructed into biologically relevant subnetworks and the probability of each subnetwork achieving apoptosis, under various regulatory conditions, is estimated via Bayesian evidence. This differs from traditional model selection and multimodel inference applications where models are typically ranked based on their fit to experimental data and high-ranking models may be averaged to obtain a composite model [47, 48, 52, 53, 54, 55]. Here, we already have a model that captures key features of programmed cell death execution. Instead, we use the differences in evidence to construct a composite picture of mechanistic evidence for apoptosis execution. To achieve this, we first tailor the objective function to represent signal execution strength, as measured by cleaved PARP concentration at the end of the simulation. The evidence derived from this objective function therefore describes the likelihood that the signal is effectively transmitted through the (sub)network. It should be noted that Bayesian evidence inherently incorporates model complexity as the objectives are integrated over normalized prior distributions [44, 52, 56]. As we will see, comparison of changes in signal strength through relevant subnetworks allows inferences to be made on the effect of the perturbed network regulator as well as various network components on the overall dynamics of the system. We focus primarily on understanding how Bayesian evidence for the caspase pathway compares to that of the complete network as these are most relevant for the analysis of Type I/II execution modes. This analysis will inform on how network components contribute to overall signal execution and provide mechanistic insights about the sensitivity of PARP cleavage to subnetwork components.

In the *Pathway Flux Analysis* (Figure 1, right path), we retain the complete network structure but instead tailor the objective functions to measure biochemical reaction flux, as described in the Methods section, through either the direct caspase or mitochondrial pathways. We primarily consider the influence of the apoptosis inhibitor XIAP on regulatory dynamics and phenotypic fate but also consider the regulatory effect of the death inducing signaling complex (DISC) and the anti-apoptotic protein Bcl-2, all of which have been found to be relevant to Type I vs Type II execution in different cell types [13, 14]. This analysis will inform on how molecular regulators modulate biochemical flux through the network and their influence on apoptosis completion as measured by PARP cleavage.

Decomposition of the extrinsic apoptosis network and reductive analysis of the effects of XIAP

To investigate the effect of network substructures on apoptosis signaling, we build a composite description of system dynamics by observing variations in signal throughput, represented by evidence values for PARP cleavage, between subnetworks (Figure 2A-F) relative to changes in

regulatory conditions. We consider relative changes in the evidence values as the number of XIAP molecules is increased where a higher evidence value indicates a stronger signal over the prior range of parameter values. XIAP was varied from 0 to 200,000 molecules per cell in increments of 250 to explore how changes in XIAP affect the likelihood of apoptosis execution. For subnetworks that include the mitochondrial pathway, Bcl-2 (an anti-apoptotic) was reduced or eliminated, to explore Type I vs Type II activity independent of inhibitors that could confound signal throughput, and more closely simulate a cells that is “primed” for death [56]. All other initial values were fixed at the levels shown in supplementary Table S1. In the absence of XIAP all six subnetworks have evidence estimates greater than 0.98, (Figure 2 A: 0.993, B: 0.998, C: 0.992, D: 0.981, E: 0.998, F: 0.981, Table S2) indicating that they can all capture a biological process that leads to PARP cleavage across the allowed range of parameters, as expected. We further explore how XIAP can modulate Type I/II apoptosis execution for a chosen subnetwork .

The results in Jost et al. [14] imply that the cellular level of XIAP determines the preferred apoptosis pathway with higher levels specific to Type II cells and lower levels specific to Type I. To hypothesize a possible mechanistic explanation for this behavior we compared the computed the Bayesian evidence values, over increasing concentrations of XIAP, for the direct caspase activation network against both the complete network and the mitochondrial network (Figures 2A and G green; 2E and G orange; 2F G blue respectively). . This mimics reported experimental strategies to study Type I/II phenotypes and allows us to gauge the effect of XIAP on networks with and without a mitochondrial component [13, 35].

As XIAP levels increase we see differential effects on all subnetworks in the form of diverging evidence estimates, indicating differences in the efficacy of XIAP induced apoptotic inhibition. The evidence values for the isolated caspase pathway (Figure 2G green) diverges from the complete network (Figure 2G orange) and mitochondrial pathway (Figure 2 blue) showing a steeper initial decline that diminishes as XIAP continues to increase. PARP cleavage evidence values for the caspase pathway falls to 0.5 at an XIAP level of roughly 32,000. However, the complete network and mitochondrial pathways require XIAP levels nearly threefold higher with the evidence value reaching 0.5 at around 92,000 and 95,000 respectively.

Because the direct caspase activation pathway (Figures 2G green) is representative of the Type I phenotype, the disproportionate drop in its evidence of PARP cleavage as XIAP concentration increases is consistent with experimental evidence showing XIAP-induced transition from a Type I to a Type II execution mode [14]. The complete network, containing the full mitochondrial subnetwork, and mitochondrial only pathway are also affected by XIAP but exhibits resistance to its anti-apoptotic effects, a difference that is most prominent at moderate levels of the inhibitor. This suggests a growing dependence on mitochondrial amplification for effective apoptosis as XIAP increases from low to moderate levels. At higher levels of XIAP the evidence values for the caspase pathway level off and the gap between it and the two mitochondrial containing networks narrows. The disproportionate effect of XIAP inhibition of apoptosis on the caspase pathway suggests that the mechanism for XIAP induced transition to a Type II pathway can be attributed to differential inhibition of the apoptotic signal through the isolated caspase pathway vs a network with mitochondrial involvement.

We note here that the small differences in evidence values between the various networks should not be surprising since every subnetwork being considered is capable of carrying apoptosis to completion, as measured by PARP cleavage. Thus, we should not expect evidence differences that would rule out any model under a model selection criterion. The log-evidence version of Figure 2G along with estimated errors generated by MultiNest are displayed in Figure S2.

The next two highest trends in evidence values after that of the direct caspase network (Figure 2G green) belong to the networks representing direct caspase activation plus mitochondrial activation and mitochondrial activation alone (Figures 2G purple and 2G brown). For most of the range with XIAP below 100,000 these two networks have largely overlapping evidence trajectories, despite the fact that the former has twice as many paths carrying the apoptotic signal. Near an XIAP level of 100,000 the two trends diverge as the decrease in evidence values for the mitochondrial activation only network accelerates. This could be explained by XIAP overwhelming the Apoptosome at these higher levels. The apoptosome is an apoptosis inducing complex (via Caspase-3 cleavage) consisting of Cytochrome C, APAF-1, and Caspase-9, and is an inhibitory target of XIAP. As XIAP increases past 125,000 the mitochondrial activation only evidence values fall below even the solo direct caspase values, possibly due to the two-pronged inhibitory action of XIAP at both the Apoptosome and Caspase-3. An interesting observation here is that the addition of the direct caspase pathway to the mitochondrial activation pathway does not appear to increase the likelihood of achieving apoptosis for lower values of XIAP.

Evidence values for the network representing direct caspase activation plus mitochondrial inhibition of XIAP are in red in Figure 2G. Below an XIAP level of 100,000 these values are consistently above the evidence values for the network of the direct caspase plus mitochondrial activation. Note that while direct caspase activation does not appear to increase the likelihood of achieving apoptosis when added to the mitochondrial activation pathway (Figure 2G purple) the amplification of the direct caspase activation via mitochondrial inhibition of XIAP leads to a higher likelihood than solo activation through the mitochondria. This suggests the possibility that the primary mechanism for mitochondrial apoptotic signal amplification, under some conditions, may be inhibition of XIAP, with direct signal transduction a secondary mechanism. Above an XIAP level of 100,000, the direct caspase with XIAP inhibition values drop to levels roughly in line with the values for direct caspase activation plus mitochondrial activation, possibly due to the fact that Smac, the mitochondrial export that inhibits XIAP, is also set to 100,000 molecules per cell. Both, however, remain more likely to attain apoptosis than direct caspase activation alone.

The two subnetworks with the highest evidence values for apoptotic signal execution are the complete network and the isolated mitochondrial pathway (Figures 2E orange and 2F blue). As previously mentioned, both of these networks contain the full mitochondrial pathway implying that this pathway supports resistance to XIAP inhibition of apoptosis. Between XIAP levels of 0 to 100,000 the two trends track very closely, with the mitochondrial only pathway showing a slight but consistent advantage for apoptosis execution. The average difference between an XIAP level of 20,000 and 80,000 is roughly 0.014, meaning we expect the average PARP cleavage to favor the mitochondrial only pathway by about 1.4 percentage points, which may seem unremarkable. Context matters however, and the context here is that the complete

network has potentially twice the bandwidth for the apoptotic signal, namely the addition of the more direct caspase pathway. Together, this raises the possibility that under some conditions the caspase pathway is not a pathway but a sink for the apoptotic signal. In such a scenario, the signal through the caspase pathway would get lost as Caspase-3 is degraded by XIAP. Not until the signal through the mitochondrial pathway begins inhibiting XIAP could the signal proceed. Around the 100,000 level of XIAP the evidence trend for the mitochondrial pathway crosses below that for the complete network. This could be due to the parity with Smac, components of the Apoptosome, or a combination of the two.

Apoptosis signal strength drives signal route through the network

The results in Scaffidi et al. [13] indicate a strong phenotypic dependence on the strength of the apoptosis signal. Here we examine hypotheses made in that work and the interplay between the DISC and XIAP regulatory axes. We once again increase XIAP from 0 to 200,000 molecules in increments of 250, but this time at a low number of DISC complexes by lowering the initial values of both the scaffold protein FADD and the initiator Caspase-8, from 130,000 to 100 molecules per cell. In addition to the Multimodel Exploration Approach used in the previous section, we also use the Pathway Flux Approach using the flux objective function (see Methods). In this way we attain a holistic view of network dynamics that incorporates both, network structure and flux cross-talk from all possible pathways. Additional analysis of caspase and mitochondrial pathway signal flux over a range of values for both XIAP and BCL-2 is displayed in Figure S3 and interpreted in Text S1.

Figure 3A displays the PARP cleavage evidence values along with their low DISC counterparts. Two things are immediately apparent. The PARP cleavage evidence values for the caspase pathway at low number of DISC is lower across the entire range of changes in the number of XIAP molecules. The complete network on the other hand shows almost no difference in low DISC conditions at lower values of XIAP. This supports the hypothesis that mitochondrial involvement is necessary to overcome weak DISC formation and that low number of DISC likely constitutes a Type II trait [13].

Figures 3B and 3C show evidence values for flux through the caspase pathway and complete network for high and low number of DISC, respectively. At higher DISC values, signal flux through the caspase pathway is consistently higher than the flux through the mitochondrial pathway. At lower DISC values the signal flux through the mitochondrial pathway exceeds the flux through the caspase pathway. Our results shed interesting mechanistic observations in the context of a previously proposed hypothesis stating that mitochondrial activation is downstream of caspase activation in Type I cells and upstream in Type II cells. If a weaker initial apoptosis cue does indeed push the signal through the mitochondrial pathway the initial activation of Caspase-8 would be weak and the amplifying activity of the mitochondria would ramp up the signal before Caspase-8 could directly activate Caspase-3. On the other hand, strong initial activation that pushes the signal through the caspase pathway would have the opposite effect. Also notable is the nearly identical trajectories of the total signal flux through the low and high DISC models. The average difference over the range of XIAP was only 0.011 (Table S3). This is consistent with observations that both Type I and Type II cells respond equally well to receptor mediated apoptosis.[13]

Overall these results set up three mechanistic explanations for apoptosis execution. On one end, high DISC formation and low XIAP results in the independence of apoptosis from the mitochondrial pathway. This behavior is consistent with Type I cells like the SKW6.4 cell lines [13]. On the other end of the spectrum is the case with low DISC formation (and by construction low Caspase-8 activity) and near complete dependence on the mitochondrial pathway. Such behavior is consistent with Type II cells like Jurkat [13]. In between these two extremes is the case where DISC formation, and subsequent Caspase-8 activation, is high but apoptosis is still dependent on mitochondrial activity. Such behavior is consistent with MCF-7 cell that are known to have traits of both phenotypes [13].

Bayes factor trends and XIAP influence on Type I/II apoptosis phenotype

Model selection methods typically calculate the evidence ratios, or Bayes factors to choose a preferred model and estimate the confidence of that choice [58, 59]. When comparing the changes in the evidence of an outcome as regulatory conditions change, the changes in the evidence ratios can provide additional information about changing network dynamics under regulatory perturbations. To characterize the effect of XIAP on the choice of apoptotic phenotype, Type I or II, we calculated the evidence ratios (Figure 4A), for each value of XIAP between the caspase pathway and both the complete network and mitochondrial pathway with a fully active mitochondrial pathway. In these calculations, the denominator represents the caspase pathway so that higher values favor a need for mitochondrial involvement. An interesting feature of both the complete and mitochondrial evidence ratio trends is the peak and reversal at a moderate level XIAP (Figure 4B). This reflects the initially successful inhibition of the caspase pathway that decelerates relatively quickly as XIAP increases, and a steadier rate of increased inhibition on networks that incorporate the mitochondrial pathway. The ratios peak between 45,000 and 50,000 molecules of XIAP (more than double the value of its target molecule Caspase-3 at 21,000) and represent the optimal level of XIAP for the requirement of the mitochondrial pathway and attainment of a Type II execution. Given the near monotonic decline of the evidence trends of both pathways, representing increasing suppression of apoptosis, the peak and decline in the evidence ratios could represent a shift toward complete apoptotic resistance. Our results therefore complement the observations in Aldridge et al. where a similar outcome was observed experimentally [60].

A common technique to study apoptosis is to knockdown Bid, overexpress Bcl-2, or otherwise shut down MOMP induced apoptosis through mitochondrial regulation. This strategy was used in Jost et al. [14] to study the role of XIAP in apoptosis and in the work of Aldridge et al. to explore Type I vs Type II execution in different cell lines [59]. Taking a similar approach, we set Bcl-2 levels to 328,000 molecules per cell, in line with experimental findings [47], to suppress MOMP activity and recreated the evidence and ratios landscapes (Figures 4C and 4D, Table S5). Under these conditions the evidence trend for the mitochondrial pathway drops well below that of the caspase pathway, which is reflected in the Bayes factor trend as a shift into negative values and indicating that the caspase pathway is favored. The evidence trend for the complete network under MOMP inhibition is shifted closer to that for the caspase pathway at higher concentrations of XIAP but the Type II pathway continues to dominate throughout the full range of XIAP. The peak for the associated Bayes factor trend is flattened as the number of XIAP increases, suggesting that increasing XIAP levels are less likely to induce a transition to a Type II

phenotype in a system with an already hampered mitochondrial pathway. We note that complete inhibition of MOMP would result in uninformative mitochondrial pathway evidence values. The evidence trend for the complete network would be indistinguishable from that for the caspase pathway alone and the complete/caspase ratio trend would simply flatline. However, our analysis shows that isolation of active biologically relevant subnetworks and direct comparison under changing molecular regulator conditions using trends in Bayesian evidence enables the extraction of information regarding the pathway interactions and differential network dynamics.

Precision vs computational cost

Increasing the precision of the evidence estimates, and tightening the evidence trendlines, is accomplished by increasing the number of live points in the nested sampling algorithm. The trade-off is an increase in the number of evaluations required to reach the termination of the algorithm and an accompanying increase in total computation time. Figures 5A and 5B display the required number of evaluations for the caspase pathway and complete network at population sizes of 500, 1000, 2000, 4000, 8000, and 16,000, when run with the PARP cleavage objective function. For both models the number of evaluations roughly doubles for every doubling in population size. Of note here is the higher number of required evaluations for the lower parameter model. The caspase pathway has only 22 parameters and required an average of 64,612 evaluations at a population size of 16,000 while the complete network, with its 56 parameters required only 53,652 evaluations, on average (Table S6). Figures 5C and 5D are the average estimated errors calculated by the MultiNest algorithm over each population size for the caspase and complete networks respectively. As expected, error estimates fall roughly as $n^{-1/2}$ [61], signifying clear diminishing returns as the number of live points is increased. The average CPU process times, as estimated by Python's `time.clock()` method, are given in Figures 5E and 5F for the caspase and complete networks respectively. Despite the greater number of required evaluations for the caspase network the average clock times for the complete network is significantly higher. At a population of 16,000 the caspase network had an average clock time of 11,964 seconds compared to 76,981 for the complete network. The difference is due to the greater simulation time for the much larger complete model. Ultimately, the choice of population size for the methods we have laid out here will depend on the networks to be compared, the objective function, and how well the evidence trends must be resolved in order to make inferences about network dynamics. For example, at a population size of 500 the evidence trend for the caspase pathway is clearly discernable from the mitochondrial pathway and the complete network, but the latter two are largely overlapping (Figure S4A). At higher population levels, however, two distinct mitochondrial and complete trends become apparent (Figure SK). If Bayes factor trends are desired then the choice of population size must take into consideration the amplification of the noise from both trends (see Figures S4(B, D, F, H, J, L) for complete/caspase Bayes factor trends).

Discussion

Characterizing information flow in biological networks, the interactions between various pathways or network components, and shifts in phenotype upon regulatory perturbations is standing challenge in molecular biology. Although comparative analysis of signal flow within a network is possible with current computational methods, the dependence of physicochemical models on unknown parameters makes the computational examination of each network component highly dependent on costly experimentation.

To take advantage of the enormous amount of existing knowledge encoded in these physicochemical networks without the dependence on explicit parameter values we take a probabilistic approach to the exploration of changes in network dynamics. By integrating an objective function that represents a simulated outcome over parameter distributions we obtain the likelihood of attaining that outcome given the available information about the signaling pathways. Qualitative exploration of network behavior for various in silico experimental setups and regulatory conditions are then attainable without explicit knowledge of every parameter value. We demonstrate the utility of the method when applied to the regulation of extrinsic apoptosis. Networks that incorporate an active mitochondrial pathway displayed a higher resistance to apoptotic inhibition from increasing levels of XIAP, consistent with experimental evidence that XIAP induces a Type II phenotype [14]. Also in line with experimental evidence [13] are the results that suggest low/high signal initiation is consistent with Type II/I phenotype respectively and that both types achieve apoptosis equally well.

A potential limitation of a Bayesian approach to study network dynamics could be the computational cost. A number of factors affect the run time of the algorithm including size of the model, the objective function, and the desired precision. Fortunately, reducing the resolution (the number of sets of initial values for which an evidence value is estimated) and the precision (the population size) can drastically reduce the cost and in many cases the method will still be viable. One aspect of the method that is severely restrictive is the number of model components that can be varied in the same run since the computational cost increases exponentially with each additional variable. Reasonable parameter ranges must also be chosen. Information regarding the parameters can be incorporated into the evidence calculations by adjusting the range and shape of the priors. Here we used generic but biologically plausible ranges with uniform distributions and produced results that were qualitatively consistent with previous experimental results. We note, however, that our results make mechanistic inferences from model experiments given existing data over a period of weeks rather than the months or years that would be required to attain this information with experimental approaches. Our results therefore support Bayesian approaches as a suitable complement to experimentation and a shift from purely deterministic models with a single optimum parameter set to a probabilistic understanding of mechanistic models of cellular processes.

Conclusions

In this paper we have developed a probabilistic approach to the qualitative analysis of the network dynamics of physicochemical models. It is designed to incorporate all available knowledge of the reaction topology, and the parameters on that topology, and calculate the likelihood of achieving an outcome of interest. Inferences on network dynamics are then made by repeating this calculation under changing regulatory conditions and various in silico experiments. We tested the method against a model of the extrinsic apoptosis system and produced results that were consistent with several lines of experimental research. To our knowledge this is the first attempt at a probabilistic analysis of network dynamics for physicochemical models. We believe this method will prove valuable for the large-scale exploration of those dynamics, particularly when parameter knowledge and data are scarce.

Acknowledgements

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Supporting information

Table S1

Table S2

Table S3

Table S4

Table S5

Table S6

Figures S1-S4

Figures Legends:

Figure 1. **General workflow for the analysis of network dynamics using trends in Bayesian evidence.** The target network is first deconstructed into all relevant subnetworks. A model for each subnetwork and each incrementing set of regulatory conditions is then created and passed to an algorithm for estimation the Bayesian model evidence. The evidence is calculated on a user-defined objective function, describing signal transduction through the network, and over a range of parameter values (the prior). The evidence trends over changing regulatory conditions are then compared to make qualitative inferences regarding network dynamics. In an alternative method, the full model is retained, but the objective function is targeted to different pathways. Inferences on network dynamics can again be made from the trends in the evidence calculations.

Figure 2. Extrinsic apoptosis subnetworks and Bayesian evidence for achieving apoptosis. (A) The direct caspase subnetwork. (B) The direct caspase + mitochondrial activation subnetwork. (C) The direct caspase + mitochondrial inhibition of XIAP subnetwork network. (D) The mitochondrial activation subnetwork. (E) The complete network. (F) the mitochondrial subnetwork. (G) The trends in Bayesian evidence for each of the networks in (A)-(F) over a range of values the apoptosis inhibitor XIAP and for an objective function that computes the proportion of PARP cleavage (a proxy for cell death) at the end of a simulated run.

Figure 3. Evidence values for PARP cleavage and pathway flux at low and high DISC values. (A) Evidence values for PARP cleavage for the caspase pathway and complete network under both low and high DISC conditions (100 and 130,000 molecules per cell of FADD and Caspase-8 respectively). (B) Signal flux through both pathways as well as the total signal flux for high DISC values. (C) Signal flux through both pathways as well as the total signal flux for low DISC values.

Figure 4. Evidence ratio trends under increasing levels of the apoptotic inhibitor XIAP for an inhibited and uninhibited mitochondrial pathway. (A) Evidence trends for the caspase pathway (green), mitochondrial pathway (blue), and complete network (orange) with no MOMP inhibition. (B) Trends for the mitochondria/caspase (blue) and the complete/caspase (orange) evidence ratios from the trends in (A). (C) Evidence trends for the caspase pathway (green), mitochondrial pathway (blue), and complete network (orange) with MOMP inhibitory protein BCL-2 at 328,000 mol. per cell. (D) Trends for the mitochondria/caspase (blue) and the complete/caspase (orange) evidence ratios from the trends in (C).

Figure 5. Precision vs. computational cost. (A) and (B) Average number of evaluations before termination of the MultiNest algorithm over a range of population sizes for the caspase pathway and complete network respectively. (C) and (D) Average of error estimates from MultiNest for each population size and the caspase and complete networks. (E) and (F) Average estimated CPU clock time over each population size for the caspase and complete networks respectively. *MultiNest was unable to estimate the error at XIAP = 0.

Box 1. Extrinsic apoptosis execution.

Extrinsic apoptosis is a receptor mediated process for programmed cell death. The Type I/II phenotypes for the extrinsic apoptosis system were first described by Scaffidi et al. [13]. In that work they examined several cell lines and classified them into those that required the mitochondrial pathway to achieve apoptosis (Type II) and those that don't (Type I). They made several interesting conclusions. They found that Type II cells had relatively weak DISC formation, that both phenotypes responded equally well to receptor mediated cell death, that there was a delay in caspase activation in Type II cells, and that caspase activation happened upstream of mitochondrial activation in Type I cells and downstream in Type II. More recently, XIAP has also been put forth as a critical regulator in the choice of apoptotic phenotype. In Jost et al. [14] they examined hepatocytes (Type II cells) and lymphocytes (Type I cells) under different conditions to examine the role XIAP plays in Type I/II determination. They made several observations upon Fas ligand or Fas-antibody induced apoptosis such as higher levels of XIAP in Type II cells and higher caspase effector activity in XIAP/Bid deficient mice versus apoptosis resistant Bid-only knockouts. In all, they concluded that XIAP is the key regulator that determines the choice of pathway.

Extrinsic apoptosis is initiated when a death inducing member of the tumor necrosis factor (TNF) superfamily of receptors (FasR, TNFR1, etc.) is bound by its respective ligand (FasL, TNF-α, etc.), setting off a sequence biochemical events that result in the orderly deconstruction of the cell [15]. The first stage of this sequence is the assembly of the DISC at the cell membrane ① and the subsequent activation of Caspase-8. Upon ligand binding and oligomerization of a receptor such as FasR or TRAIL, an adapter protein, like FADD (Fas-associated protein with death domain), is recruited to the receptors cytoplasmic tail [16, 17, 18]. FADD, in turn, recruits Caspase-8 via their respective death effector domains (DEDs), thus completing DISC formation [17, 18]. Other DISC components could also be included here, such as the regulator cFlip [19]. Once recruited, proximal Procaspase-8 monomers dimerize, inducing their autoproteolytic activity and producing active Caspase-8 [20, 21, 22].

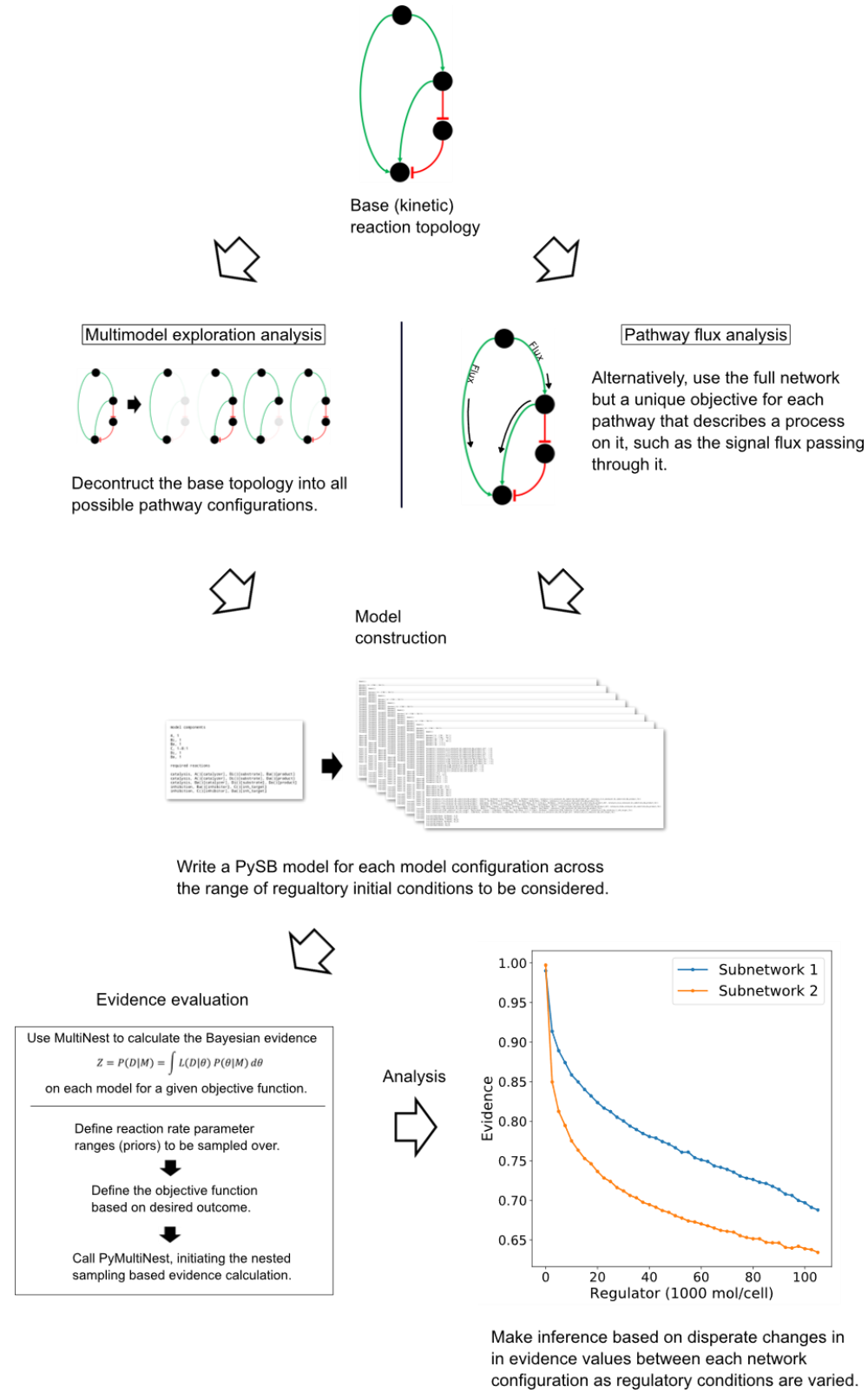
After Caspase-8 activation the apoptotic signal can progress down two distinct pathways that both lead to the activation of Caspase-3 and the ensuing proteolysis of downstream targets. One pathway consists of a caspase cascade in which active Caspase-8 directly cleaves and activates Caspase-3 ② [23], while another, more complex pathway is routed through the mitochondria. In the mitochondrial pathway Caspase-8 cleaves the pro-apoptotic Bcl-2 family protein Bid in the cytosol, which then migrates to the mitochondria ③ where it initiates mitochondrial outer membrane permeabilization (MOMP) and the release of pro-apoptotic factors that lead to Caspase-3 activation [24, 25].

MOMP has its own set of regulators that govern the strength of apoptotic signaling through the mitochondria ④. After Caspase-8 activated Bid, (tBid), migrates to the mitochondria it activates proteins in the outer mitochondrial membrane, such as Bax, that subsequently self-aggregate into membrane pores and allow exportation of Cytochrome-c and Smac/DIABLO to the cytosol [26]. Bid and Bax are examples of pro-apoptotic proteins from the Bcl-2 family, all of which share BH domain homology [27]. Other members of this family act as MOMP regulators; the anti-apoptotic Bcl-2, for example, binds and inhibits both Bid and Bax while the pro-apoptotic Bad similarly binds and inhibits its target, Bcl-2 [28, 29, 30, 31]. Many other pro- and anti-apoptotic members of the Bcl-2 family have been discovered and together regulate MOMP [32].

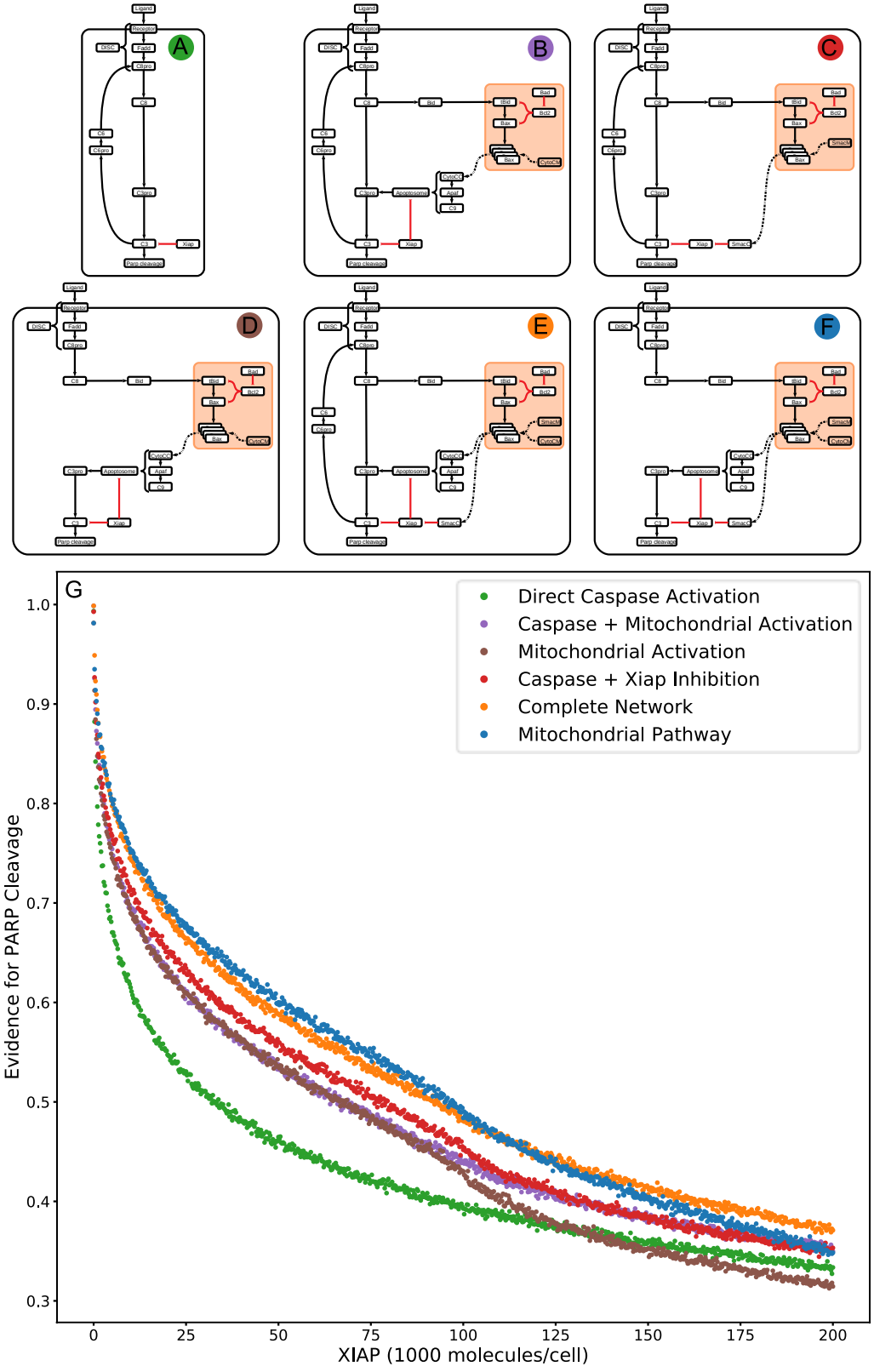
Regardless of which pathway is chosen, the intermediate results are Caspase-3 activation and subsequent cleavage of PARP ⑧, a proxy for cell death in the analyses here [33, 34]. XIAP (X-linked inhibitor of apoptosis protein) is an inhibitor of Caspase-3 and has been proposed to be a key regulator in determining the Type I/II apoptotic phenotype of a cell [35]. XIAP sequesters Caspase-3 but also contains a ubiquitin ligase domain that directly targets Caspase-3 for degradation. The inhibitor also sequesters and inhibits the Caspase-3 activating Caspase-9 residing within the apoptosome complex [36, 37, 38]. Apoptosome formation is initiated by Cytochrome-c exported from the mitochondria during MOMP ⑤. Cytochrome-c induces the protein APAF-1 to oligomerize and subsequently recruit and activate Caspase-9, thus forming the complex [39]. Another MOMP export, the protein Smac/DIABLO ⑥, binds and inhibits XIAP, working in tandem with Cytochrome-c to oppose XIAP and carry out the apoptosis inducing activity of the Type II pathway [40]. Finally, Procaspase/Caspase-6 constitutes a feed forward loop between Caspase-3 and Caspase-8 ⑦ [41].

Figures:

Figure 1.



790 **Figure 2.**



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792 **Figure 3.**

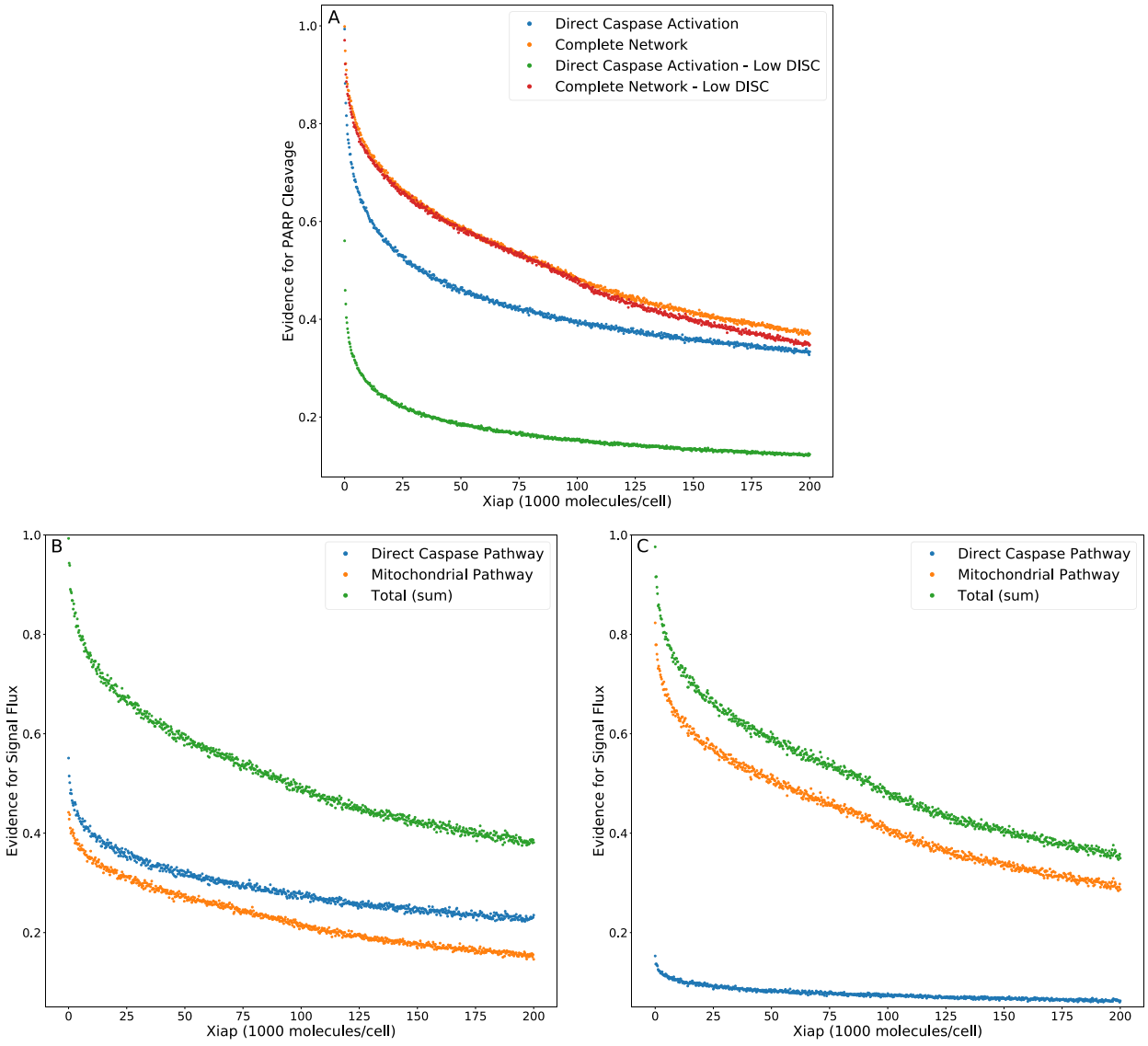

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Figure 4.

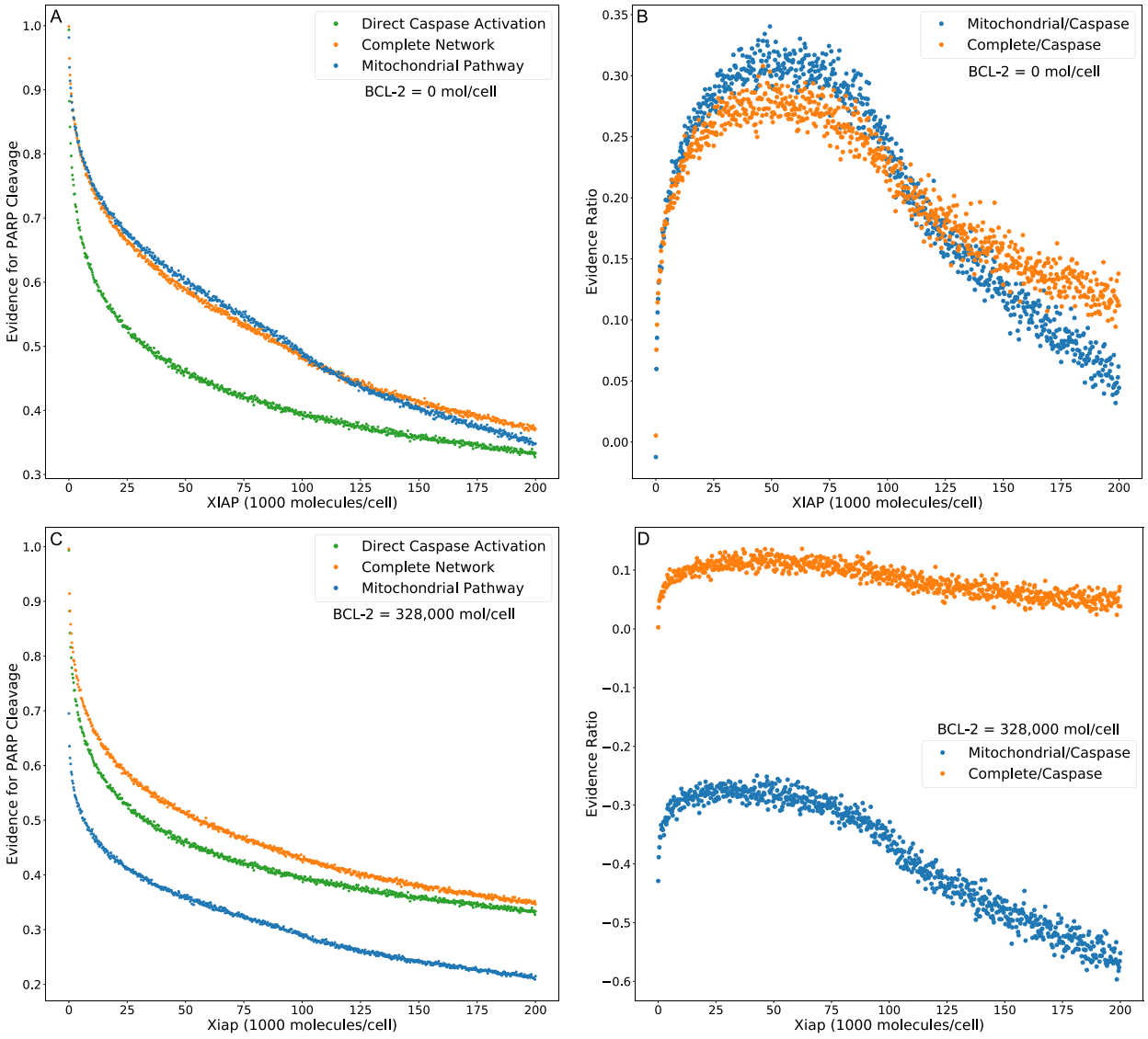
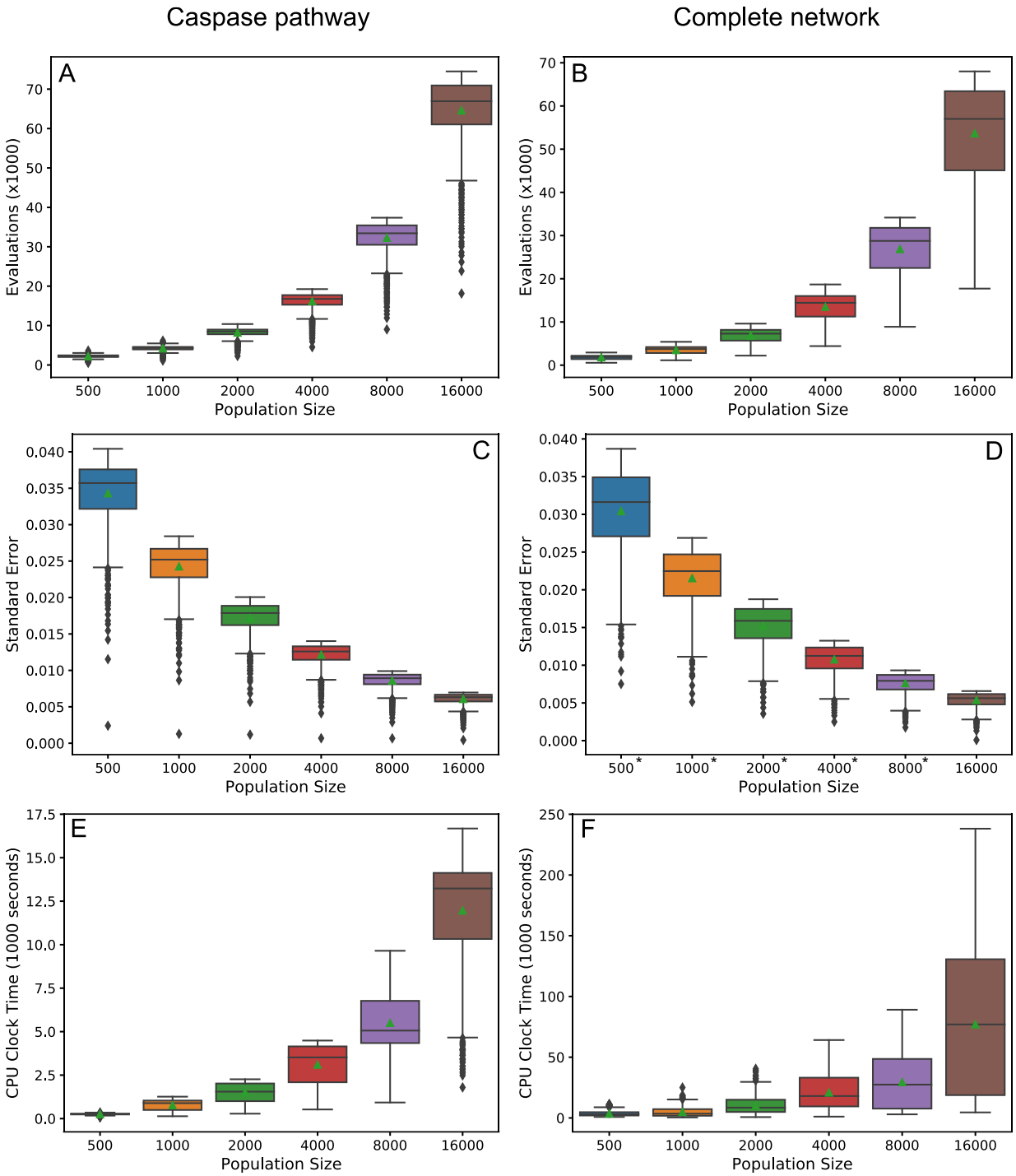


Figure 5.



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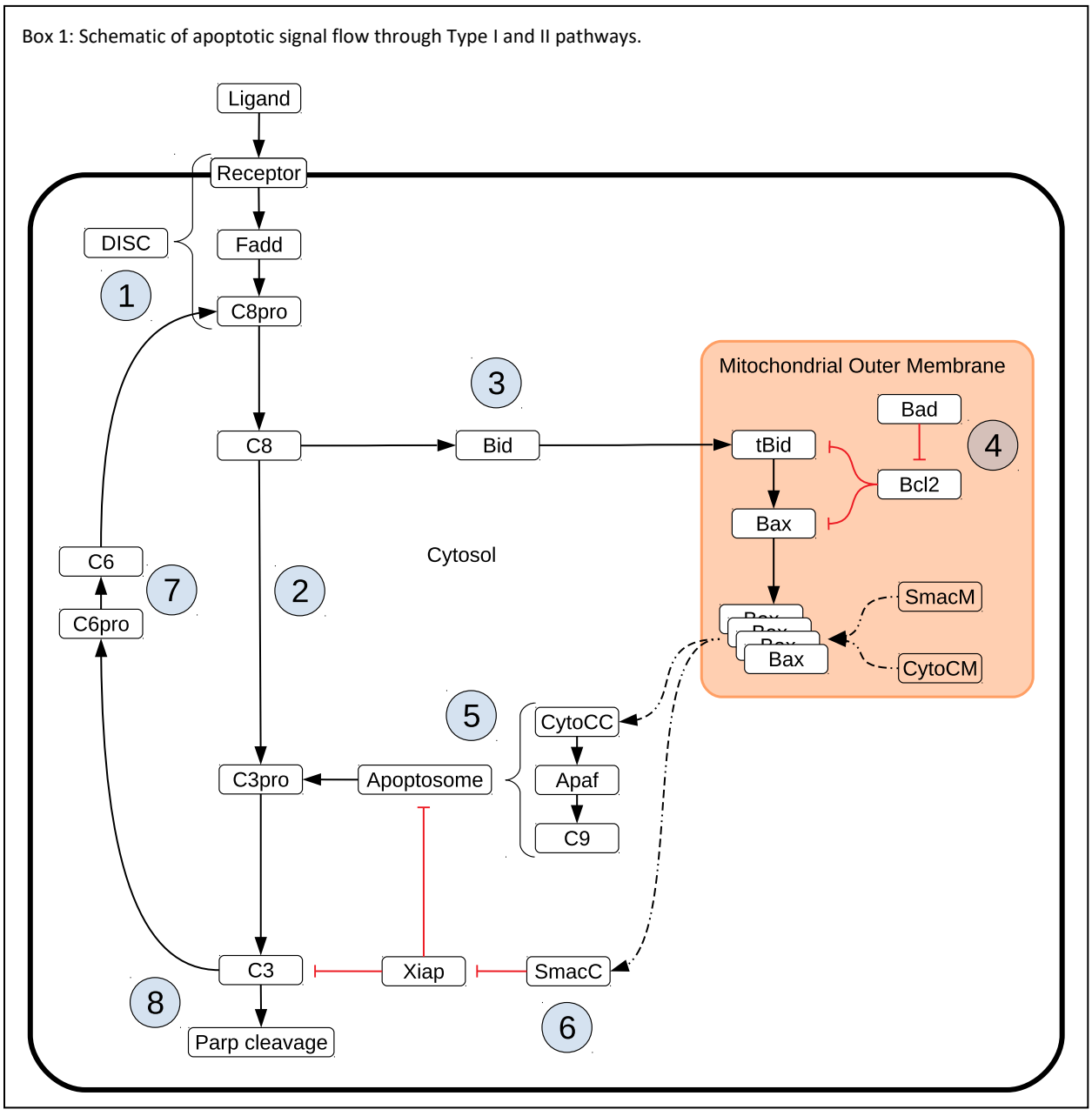
810 **Box 1.**



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