

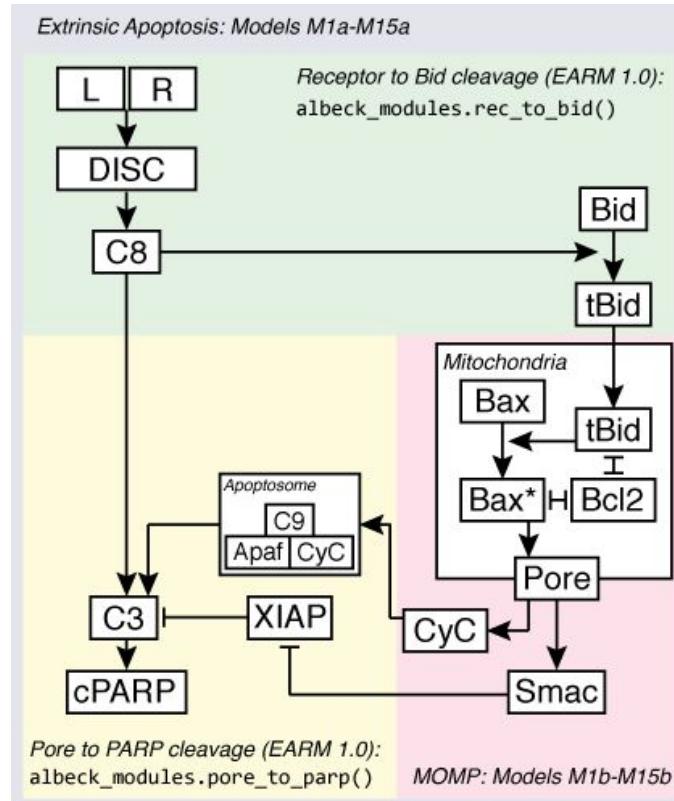
Usage of Modules

Introduction to EARM modules

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EARM (extrinsic apoptosis reaction model)

- Programmed cell death mediated by death receptor
- Ligands dock at the death inducing signaling complex (DISC)
- Signals lead to permeabilization of the outer mitochondrial membrane (MOMP)
- MOMP permeabilization is controlled by the Bcl-2 family of proteins
 - BH3-only proteins monitor cellular fitness & signal execution (e.g., Bid)
 - MOMP mediation proteins (e.g., Bax, Bak)
 - Inhibitory proteins for apoptosis (e.g., Bcl2, Bcl-XL, Mcl-1)
- Intermembrane space proteins from the mitochondria are released
- Apoptosis inhibitors are inhibited
- Caspase 3 cleaves PARP



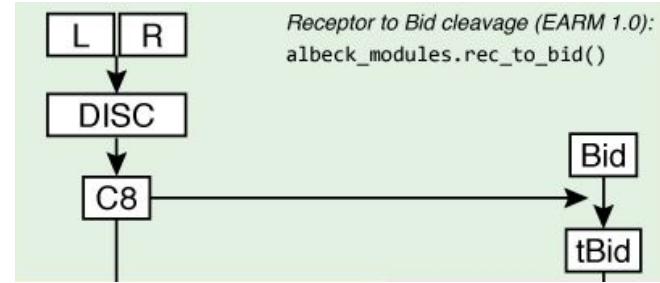
EARM: Receptor to Bid cleavage

- Ligands (L) dock to DISC receptor (R)

```
# L + R <--> L:R --> DISC
# 2-step catalytic-type process, but the "catalyst" is effectively consumed
catalyze_convert(L(), R(), DISC(bf=None), [4e-7, KR, 1e-5])
```
- DISC activates initiator caspases for apoptosis
Caspase 8 (C8)

```
# pro-C8 + DISC <--> DISC:pro-C8 --> C8 + DISC
catalyze(DISC(), C8(state='pro'), C8(state='A'), [KF, KR, KC])
```
- BH3-only proteins (Bid) is truncated by C8

```
# Bid + C8 <--> Bid:C8 --> tBid + C8
catalyze(C8(state='A'), Bid(state='U'), Bid(state='T'), [KF, KR, KC])
```
- tBid translocates to mitochondria
(code in MOMP-section)



EARM: MOMP

- MOMP is the commitment step in apoptosis
- Activated BH3 protein (tBid) bind to one or more of the multidomain Bcl-2 family proteins
- tBid inhibits the anti-apoptotic protein Bcl2
- tBid activates the membrane permeabilizing proteins Bak & Bax
- The pores in the mitochondrial membrane enable intermembrane space proteins to be released in the cytoplasm
- EARM facilitates 15 different types of MOMP models M1b - M15b (details of these modules are described in the later section)

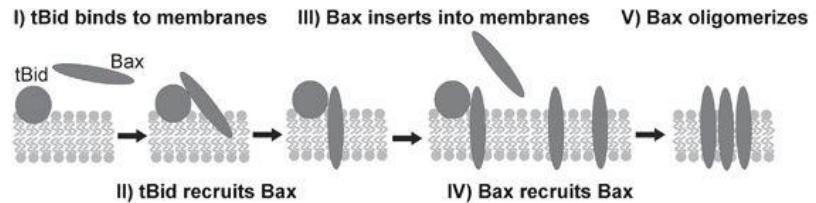
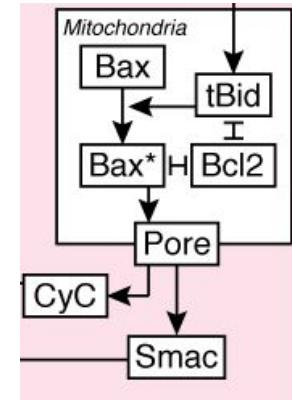


Figure: Andrews et al, Oncogene, 2010



MOMP: Models M1b-M15b

EARM: Pore to PARP cleavage

- Active C8 activates Caspase 3

```
# pro-C3 + C8 <=> pro-C3:C8 --> C3 + C8 CSPS
catalyze(C8(state='A'), C3(state='pro'), C3(state='A')), [1e-7, KR, KC])
```

- Intermembrane space proteins (CytoC and Smac) are activated after released from the mitochondria

```

equilibrate(Smac(bf=None, state='C'), Smac(bf=None, state='A'), transloc_rates)
equilibrate(CytoC(bf=None, state='C'), CytoC(bf=None, state='A'), transloc_rates)

```

- Active CytoC and Smac form apoptosomes; Smac inhibits the X-linked inhibitor of apoptosis protein XIAP

```

# Apaf + cCytoC <=> Apaf:cCytoC --> active Apaf + cCytoC
# active Apaf + pro-C9 <=> Apop
# Apop + pro-C3 <=> Apop:pro-C3 --> Apop + C3
catalyze(CytoC(state='A'), Apaf(state='I'), Apaf(state='A'), [5e-7, KR, KC])
one_step_conv(Apaf(state='A'), C9(), Apop(bf=None), [5e-8, KR])
catalyze(Apop(), C3(state='pro'), C3(bf=None, state='A'), [5e-9, KR, KC])

# Apop + XIAP <=> Apop:XIAP
# cSmac + XIAP <=> cSmac:XIAP
bind(Apop(), XIAP(), [2e-6, KR])
bind(Smac(state='A'), XIAP(), [7e-6, KR])

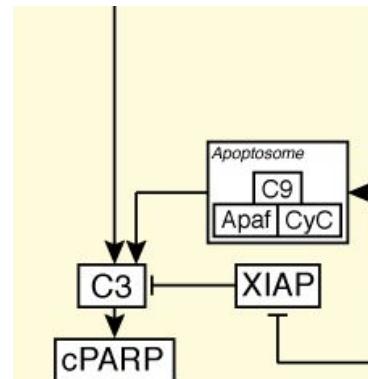
```

- C3 no longer inhibited by XIAP cleaves PARP

```

# XIAP + C3 <=> XIAP:C3 --> XIAP + C3_U CSPS
# PARP + C3 <=> PARP:C3 --> CPARP + C3 CSPS
catalyze(XIAP(), C3(state='A'), C3(state = 'ub'), [2e-6, KR, 1e-1])
catalyze(C3(state='A'), PARP(state='U'), PARP(state='C'), [KF, 1e-2, KC])

```

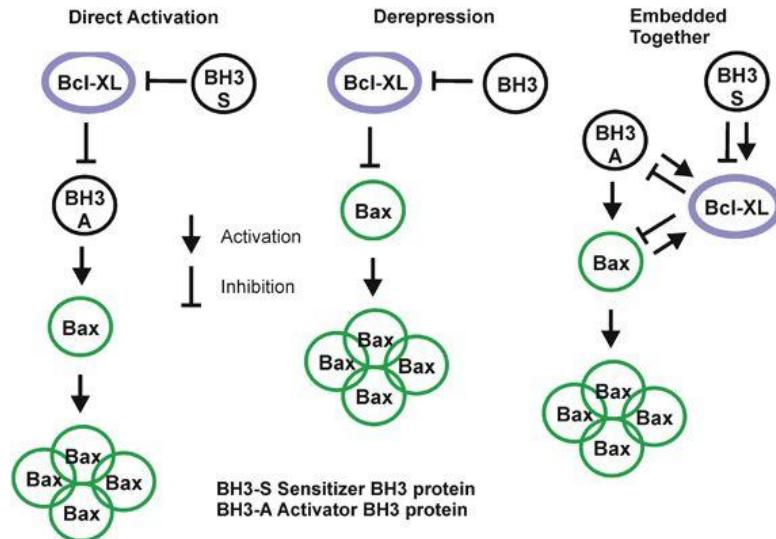


Pore to PARP cleavage (EARM 1.0)
albeck modules.pore to parp()

Use modules for hypothesis testing

In Andrews et al, Oncogene, 2010 the following hypotheses are proposed for MOMP:

- Direct activation
 - Bax/Bak are inactive
 - Bax/Bak must be activated by BH3-only proteins
 - Sensitizers lead to Bax/Bak activation indirectly
- Derepression
 - Bax/Bak are constitutively active
 - Bax/Bak must be repressed by anti-apoptotics
- Embedded together
 - BH3-only proteins can both inhibit & activate functions of anti-apoptotic Bcl-2 proteins

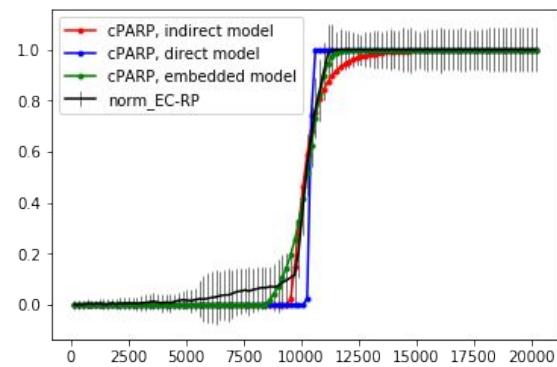
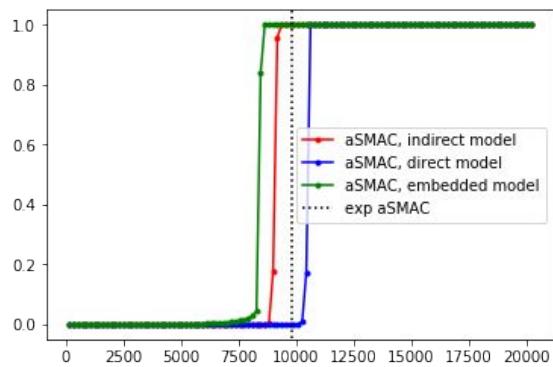
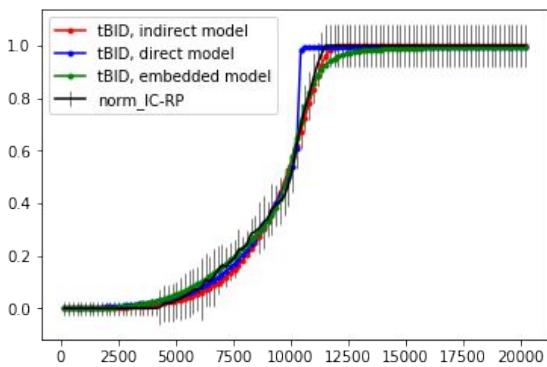


Exercise

(more detailed information about the various modules provided by *earm* as well as the structure of the code can be found in the following pages)

- Use the provided data for the TRAIL drug treatment; the measured quantities are the protein signals
 - IC-RP (tBid)
 - IMS-RP (Smac release)
 - EC-RP (PARP cleavage)
- Load the *lopez_modules* provided by *earm*
 - direct (corresponding to the direct activation hypothesis)
 - indirect (corresponding to the derepression hypothesis)
 - embedded (corresponding to the embedded together hypothesis)
- Use the parameters hardcoded in the model and compare the observables (mBid, aSmac, cPARP) to the experimental data - what is off?
- Use Particle Swarm Optimization (PSO) provided by *simplepso* to train the model to the data
 - the rate constants are re-evaluated to fit the measured data
 - are your PSO results similar to your neighbors? (PSO is a stochastic algorithm)
- Use the new rate constants in your original models and compare again to the experimental data

result with the best likelihood value after 50 PSO runs for each model
best_{indirect}=0.151, best_{direct}=0.401, best_{embedded}=0.155



EARM structure (M1a-M15a)

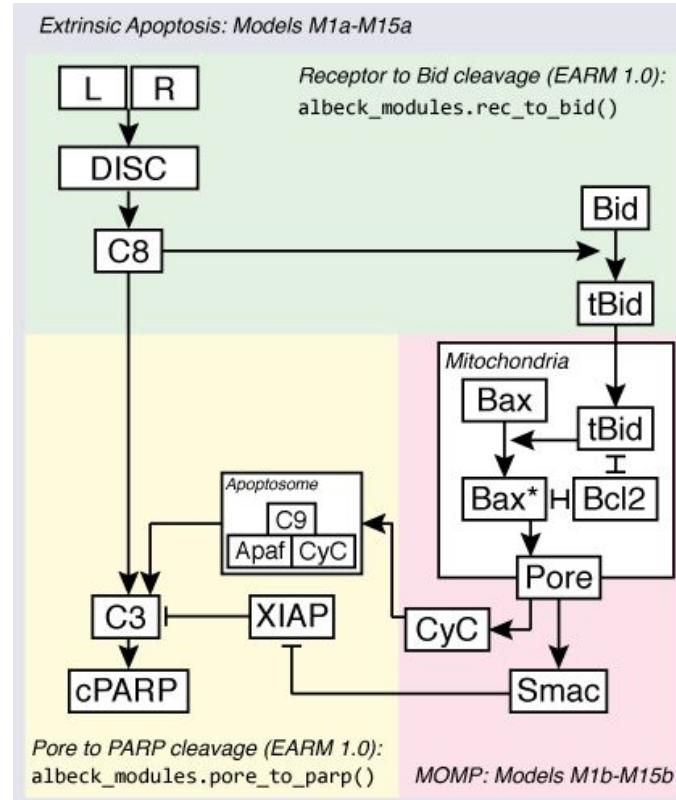
Receptor ligation to Bid cleavage
albeck_modules.rec_to_bid()

Mitochondrial Outer Membrane Permeabilization (MOMP)
Models M1b-M15b

Pore transport to effector caspase activation and PARP cleavage
albeck_modules.pore_to_parp()

Import a model:
from earm.lopez_embedded import model

Documentation: <https://earm.readthedocs.io>



lopez_modules (M1a-M3a)

Load monomers

Albeck: ligand_to_C8_monomers(), apaf1_to_parp_monomers()

Lopez: momp_monomers()

Include upstream & downstream section

Albeck: rec_to_bid(), pore_to_parp()

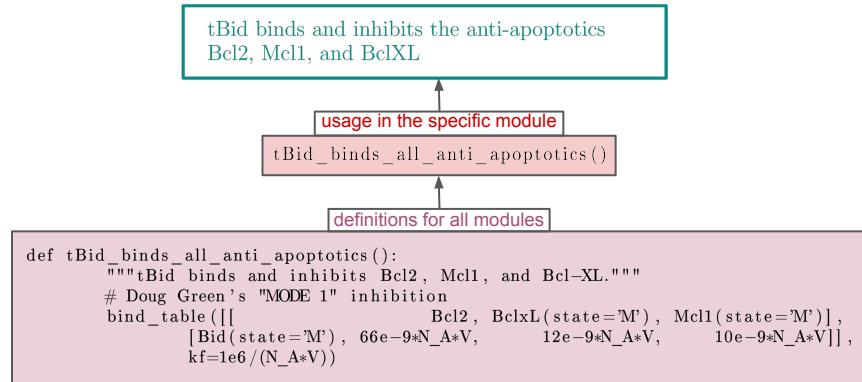
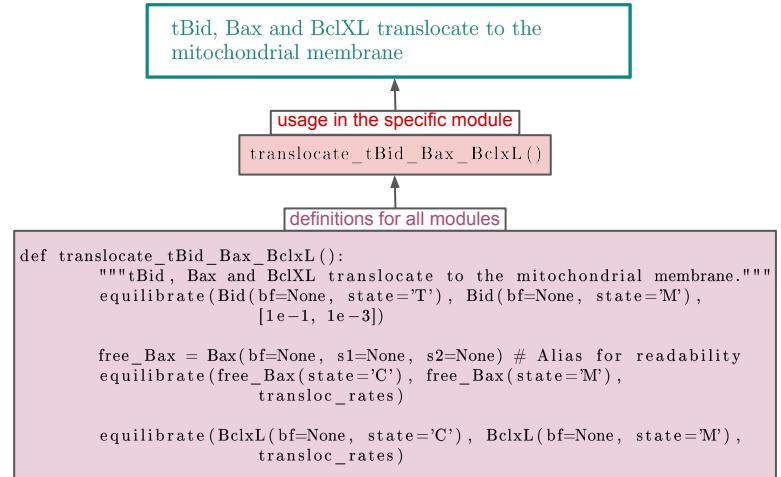
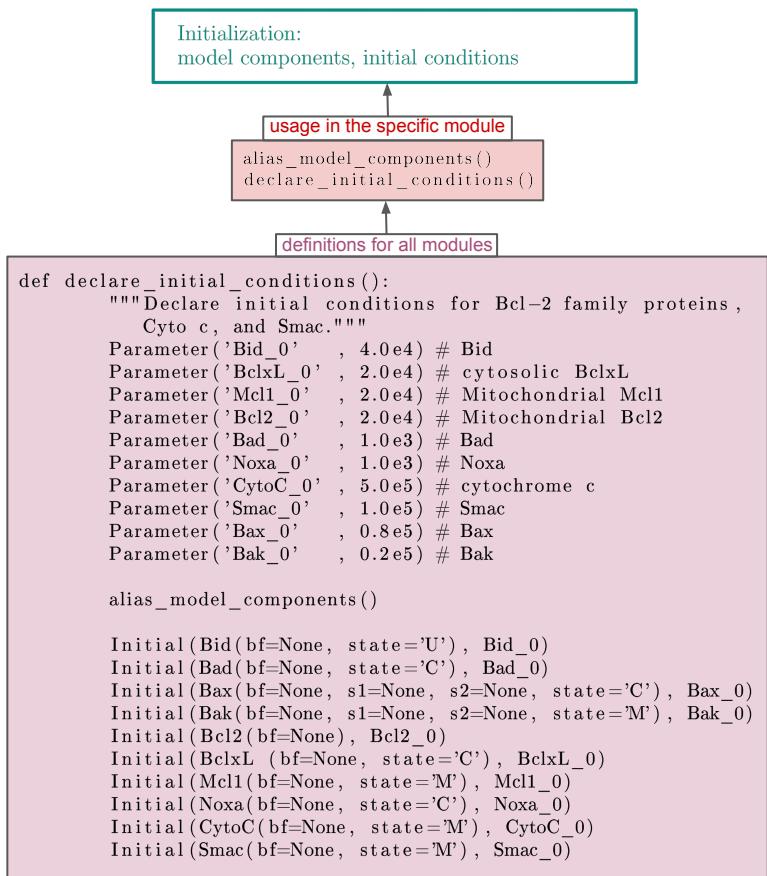
Specify MOMP module

Lopez: embedded() (M1a), direct() (M2a), indirect() (M3a)

Declare observables

Shared: observables()

lopez_modules() legend (used by all lopez_modules)



lopez_modules() legend

(used by all lopez_modules)

```
Pore formation and transport process
usage in the specific module
lopez_poreFormation(do_pore_transport=do_pore_transport)
definitions for all modules

def lopez_poreFormation(do_pore_transport=True):
    """ Pore formation and transport process used by all modules."""
    alias_model_components()

    # Rates
    pore_max_size = 4
    pore_rates = [[2.040816e-04, # 1.0e-6/v**2
                   1e-3]] * (pore_max_size - 1)
    pore_transport_rates = [[2.857143e-5, 1e-3, 10]] # 2e-6 / v?

    #Pore formation by effectors
    assemble_pore_sequential(Bax(bf=None, state='A'), pore_max_size, pore_rates)
    assemble_pore_sequential(Bak(bf=None, state='A'), pore_max_size, pore_rates)

    # CytoC, Smac release
    if do_pore_transport:
        pore_transport(Bax(bf=None, state='A'), 4, CytoC(state='M'), CytoC(state='C'),
                      pore_transport_rates)
        pore_transport(Bax(bf=None, state='A'), 4, Smac(state='M'), Smac(state='C'),
                      pore_transport_rates)
        pore_transport(Bak(bf=None, state='A'), 4, CytoC(state='M'), CytoC(state='C'),
                      pore_transport_rates)
        pore_transport(Bak(bf=None, state='A'), 4, Smac(state='M'), Smac(state='C'),
                      pore_transport_rates)
```

```
sensitizers Bad and Noxa bind anti-apoptotics
Bcl2, Mcl1, and BclXL
usage in the specific module
sensitizers_bind_anti_apoptotics()
definitions for all modules

def sensitizers_bind_anti_apoptotics():
    """Binding of Bad and Noxa to Bcl2, Mcl1, and Bcl-XL."""
    bind_table([[Bcl2, BclXL(state='M'), Mcl1(state='M')], [Bad(state='M'), 11e-9*N_A*V, 10e-9*N_A*V, None], [Noxa(state='M'), None, None, 19e-9*N_A*V]], kf=1e6/(N_A*V))
```

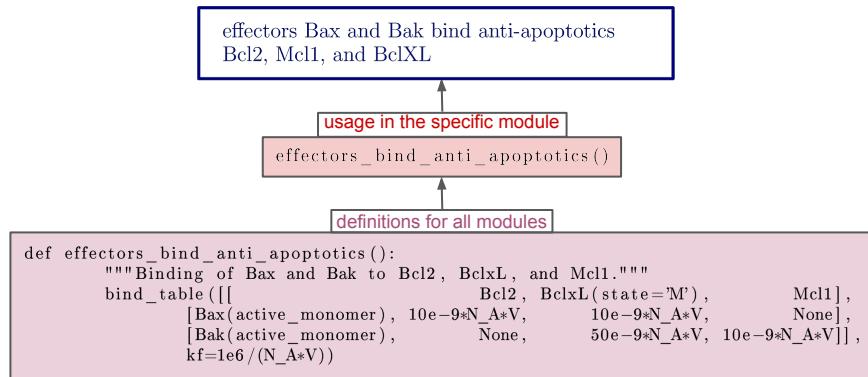
(used by embedded, direct)

```
tBid activates Bax and Bak
usage in the specific module
tBid_activates_Bax_and_Bak()
definitions for all modules

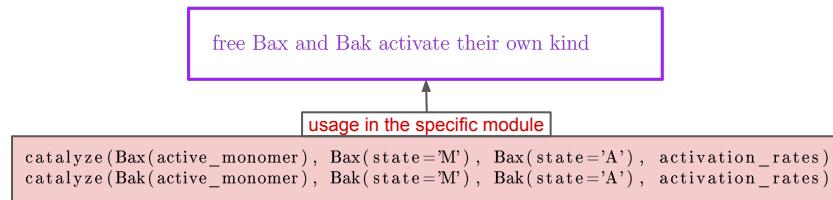
def tBid_activates_Bax_and_Bak():
    """tBid activates Bax and Bak."""
    catalyze(Bid(state='M'), Bax(state='M'), Bax(state='A'), activation_rates)
    catalyze(Bid(state='M'), Bak(state='M'), Bak(state='A'), activation_rates)
```

lopez_modules() legend

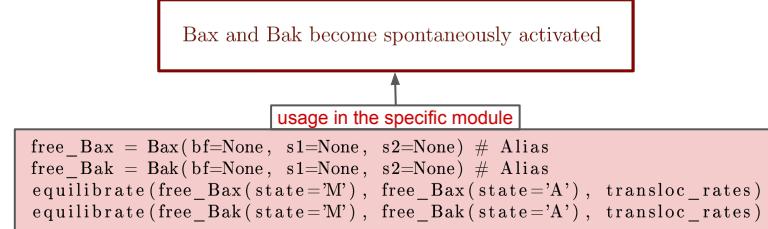
(used by embedded, indirect)



(used by embedded)



(used by indirect)



lopez_modules (M1b-M3b)

Incorporates a larger repertoire of Bcl-2 family members including

one “activator” Bid

two “sensitizers” Bad & Noxa

two effectors Bax & Bak

three “anti-apoptotics” Bcl-2, Bcl-xL, and Mcl-1

Dissociation constants taken from *Certo, M., Del Gaizo Moore, V., Nishino, M., Wei, G., Korsmeyer, S., Armstrong, S. A., & Letai, A. (2006). Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell, 9(5), 351-365. doi:10.1016/j.ccr.2006.03.027*

Affinities of Bak for Bcl-xL and Mcl-1 are taken from *Willis, S. N., Chen, L., Dewson, G., Wei, A., Naik, E., Fletcher, J. I., Adams, J. M., et al. (2005). Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes & Development, 19(11), 1294-1305. doi:10.1101/gad.1304105*

Preferential affinity of Bax for Bcl-2 and Bcl-xL were taken from *Zhai, D., Jin, C., Huang, Z., Satterthwait, A. C., & Reed, J. C. (2008). Differential regulation of Bax and Bak by anti-apoptotic Bcl-2 family proteins Bcl-B and Mcl-1. The Journal of biological chemistry, 283(15), 9580-9586. doi:10.1074/jbc.M708426200*

M1b: lopez_embedded()

- combines elements of both direct and indirect: tBid activates Bax and Bak; the anti-apoptotics bind tBid, sensitizers and Bax and Bak. In addition, Bax and Bak are able to auto-activate
- direct and indirect modes of action, occurring at the membrane.
- anti-apoptotics bind activator tBid:
Doug Green's "MODE 1" inhibition
- anti-apoptotics bind activated effectors:
Doug Green's "MODE 2" inhibition

Initialization:
model components, initial conditions

tBid, Bax and BclXL translocate to the mitochondrial membrane

tBid activates Bax and Bak

free Bax and Bak activate their own kind

tBid binds and inhibits the anti-apoptotics Bcl2, Mcl1, and BclXL

effectors Bax and Bak bind anti-apoptotics Bcl2, Mcl1, and BclXL

sensitizers Bad and Noxa bind anti-apoptotics Bcl2, Mcl1, and BclXL

Pore formation and transport process

M2b: lopez_direct()

- tBid directly activates both Bax and Bak; the anti-apoptotics bind tBid and the sensitizers (Bad and Noxa) but not Bax and Bak
- anti-apoptotics prevent BH3-onlies from activating Bax and Bak
- Bax and Bak require activation to be able to form pores the anti-apoptotics don't inhibit activated Bax and Bak; their only role is to bind BH3-onlies
- anti-apoptotics bind activator tBid
Doug Green's "MODE 1" inhibition

Initialization:
model components, initial conditions

tBid, Bax and BclXL translocate to the mitochondrial membrane

tBid activates Bax and Bak

tBid binds and inhibits the anti-apoptotics Bcl2, Mcl1, and BclXL

sensitizers Bad and Noxa bind anti-apoptotics Bcl2, Mcl1, and BclXL

Pore formation and transport process

M3b: lopez_indirect()

- Bax and Bak are not explicitly activated by tBid, but rather are in an equilibrium between inactive and active states. The anti-apoptotics bind tBid, sensitizers, and Bax and Bak
- Bax and Bak spontaneously form pores without activation; the "activator" tBid binds all of the anti-apoptotics
- Anti-apoptotics bind activator tBid
Doug Green's "MODE 1" inhibition
- Anti-apoptotics bind activated effectors
Doug Green's "MODE 2" inhibition

Initialization:
model components, initial conditions

tBid, Bax and BclXL translocate to the mitochondrial membrane

Bax and Bak become spontaneously activated

tBid binds and inhibits the anti-apoptotics Bcl2, Mcl1, and BclXL

effectors Bax and Bak bind anti-apoptotics Bcl2, Mcl1, and BclXL

sensitizers Bad and Noxa bind anti-apoptotics Bcl2, Mcl1, and BclXL

Pore formation and transport process

albeck_modules (M4a-M8a)

Load monomers

Albeck: all_monomers()

Include upstream & downstream section

Albeck: rec_to_bid(), pore_to_parp()

Specify MOMP module

Albeck: albeck_11b() (M4a), albeck_11c() (M5a), albeck_11d() (M6a), albeck_11e() (M7a),
albeck_11f() (M8a)

Declare observables

Shared: observables()

+ active Bax

albeck_modules (M4b-M8b)

Five variants of MOMP introduced in *Albeck, J., Burke, J. M., Spencer, S. L., Lauffenburger, D. A., Sorger, P. K. (2008). Modeling a Snap-Action, Variable-Delay Switch Controlling Extrinsic Cell Death. PLoS biology, 6(12), e299. doi:10.1371/journal.pbio.0060299'*

M4b: albeck_11b()

Minimal MOMP model

- Bid activates Bax
- Active Bax is inhibited by Bcl2
- Free active Bax binds to and transports Smac to the cytosol

Initialization:
model components, initial conditions

Bid activates Bax

active Bax is inhibited by Bcl2

free active Bax binds to and transports
Smac to the Cytosol

M5b: albeck_11c()

Model incorporating Bax oligomerization

- Bid activates Bax
- Active Bax dimerizes; Bax dimers dimerize to form tetramers
- Bcl2 binds/inhibits Bax monomers, dimers, and tetramers
- Bax tetramers bind to and transport Smac to the cytosol

Initialization:
model components, initial conditions

Bid activates Bax

active Bax dimerizes,
Bax dimers form tetramers

active Bax, Bax dimers and Bax tetramers
are inhibited by Bcl2

Bax tetramers
bind to and transport Smac to the Cytosol

M6b: albeck_11d()

Model incorporating mitochondrial transport

- Bid activates Bax
- Active Bax translocates to the mitochondria
- All reactions on the mito membrane have increased association rates
- Mitochondrial Bax dimerizes; Bax dimers dimerize to form tetramers
- Bcl2 binds/inhibits Bax monomers, dimers, and tetramers
- Bax tetramers bind to and transport Smac to the cytosol

Initialization:
model components, initial conditions

Bid activates Bax
in the Cytosol

active Bax translocates to mitochondria

active Bax dimers and tetramers
form in the mitochondria

active Bax, Bax dimers and Bax tetramers
are inhibited by Bcl2 in the mitochondria

Bax tetramers in the mitochondria
bind to and transport Smac to the Cytosol

M7b: albeck_11e()

Model incorporating mitochondrial transport and pore "insertion"

- Bid activates Bax
- Active Bax translocates to the mitochondria
- All reactions on the mitochondria have increased association rates
- Mitochondrial Bax dimerizes; Bax dimers dimerize to form tetramers
- Bcl2 binds/inhibits Bax monomers, dimers, and tetramers
- Bax tetramers bind to mitochondrial "sites" and become active pores
- Active pores bind to and transport Smac to the cytosol

use module 11d without pore transport

Bax tetramers bind to mitochondrial sites and become active pores that bind to and transport Smac to the Cytosol

M8b: albeck_11e()

Model as in 11e, but with cooperative assembly of Bax pores

association rate constants for Bax dimerization, tetramerization, and insertion are set so that they increase at each step (from 1e-8 to 1e-7 and then 1e-6), thereby creating cooperative assembly

use module 11e (including the new pores)

set parameter values for cooperative pore formation

shen_modules (M9a-M15a)

Load monomers

Albeck: ligand_to_C8_monomers(), apaf1_to_parp_monomers()

Shen: momp_monomers()

Add initial condition

Set initial Bid-value

Include upstream & downstream section

Albeck: rec_to_bid(), pore_to_parp()

Specify MOMP module

Shen: chen_biophys_j() (M9a), chen_feb5_direct() (M10a), chen_feb5_indirect() (M11a),
cui_direct() (M12a), cui_direct1() (M13a), cui_direct2() (M14a), howells() (M15a)

Declare observables

Shared: observables()

+ active Bax, Bcl2, Bcl2_Bid, Bcl2_Bax

shen_modules (M9b-M15b)

PySB implementations of Bcl2-models from the group of Pingping Shen, along with other derived, closely related models.

In a series of papers from 2007-2010, the research group of Pingping Shen implemented and investigated models of Bcl-2 family interactions.

This file also includes a model from Howells et al. which is a fairly straightforward extension of a Shen group model.

M9b: chen_biophys_j(True,False)

Drawn from *Chen, C., Cui, J., Lu, H., Wang, R., Zhang, S., & Shen, P. (2007). Modeling of the role of a Bax-activation switch in the mitochondrial apoptosis decision. Biophysical Journal, 92(12), 4304-4315. doi:10.1529/biophysj.106.099606'*

- Activation of Bax by an activator (tBid) in a one-step, hit-and-run manner; Bax activation is reversible
- Bcl2 binds both tBid and Bax Bax can displace tBid from Bcl-2 (but not the reverse)
- If Bax oligomerization is incorporated into the model (see `do_pore_assembly` argument), then this occurs as a spontaneous, order 4 reaction.
- This model combines both "direct" type and "indirect" type elements in that Bcl-2 is capable of binding both Bid and Bax

tBid activates Bax

Bcl2 binds tBid and Bax
Bax can displace tBid from Bcl2 (but not the reverse)

do_pore_assembly	
TRUE	adds the formation of Bax oligomers to the model
FALSE	
model's most downstream element: Bax activation	

release Cytochrome C and Smac

M10b: chen_febs_indirect(True,False)

Drawn from *Chen, C., Cui, J., Zhang, W., & Shen, P. (2007).*

Robustness analysis identifies the plausible model of the Bcl-2 apoptotic switch. FEBS letters, 581(26), 5143-5150.

'doi:10.1016/j.febslet.2007.09.063'

- There is no activation of Bax by tBid. Bax starts out constitutively "active" in that in its initial state, it is able to form oligomers
- Bcl-2 can bind tBid and Bax

no activation of Bax; Bax is in the active state by default; it is able to form oligomers

Bcl2 binds tBid and Bax
Bax can displace tBid from Bcl2 (but not the reverse)

do_pore_assembly	
TRUE	FALSE
adds the formation of Bax oligomers to the model	model's most downstream element: Bax activation

release Cytochrome C and Smac

M11b: chen_febs_direct(True,False)

Drawn from *Chen, C., Cui, J., Zhang, W., & Shen, P. (2007).*

Robustness analysis identifies the plausible model of the Bcl-2 apoptotic switch. FEBS letters, 581(26), 5143-5150.

'doi:10.1016/j.febslet.2007.09.063'

- Activation of Bax by an activator (tBid) in a one-step, hit-and-run manner; Bax activation is reversible
- Bcl-2 can bind tBid, but not Bax
- Pore assembly with tetrameric pores

tBid activates Bax

Bcl2 binds tBid
and the sensitizer Bad but not Bax

do_pore_assembly

TRUE

FALSE

adds the formation of Bax oligomers to the model

model's most downstream element: Bax activation

release Cytochrome C and Smac

M12b: cui_direct(False,False)

Drawn from *Cui, J., Chen, C., Lu, H., Sun, T., & Shen, P. (2008). Two independent positive feedbacks and bistability in the Bcl-2 apoptotic switch. PLoS One, 3(1), e1469. doi:10.1371/journal.pone.0001469'*

- Builds on the chen_febs_direct model
- do_pore_assembly is set to False: dimeric pores instead of tetrameric pores

use module chen_febs_direct
without pore assembly and pore transport

adjust parameter values
add Bad-for-Bid displacement reaction
add simplified MAC formation (Bax dimerization)
add synthesis and degradation reactions

release Cytochrome C and Smac
different parameters than chen_febs_direct

M13b: cui_direct1(False)

Drawn from *Cui, J., Chen, C., Lu, H., Sun, T., & Shen, P. (2008). Two independent positive feedbacks and bistability in the Bcl-2 apoptotic switch. PLoS One, 3(1), e1469. doi:10.1371/journal.pone.0001469'*

- Builds on the (base) direct model

```
use module cui_direct
```

```
add inhibition of Bax by Bcl2  
add associated displacement reactions  
add degradation of the active Bax:Bcl2 complex
```

M14b: cui_direct2(False)

Drawn from *Cui, J., Chen, C., Lu, H., Sun, T., & Shen, P. (2008). Two independent positive feedbacks and bistability in the Bcl-2 apoptotic switch. PLoS One, 3(1), e1469. doi:10.1371/journal.pone.0001469'*

- Builds on the direct 1 model

use module cui.direct1

add simultaneous auto-activation and dimerization of Bax

M15b: howells(True,False)

Drawn from *Howells, C. C., Baumann, W. T., Samuels, D. C., & Finkielstein, C. V. (2011). The Bcl-2-associated death promoter (BAD) lowers the threshold at which the Bcl-2-interacting domain death agonist (BID) triggers mitochondria disintegration. Journal of theoretical biology, 271(1), 114-123. doi:10.1016/j.jtbi.2010.11.040'*

- Builds on the chen_biophys_j model
- Modifies some parameter values
- Add a number of Bad-related reactions (incl. unphosphorylated Bad spontaneously translocates between cytosol and mitochondria, Bad binds Bcl-2, Bad displaces tBid from Bcl-2, Cytosolic, mitochondrial, and Bad in a mitochondrial Bad:Bcl2 complex can be phosphorylated at various rates, Bad can be sequestered by, and released from, 14-3-3 domains in the cytosol)

```
use module chen.biophys_j
```

```
adjust parameter values  
translocation equilibrium between unphosphorylated  
cytosolic and mitochondrial Bad  
Bad binds Bcl2  
Bad displaces tBid from Bcl2  
phosphorylation of Bad  
sequester phospho-Bad by binding 14-3-3 domains  
release of Bad from 14-3-3 domains
```