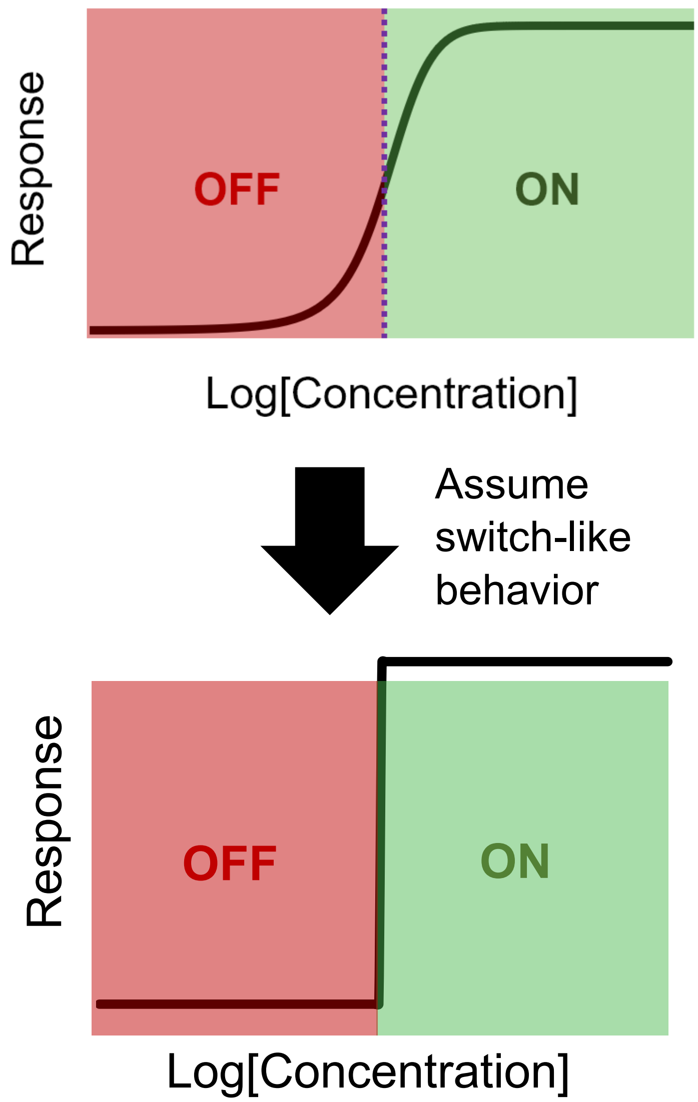


Figure 1. When considering how proteins can be considered on or off, assume that below a set value the concentration of protein in a system is insufficient to evoke a physiological response. When the protein accumulates to levels above that value, a response is observed. To incorporate molecular components into a model Boolean, molecular kinetics are simplified to a switch like function with either an on or off state.

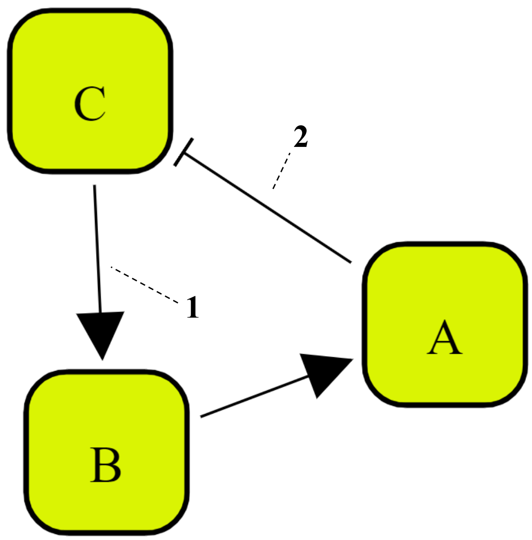


Figure 2. A three node Boolean network. Arrows leading from one node to another represent an identity edge, whereas flat-ended arrows represent an inverse edge. 1) The relationship between node C and B is described by the identity edge: that after a timestep, node B will have the same state as node C at the prior timestep. 2) The relationship between node A and C is described by an inverse edge: after a timestep node C will be in the opposite state of what node A at the prior timestep.

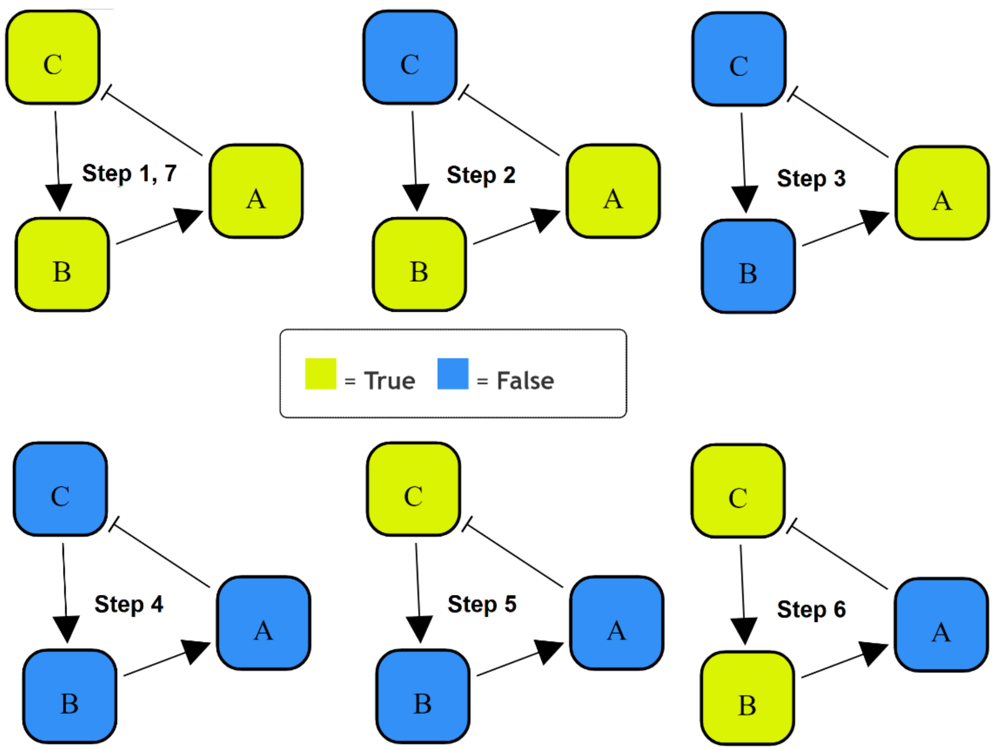


Figure 3. When the starting states of the network are set to true, allowing the three node Boolean network to iterate through states results in an infinite cycle, termed limit cycle, where the cycle reaches its beginning state after every 6 iterations.

A

C = A and not B		
A	B	C
TRUE	TRUE	FALSE
TRUE	FALSE	TRUE
FALSE	TRUE	FALSE
FALSE	FALSE	FALSE

B

C = A or not B		
A	B	C
TRUE	TRUE	TRUE
TRUE	FALSE	TRUE
FALSE	TRUE	FALSE
FALSE	FALSE	TRUE

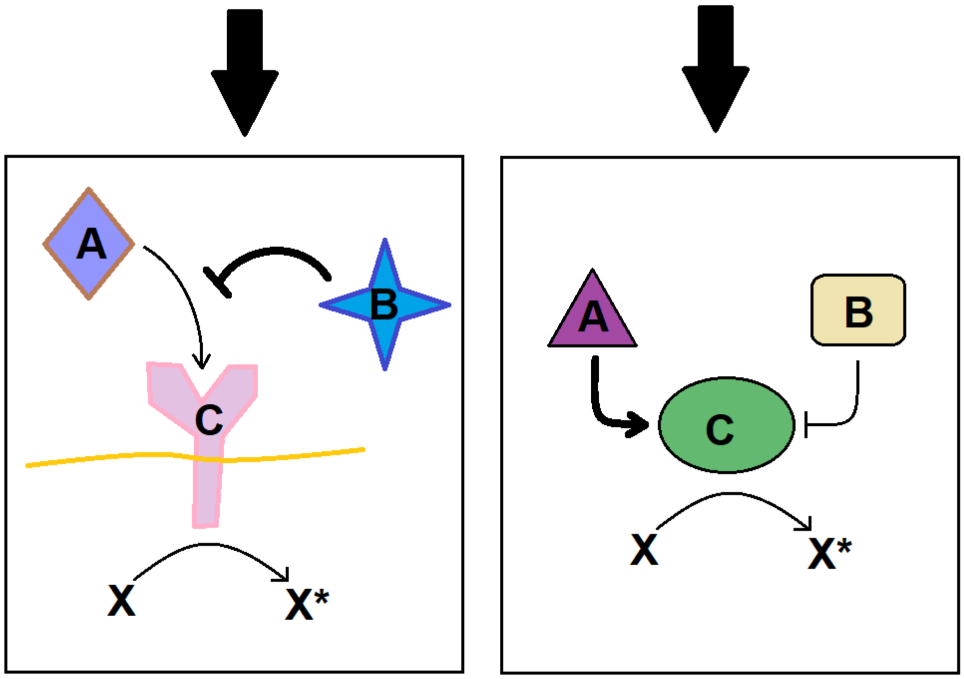


Figure 4. Using the 'not' operation with the 'and' and 'or' gates allows behavior like that seen in intracellular environments. A) Replicates a relationship where the inhibitor B is dominant over agonist A. The receptor C will not generate product X* from X unless agonist A is present and there is no inhibition from B. B) Replicates a constitutively active enzyme C converting X to X* that is under control of an activator A that achieves its action through blocking the action of inhibitor B. In this case, only when no activator is present will enzyme C cease activity, so activator A is dominant over inhibitor B.

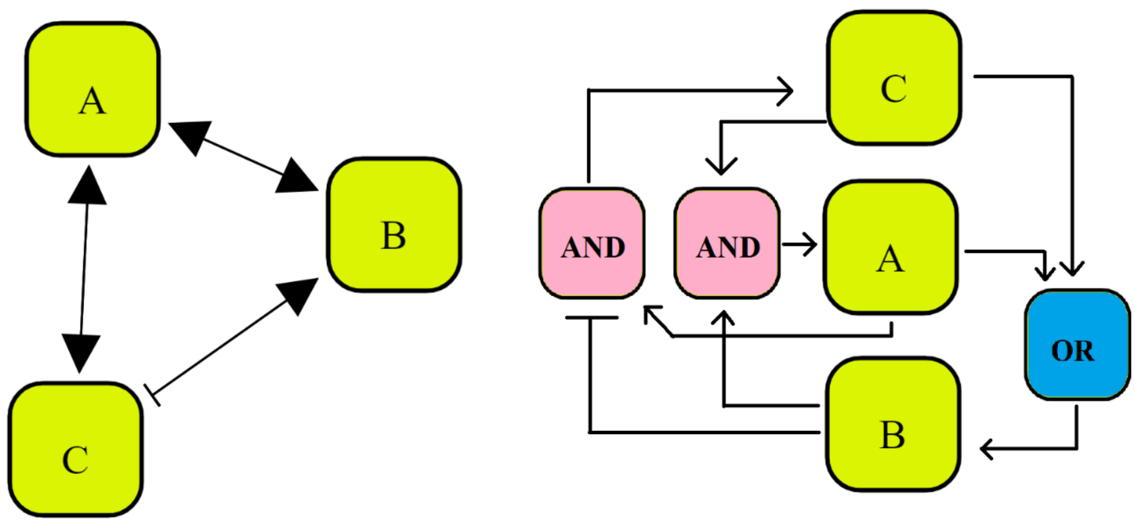
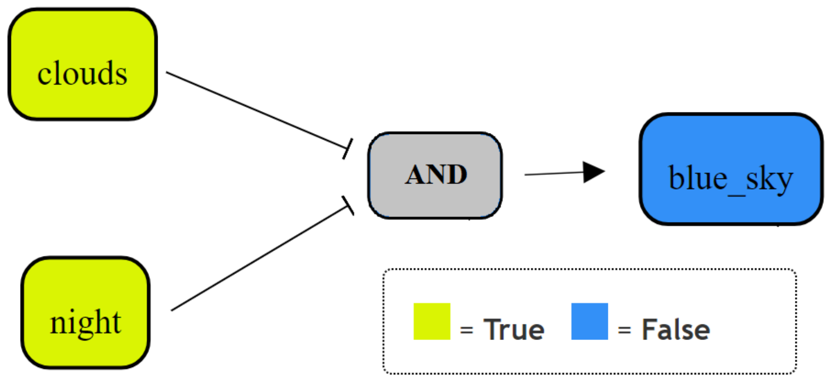


Figure 5. Two representations of the same Boolean network. Which is clearer?

A



B

Node	Rule
blue_sky	blue_sky^{T+1} = not clouds^{T} and not night^{T}
clouds	clouds^{T+1} = clouds^{T}
night	night^{T+1} = night^{T}

C

Truth Table		
clouds	night	blue_sky
TRUE	TRUE	FALSE
TRUE	FALSE	FALSE
FALSE	TRUE	FALSE
FALSE	FALSE	TRUE

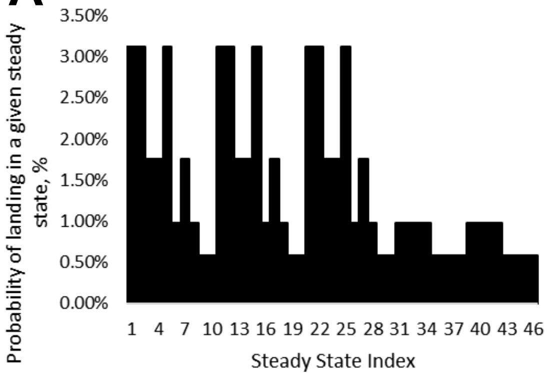
Figure 6. Utilizing the ‘AND’ logical operator allows more complicated behavior to emerge from simple rulesets. A) A three node Boolean network that roughly approximates what weather is conditions are necessary to observe a blue sky. B) A list of rules derived from the flowchart in A that demonstrate in writing the logical rules that govern the state of all nodes. The rules are in the form such that the right-hand side of the equation represents the state of nodes at time T, and on the left-hand side of the equation represent a node at T+1 where T is an abstract time step. C) A truth table that demonstrates that the only way for the node ‘blue_sky’ at T+1 to be TRUE is if the nodes ‘clouds’ and ‘night’ were false at T.

Map Rules	
Number	RULE
Input Rules	
1	ALK* = ALK
2	MDK* = MDK
3	TrkA* = not MYCN
4	NGF* = NGF
5	TrkB* = TrkB
6	BDNF* = BDNF
Internal Rules	
7	DNADamage* = DNADamage
8	p53* = DNADamage and not MDM2
9	MDM2* = p53
10	MAPK* = (MDK and ALK) or Ras
11	p27* = FoxO or not MYCN
12	FoxO* = not AKT
13	AKT* = (MDK and ALK) or (BDNF and TrkB)
14	Ras* = NGF and TrkA
15	MYCN* = (AKT or Ras) and not TrkA
16	MTOR* = AKT
17	IP3* = BDNF and TrkB
Outcome Rules	
18	Differentiation* = MAPK
19	Apoptosis* = (p53 and not AKT) or (TrkA and not NGF)
20	Proliferation* = (not p27 and not p53) or IP3
21	Angiogenesis* = MTOR

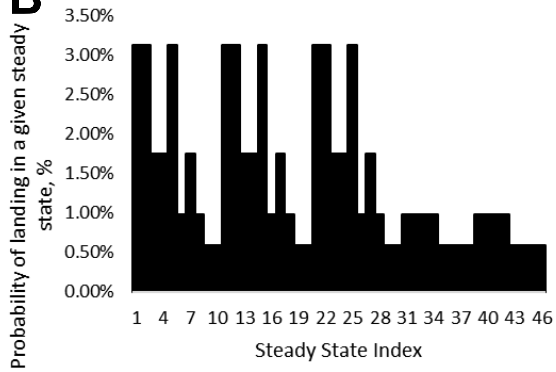
List Rules	
Number	RULE
Input Rules	
1	TrkB* = TrkB
2	NGF* = NGF
3	TrkA* = not MYCN
4	MDK* = MDK
5	ALK* = ALK
6	BDNF* = BDNF
Internal Rules	
7	Ras* = NGF and TrkA
8	AKT* = (BDNF and TrkB) or (MDK and ALK)
9	MYCN* = (Ras or AKT) and not TrkA
10	FoxO* = not AKT
11	p27* = FoxO or (not MYCN)
12	MDM2* = p53
13	p53* = p53
14	IP3* = BDNF and TrkB
Outcome Rules	
15	Differentiation* = Ras or (MDK and ALK)
16	Apoptosis* = (p53 and not AKT) or (TrkA and not NGF)
17	Angiogenesis* = AKT
18	Proliferation*= IP3 or (not p27 and not p53)

Figure 7. The updating rules for the full (A) and reduced (B) models of neuroblastoma proposed by Kasemeier-Kulesa *et al.* (20). Input rules are the rules whose initial states would be determined from samples of patient tissue. Internal rules states are not set based upon patient information but are simulated. Outcome rules indicate the broadly defined outcome for the neuroblastoma of the individual patient. The asterisk on the left-hand side of the update rule equation indicates that both the denoted node is the node that is being updated and is equivalent to x^{t+1}, where x is a rule and t is the timestep. Using asterisk notation allows rules to be specified both for Boolean networks updating with a SU scheme in which x^{t+1} notation is fundamentally correct, and one with a ROAU scheme, where the transitions between one time step and another is less clear.

A



B



C

		Outcome Node 'On' Probability				
Rules Used	Total Nodes	Apoptosis	Proliferation	Angiogenesis	Differentiation	# FPA
List Rules	18	41.80	32.03	43.75	50.78	46
List Rules - MDM2	17	41.80	32.03	43.75	50.78	46
List Rules - MDM2, IP3	16	41.80	32.03	43.75	50.78	46

Figure 8. Removing IP3 and MDM2 nodes from the 18-node model of neuroblastoma proposed by Kasemeier-Kulesa *et al.* (20) creates a model with identical behavior while taking a quarter of the time of the 18-node model to compute. A) A bar graph showing the probability of entering a specific steady state (numbered 1-46) from a random starting state when utilizing the 18-node model from Kasemeier-Kulesa *et al.* B) The same results as (A) but computed using the new 16 node model that has IP3 and MDM2 removed. There was no change in the probability of entering a given steady state between the two models, and the specific steady states (1-46) in each model were verified to be identical. C) A table that shows that reducing the Kasemeier-Kulesa *et al.* model ("List Rules") to an intermediate 17-node, then finally 16-node version had no impact on the on-probability of the four outcome nodes. The 16-node model also had an identical number of fixed-point attractors as the 18-node model. Green shading indicates that the outcome nodes were identical between models.

A

Node	Rule
A	$A^{T+1} = A^{T}$
B	$B^{T+1} = B^{T}$
C	$C^{T+1} = A^{T}$ or B^{T}

C

Node	Rule
A	$A^{T+1} = A^{T}$
B	$B^{T+1} = B^{T}$
C	$C^{T+1} = A^{T}$ or B^{T} or D^{T}
D	$D^{T+1} = D^{T}$

B

Truth Table		
A	B	C
TRUE	TRUE	TRUE
TRUE	FALSE	TRUE
FALSE	TRUE	TRUE
FALSE	FALSE	FALSE

D

Truth Table			
A	B	D	C
TRUE	TRUE	TRUE	TRUE
TRUE	TRUE	FALSE	TRUE
FALSE	TRUE	FALSE	TRUE
FALSE	TRUE	TRUE	TRUE
FALSE	FALSE	TRUE	TRUE
FALSE	TRUE	FALSE	TRUE
TRUE	FALSE	FALSE	TRUE
FALSE	FALSE	FALSE	FALSE

Figure 9. Adding nodes to the Boolean network increases the total number of possible outcomes for node C by 2^{N-1} Where N is the number of nodes but did not increase the number of system states where node C was True. A) The logical rules governing the three node Boolean network. B) Utilizing the 'OR' logical operator in this three node Boolean network results in three out of four possible states for node C to be True. C) A four node Boolean network expanded from panel A. D) Adding another node to the network and incorporating that node into the rule governing node C increases the number of possible states for node C by a factor of two. Note that the total possible number of states in a Boolean network with N nodes is 2^{N} .

$$MTOR = AKT$$

$$Angiogenesis = MTOR$$

$$MTOR = AKT = Angiogenesis$$

$$Thus,$$

$$AKT = Angiogenesis$$

Equation 1. Removal of the MTOR node from Figure X1-A using Boolean algebra.

$$Differentiation = MAPK$$

$$MAPK = (MDK\ and\ ALK)\ or\ Ras$$

$$Differentiation = MAPK = (MDK\ and\ ALK)\ or\ Ras$$

$$Thus,$$

$$Differentiation = (MDK\ and\ ALK)\ or\ Ras$$

Equation 2. Removal of the MAPK node from Figure X1-A using Boolean algebra.

$$IP3 = BDNF\ and\ TrkB$$

$$Proliferation = IP3\ or\ (not\ p27\ and\ not\ p53)$$

$$Thus,$$

$$Proliferation = (BDNF\ and\ TrkB)\ or\ (not\ p27\ and\ not\ p53)$$

Equation 3. Substitution of (BDNF and TrkB) for IP3 is justified by Boolean algebra.