Computational Systems Biology

…Biology X – Lecture 2…

Bud Mishra

Professor of Computer Science, Mathematics, & Cell Biology

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Syllabus: Biology X

- Evolutionary Biology
- Computability in Biology
- Reconstructibility in Biology
- Biology of Cancer
- Biology of Aging

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Evolutionary Biology

- Basic Biology
- Genome Structure:
  - Retro-Elements and their distributions
  - Physical Properties of a genome
  - Large Segmental Duplications
  - Models of Segmental Duplications
- Genome Evolution:
  - Point Mutations
  - Rearrangements
  - Evolution by Duplication
- RNA evolution Model
- Evidence for Evolution
  - Luria-Delbruck JAckpot
- Polymorphisms
  - SNPS & CNPS
  - Haplotyping and Haplotype phasisng
- Genetics
  - Linkage Analysis
  - Association Studies
- Phylogeny
  - Algorithms for Phylogenetic Trees.

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Computability in Biology

- Models in Biology
  - Regulatory Networks
  - Metabolic Networks
  - Signaling Networks
- KMA Models:
  - ODE's describing Regulatory Networks
  - How to create such models
  - Questio of Reachability
- Hybrid Models
  - Basic Definitions
  - Classes of Hybrid Models
- Model Checking:
  - CTL and Basic Model Checking algorithms
  - TCTL and RTL
- Algorithmic problems
- Examples

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Reconstructibility in Biology

- Biological Networks
- Protein-DNA and Protein-Protein Interactions
  - Two hybrid experiments
  - Motifs and Scale-Free Networks
  - Origi of strctures
- Network Reconstruction
  - From Microarray Data
  - Techniques based on Linear and non-linear regression
  - The issue of sparsity
- Theory of Information Bottleneck
- Clustering using IB, side-information and ontology
- Analysis of Time-Course Data
- GOALIE

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Biology of Cancer

- Cancer
  - A Genomic Disease
- Cancer Data Analysis
  - Genomic Data
  - Transcriptomic Data
  - Proteomic Data
- Cancer Gene Discovery
- Somatic Evolution n Cancer
- Theories of Origin of Cancer

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Biology of Aging

- Theories of Aging:
  - Hayflick's model
  - Mitochodria and Oxidative Stress
  - Stem Cells & Niche-Clonality
  - Genome Evolution
  - Proteomic Explanation: E.g, Protein Degradation
- Key Experiments in Animals
- Examples:
  - Deinococcus radiodurans
  - Tradigrades
  - C. Elgans
- Anti-Aging
- Immortality

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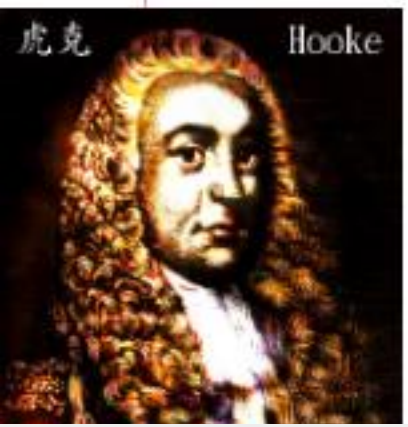
Hooke

Thursday 25 May 1676

Damned Doggs.

Vindica me deus.

- Commenting on
Sir Nicholas Gimcrack character in
The Virtuoso, a play by Thomas Shadwell.



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Hooke

in the Royal Society, 26 June 1689

- "I have had the misfortune either not to be understood by some who have asserted I have done nothing…
- "Or to be misunderstood and misconstrued (for what ends I now enquire not) by others…
- "And though many things I have first Discovered could not find acceptance yet I finde there are not wanting some who pride themselves on arrogating of them for their own…
- "—But I let that passe for the present."

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Hooke…

- "So many are the links, upon which the true Philosophy depends, of which, if any can be loose, or weak, the whole chain is in danger of being dissolved;
- "it is to begin with the Hands and Eyes, and to proceed on through the Memory, to be continued by the Reason;
- "nor is it to stop there, but to come about to the Hands and Eyes again, and so, by a continuall passage round from one Faculty to another, it is to be maintained in life and strength."

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Application: Modeling Apoptosis

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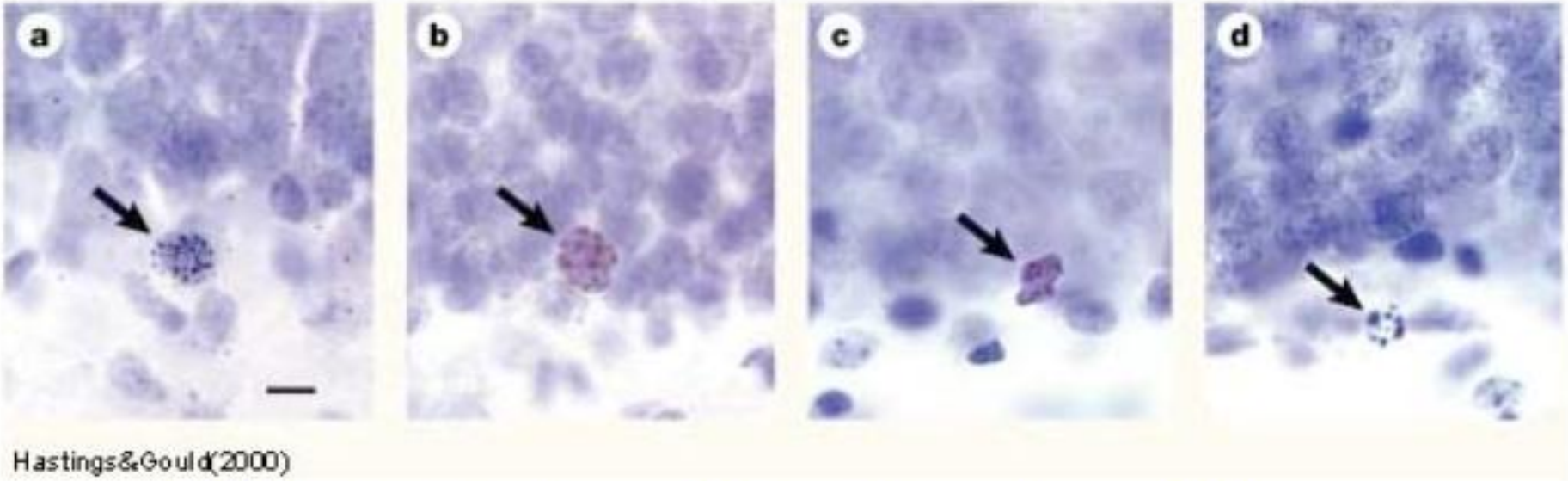
Cell Death



Hastings&Gould(2000)

Cell shrink, organelles swell, chromatin condenses, DNA fragmented, cell junctions disintegrated, membrane blabbing, finally get engulfed—all within 30 minutes.

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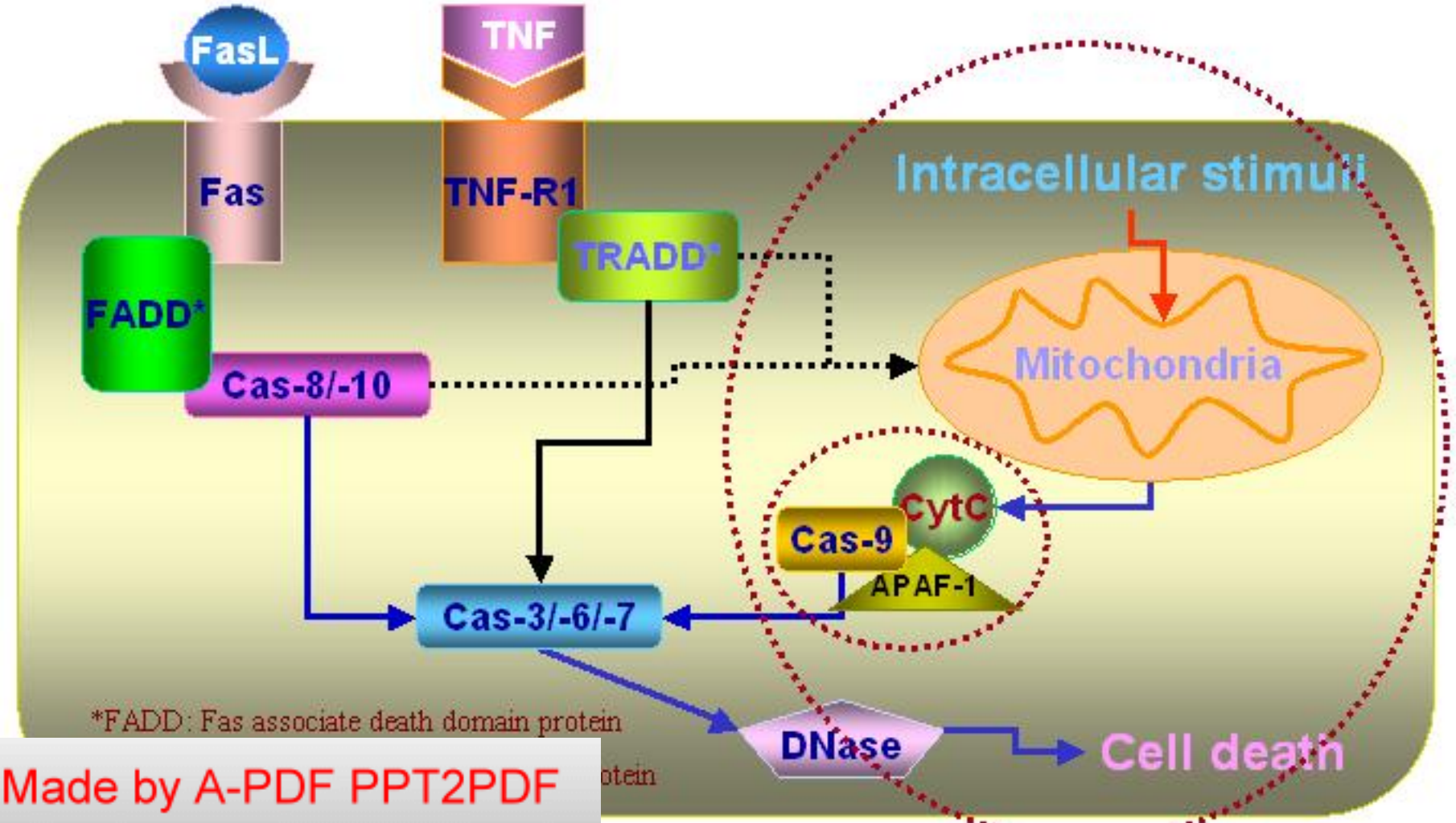
Simplified Apoptotic Pathway



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What are Caspases?

- First caspase was found in C. elegans, ced-3 gene (1993). An acronym for:
  - Cystein aspartate-specific protease
  - Activated by proteolysis; Many substrates (~40 and increasing) such as PARP (Poly (ADP-ribose) polymerase, BID (Bcl2-interacting domain)
- Identified genes so far:
  - C. elegans (4), Drosophila (7), Human and mouse (11)
  - Number of caspases over phylogenetic time seems to have been increasing

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Basic Structures of Caspases

Procaspase-8,10 (= initiator caspases)

Zymogens

Procaspase-3,6,7 (= effector caspases)

Proteolytic process

Active site
QA**C**XG

Prodomain

Death effector domains (DED): Protein interaction domain

Active tetramer

D): Protein interaction domain

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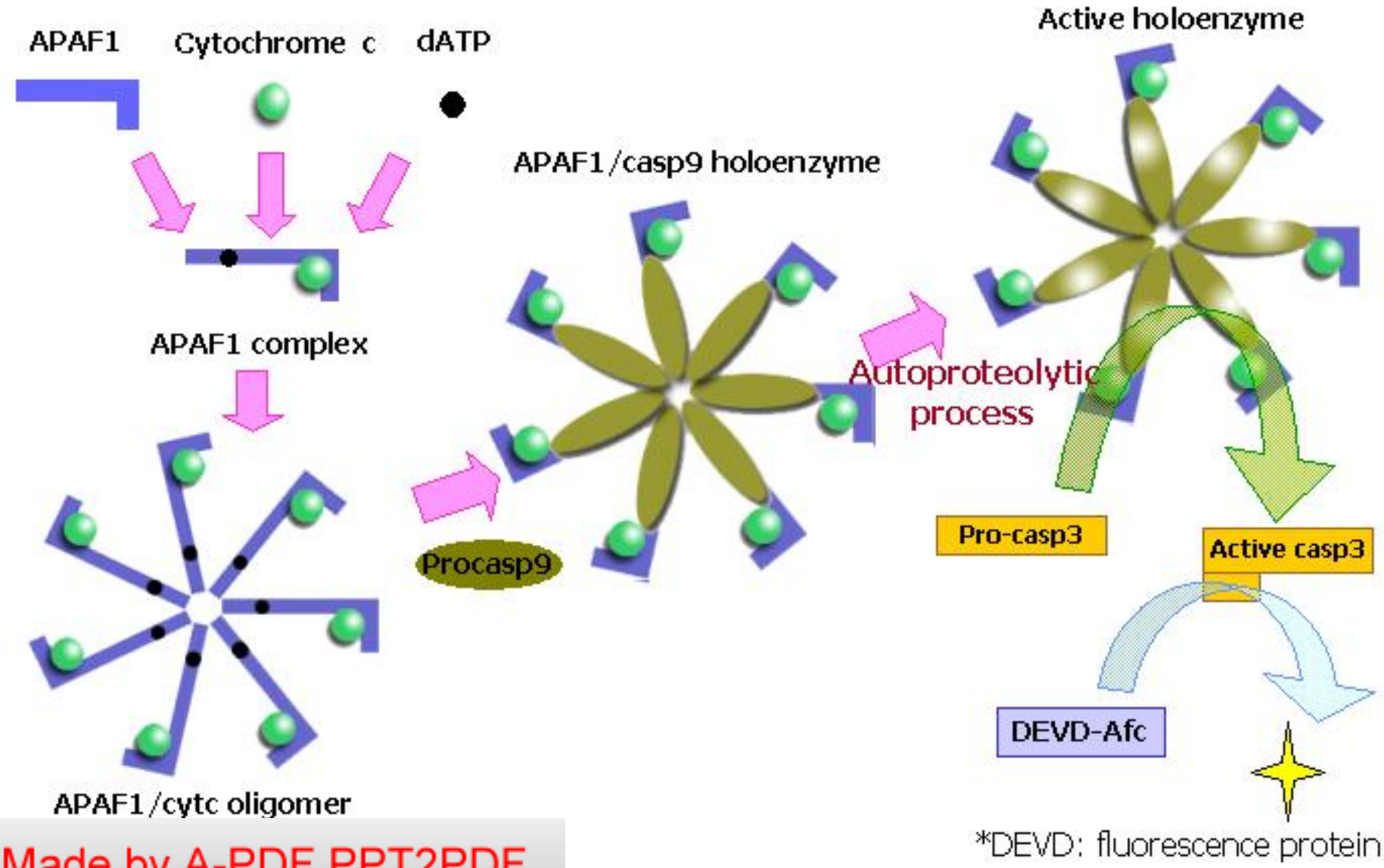
Formation of Holoenzyme Complex



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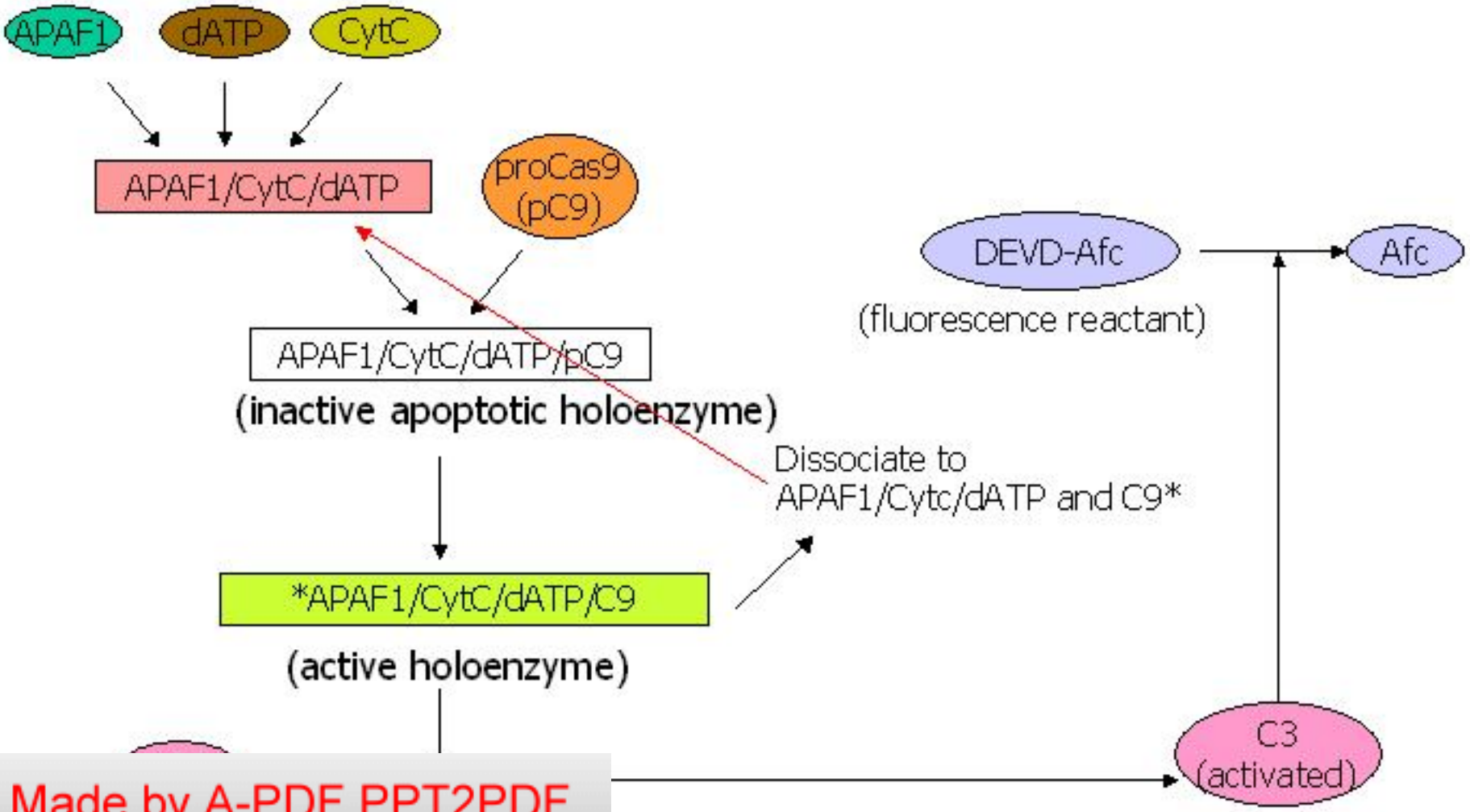
Scheme of the Analysis



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Determine reaction parameters based on the experimental data

Step	What are the parameters	Exp. Data.	References.	Note
0	[ATP]=2~10nM [Mg^{++}]=?mM (near physiological concentration) [dATP]=10uM [Ca^{++}]=?mM(near physiological concentration) [A1]=2~5nM [C9]=20nM [cytc]=1.2uM [C3]=? APAF1 can bind dATP without cytc	^{32}p	Zubay's Biochemistry 3/e p579 JBC(249)p6434 Joe and Jack JBC(274)p8359 and Jack's western Jack's ELISA JBC(275)p31199	[dATP] changes through cell cycle
1	cytc can binds to APAF1 at ratio 1~2, no neucleotide required.	K_{on} K_{off} K	JACS(121)p7435	recomb
2	A1/cytc get oligomerized despite of ATP/dATP presence.	gel-filtration	Jack	cellfree
3	cytc binding induce A1 binds to dATP/ATP dATP hydrolysis is not required for c9 activity. C9 binding improve affinity of dATP ATP and dATP has different k_D with holoenzyme	K_D of dATP/ATP	JBC(275)p31199	recomb
4	The A1/cytc apoptosome is heptamer, some apoptosome may form dimer	EM with dATP	Mol.Cell(9)p423	recomb
5	C9 binds to A1 in the presence of cytc by the ratio of 1:1 through CARD-CARD interaction apoptosome undergo conformational change, the tail of bounded c9 is flexible.	IP? crystal computer compare/EM	XDW 2nd A1 / 1st C9 paper Nature(399)p549 Mol.Cell(9)p423	cellfree recomb recomb
6	The bounded c9 in apoptosome recruit 2nd c9, like dimer, but only one site is active.	image	Mol.Cell(9)p423 PNAS(98)p14250	
7	C9 in A1/C9/cc holoenzyme got cleaved in the presence of ATP, but this cleavage is not required for it's protease activity. The cleavage is intra?	gradient.	Joe's paper JBC(275)p31199 Nature (410)p112 XDW?	cellfree recomb
8	cytc is still in A1/C9 holoenzyme	gel-filtration and gradient.	Joe's paper and JBC(275)p31199	recomb cellfree
9	XIAP binds to 2nd c9 N-teminal. It regulates the Caspase9's activity.	IP Total recomb activity	Nature(410)p112 Nature Structural Biol.(8)p394 JBC(276)p091	
10	apoptosome recruit Caspase3 as well	Jack's gel-filtration IP?		cell free
11	caspase9 cleave caspase3			
		K_{cat} and K_M	JBC(272)p9677	
				cell free

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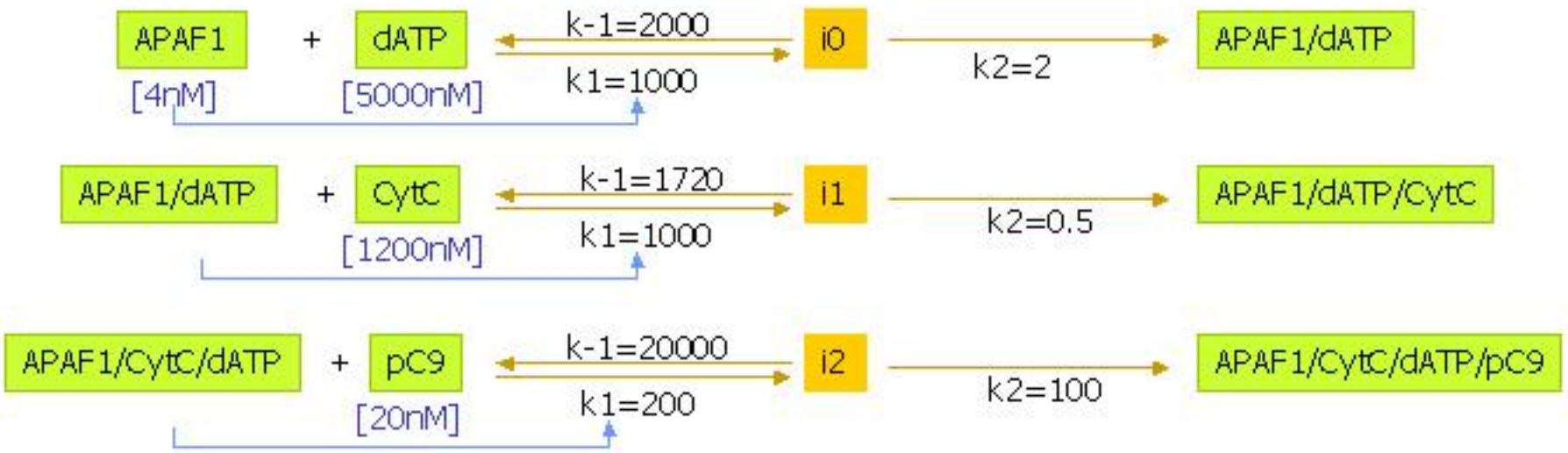
Individual steps in Caspase-9 pathway



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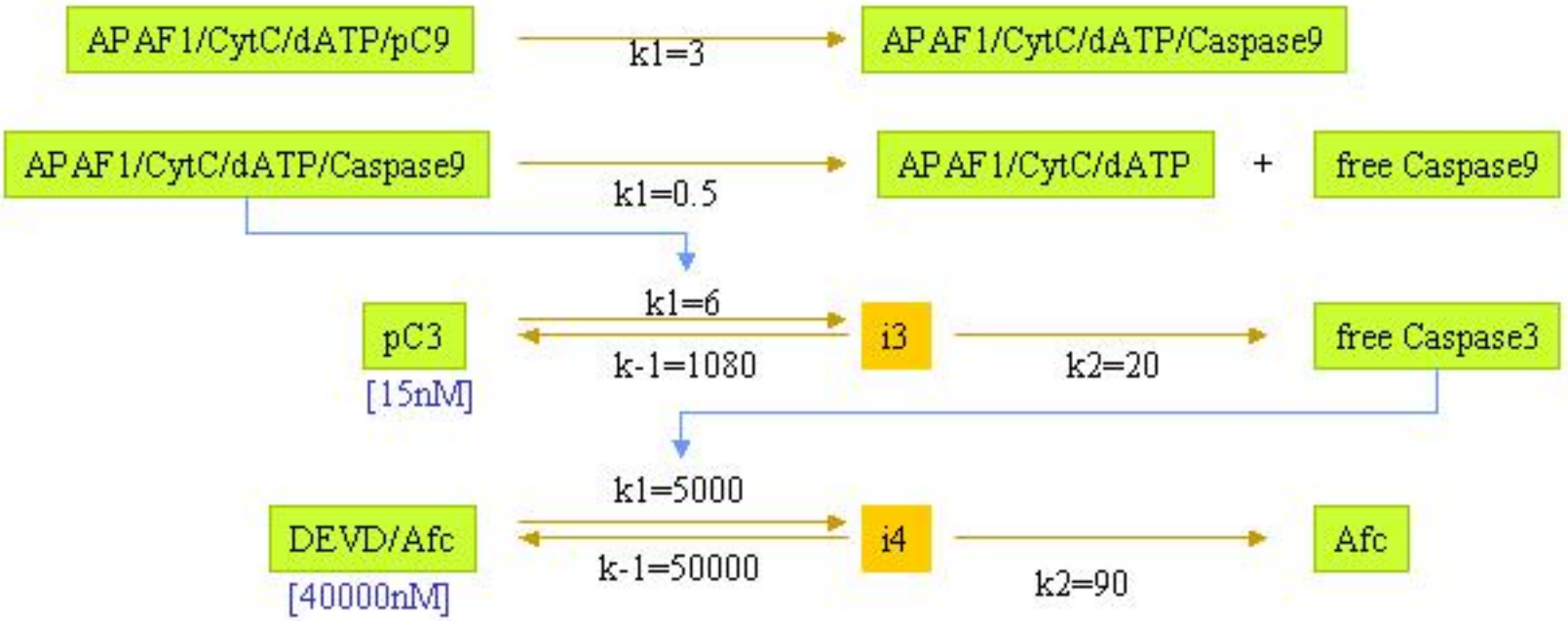
Individual steps in Caspase-9 pathway



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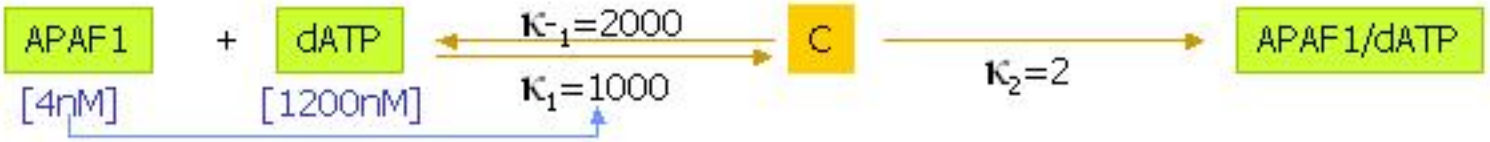
Enzyme Kinetics

First reaction

1. APAF1 (α): (Initial concentration (α_0) = **4nM**)
2. dATP (δ): (Initial concentration (δ_0) = **5000nM**)
3. C: Intermediate of APAF1/dATP complex (Initial concentration (C_0) = 0)
4. APAF1/dATP (ω): Initial concentration (ω_0)= 0



$$d\omega/dt = -\kappa_1 * [\alpha] * [\delta]\,\kappa_0 + \kappa_{-1} * [C] - 2 * \kappa_2$$

$$d\delta/dt = -\kappa_1 * [\alpha] * [\delta]\,\kappa_0 + \kappa_{-1} * [C] - 2 * \kappa_2$$

$$dC/dt = +\kappa_1 * [\alpha] * [\delta]\,\kappa_0 - \kappa_{-1} * [C] - 2 * \kappa_2$$

$$d\omega/dt = +\kappa_1 * [\alpha] * [\delta]\,\kappa_0 - \kappa_{-1} * [C] + 2 * \kappa_2$$

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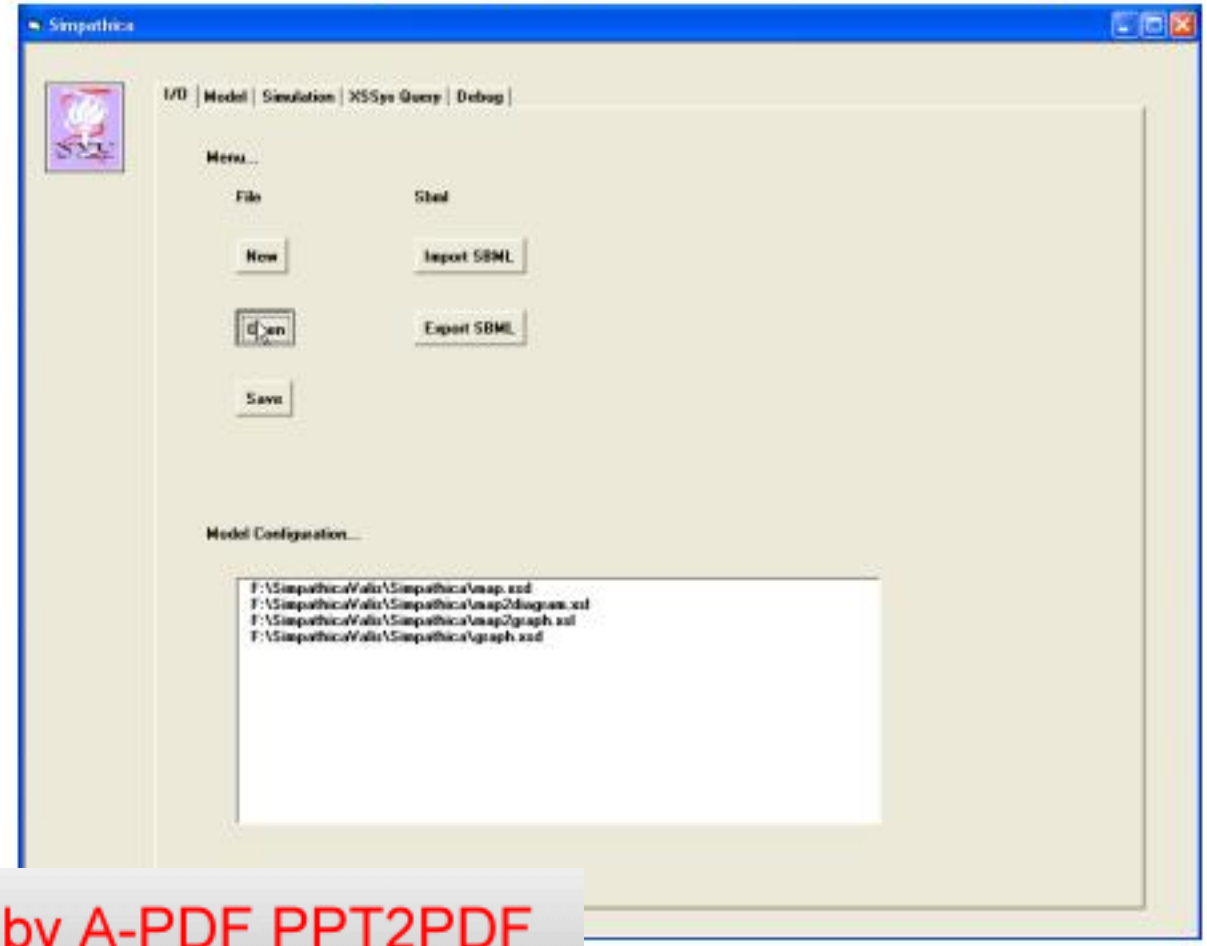
Simpathica: Simulation for the Biological Pathway

- http://www.bioinformatics.nyu.edu/Projects/Simpathica/



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Simpathica generates differential equations

```
from scipy import *
from scipy.integrate import *
from Numeric import *
class __simpathica:
        def jac110271AIP1(self, X, t):
                rslot = []
                rslot.append( + -1 *(+600000000 *X[1] **(+1 )*X[0] **(+1 )) + +1 *(+.006 *X[13] **(+1)) )
                rslot.append( + -1 *(+600000000 *X[1] **(+1 )*X[0] **(+1 )) + +1 *(+.006 *X[13] **(+1)) )
                rslot.append( + +1 *(+2 *X[13] **(+1 )) + -1 *(+1000 *X[3] **(+1)*X[2] **(+1)) + +1 *(+1720 *X[14] **(+1 )) )
                rslot.append( + -1 *(+1000 *X[3] **(+1 )*X[2] **(+1 )) + +1 *(+1720 *X[14] **(+1)) )
                rslot.append( + +1 *(+.5 *X[7] **(1)) + +1 *(+0.5 *X[14] **(+1 )) + -1 *(+200 *X[5] **(+1)*X[4] **(+1)) + +1 *(+20000 *X[15] **(+1 )) )
                rslot.append( + -1 *(+200 *X[5] **(+1 )*X[4] **(+1 )) + +1 *(+20000 *X[15] **(+1)) )
                rslot.append( + -1 *(+3 *X[6] **(1)) + +1 *(+100 *X[15] **(+1 )) )
                rslot.append( + +1 *(+3 *X[6] **(1)) + -1 *(+.5 *X[7] **(1)) + -1 *(+6 *X[8] **(+1 )*X[7] **(+1 )) + +1 *(+1080 *X[16] **(+1)) +
                        +1 *(+20 *X[16] **(+1)) )
                rslot.append( + -1 *(+6 *X[8] **(+1 )*X[7] **(+1 )) + +1 *(+1080 *X[16] **(+1)) )
                rslot.append( + +1 *(+20 *X[16] **(+1 )) + -1 *(+5000 *X[11] **(+1)*X[9] **(+1)) + +1 *(+50000 *X[17] **(+1 )) + +1 *(+90 *X[17] **(+1)) )
                rslot.append( + +1 *(+.5 *X[7] **(1)) )
                rslot.append( + -1 *(+5000 *X[11] **(+1 )*X[9] **(+1 )) + +1 *(+50000 *X[17] **(+1)) )
                rslot.append( + +1 *(+90 *X[17] **(+1 )) )
                rslot.append( + +1 *(+600000000 *X[1] **(+1 )*X[0] **(+1 )) + -1 *(+.006 *X[13] **(+1)) + -1 *(+2 *X[13] **(+1 )) )
                rslot.append( + +1 *(+1000 *X[3] **(+1 )*X[2] **(+1 )) + -1 *(+1720 *X[14] **(+1)) + -1 *(+0.5 *X[14] **(+1 )) )
                rslot.append( + +1 *(+200 *X[5] **(+1 )*X[4] **(+1 )) + -1 *(+20000 *X[15] **(+1)) + -1 *(+100 *X[15] **(+1 )) )
                rslot.append( + +1 *(+6 *X[8] **(+1 )*X[7] **(+1 )) + -1 *(+1080 *X[16] **(+1)) + -1 *(+20 *X[16] **(+1 )) )
                rslot.append( + +1 *(+5000 *X[11] **(+1 )*X[9] **(+1 )) + -1 *(+50000 *X[17] **(+1)) + -1 *(+90 *X[17] **(+1 )) )
                return rslot

initial = [4,1200,0,5E+6,0,20,0,0,15,0,0,40000,0,0,0,0,0,0]
compoundsNames = ["APAF1", "cytc", "APAF1/cytc", "dATP", "APAF1/cytc/dATP", "pC9", "APAF1/cytc/dATP/pC9", "APAF1/cytc/dATP/casp9",
+                    "X0", "X1", "X2", "X3", "X4"]
```

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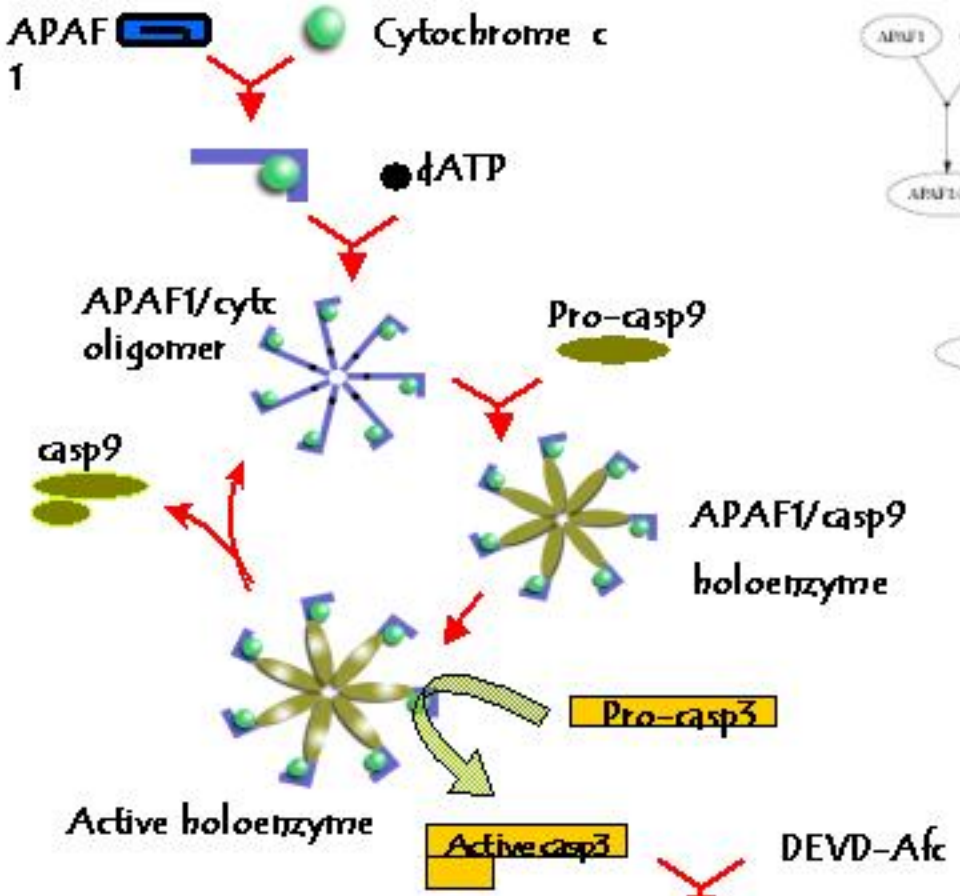
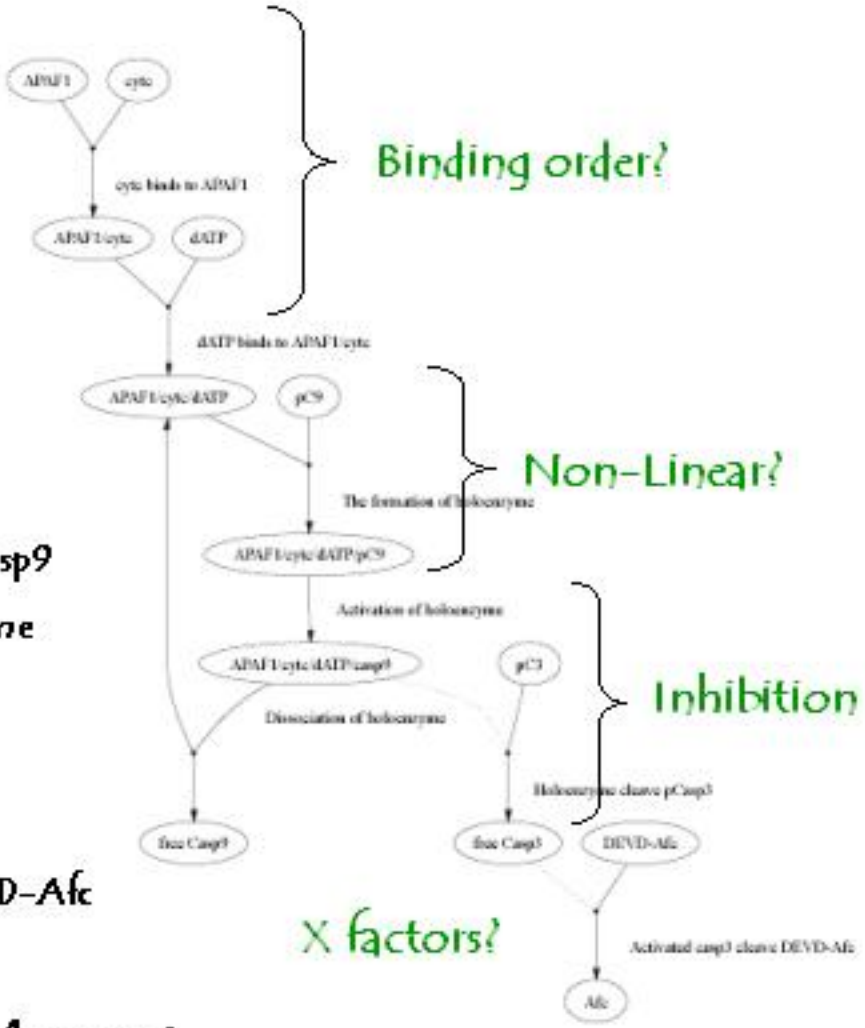
Questions that can be answered by Simpathica





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Model Checking: Recombinant System

Use purified recombinant components

Easy to determine rate changes (synthesis or degradation)

	APAF1	Casp9	cytc	dATP	DEVD-Afc
Initial conc.	100nM	200nM	12uM	5mM	40uM
Final conc.	98.365 nM	130.03 nM	3.9346 uM	0.2 mM	?

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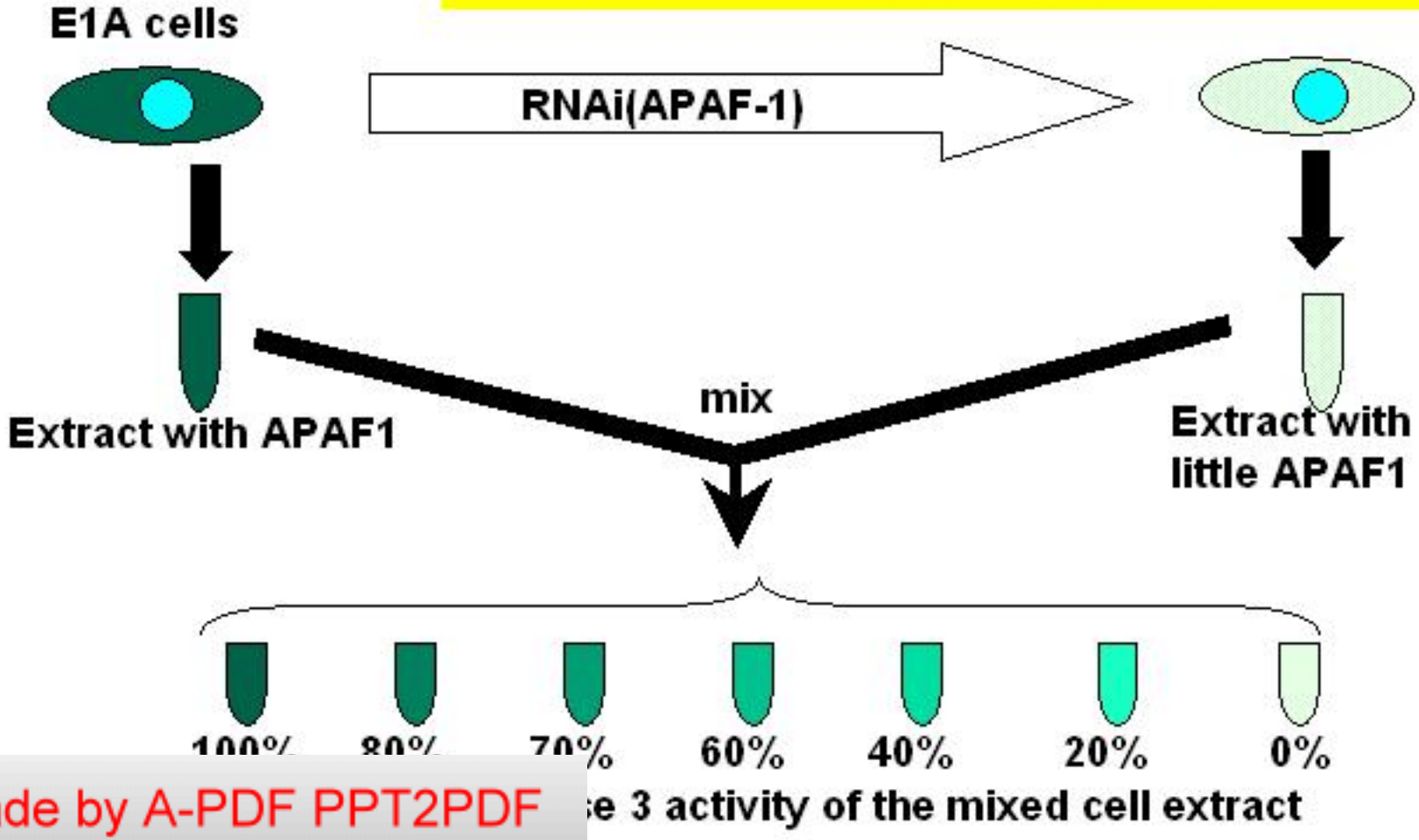
Model Checking: In Vitro Assay

•Mix RNAi(APAF-1) treated and untreated E1A cell extract.



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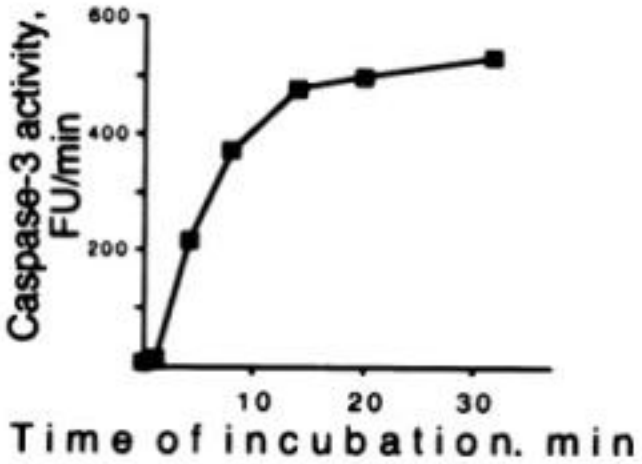
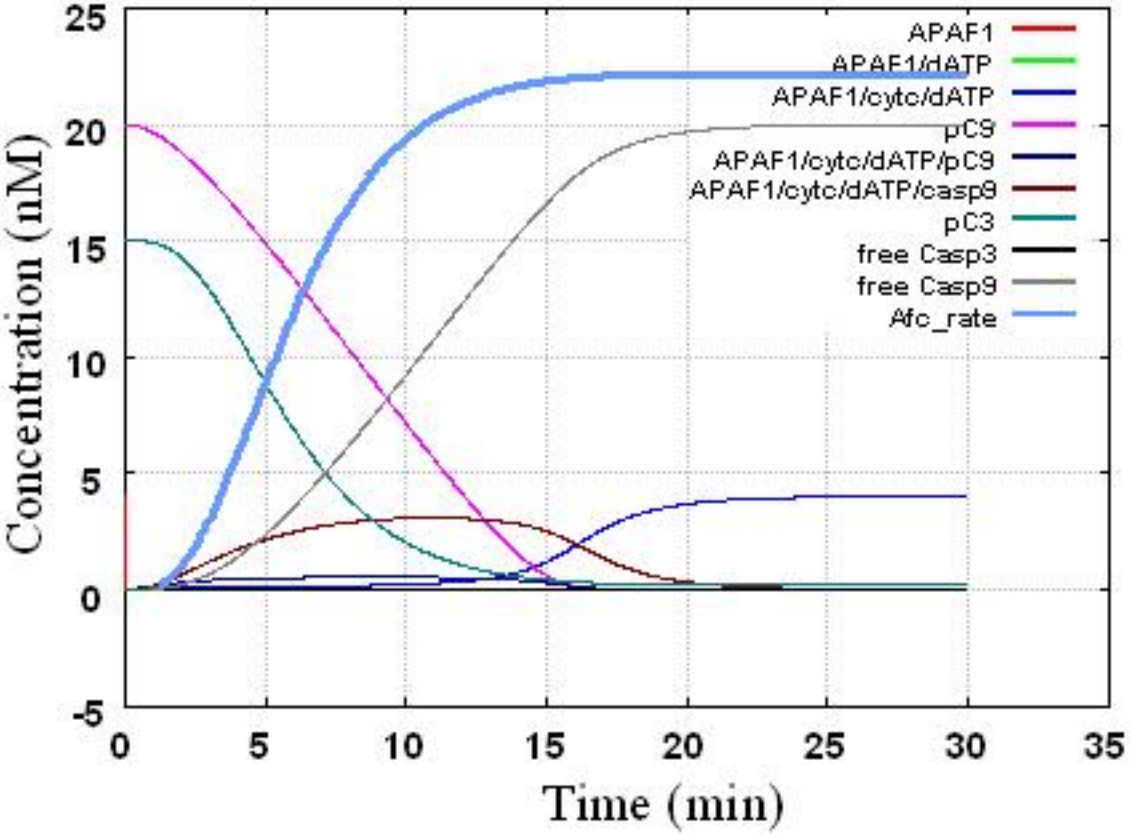
Simpathica recapitulates the holoenzyme formation process



Rodriguez and Lazebnik (1999)



Simulation in Simpathica

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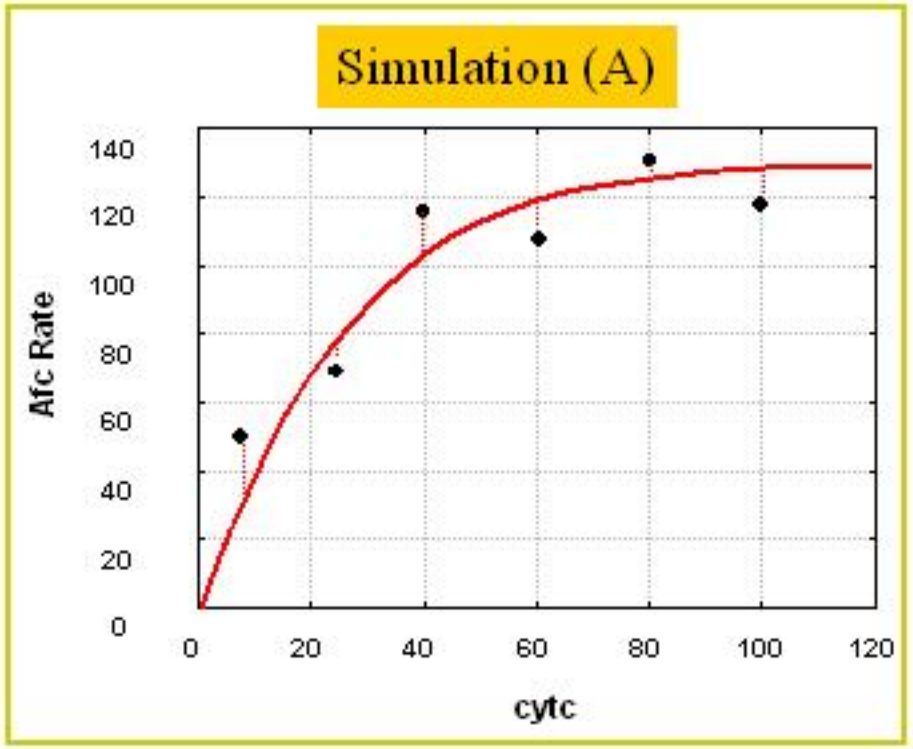
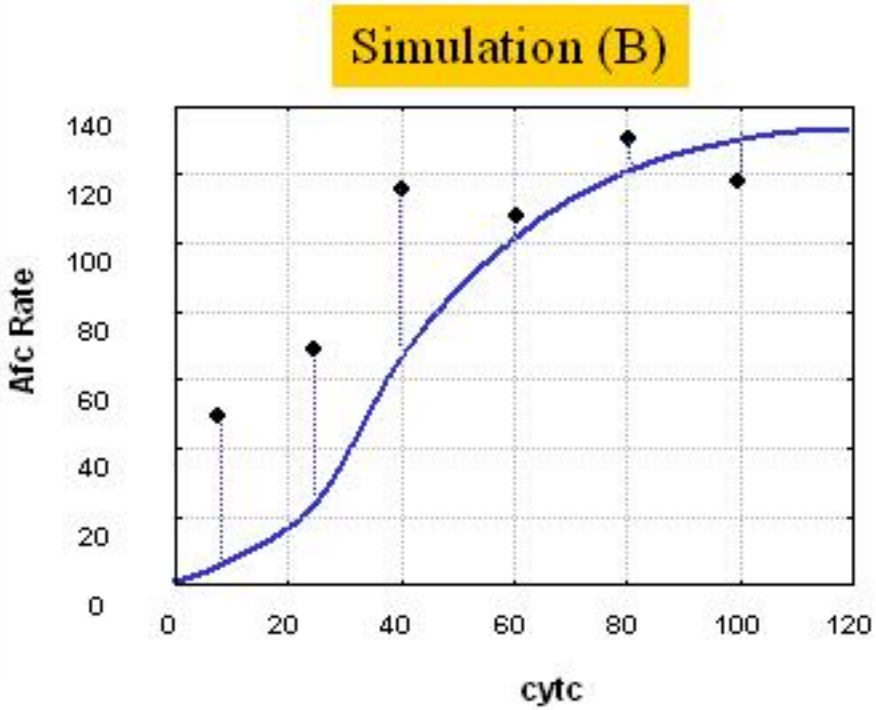
Least-Square Method





Minimize $\left(\sum_{i=1}^{n} \sqrt{x_i^2 - f(y_i)^2}\right)$

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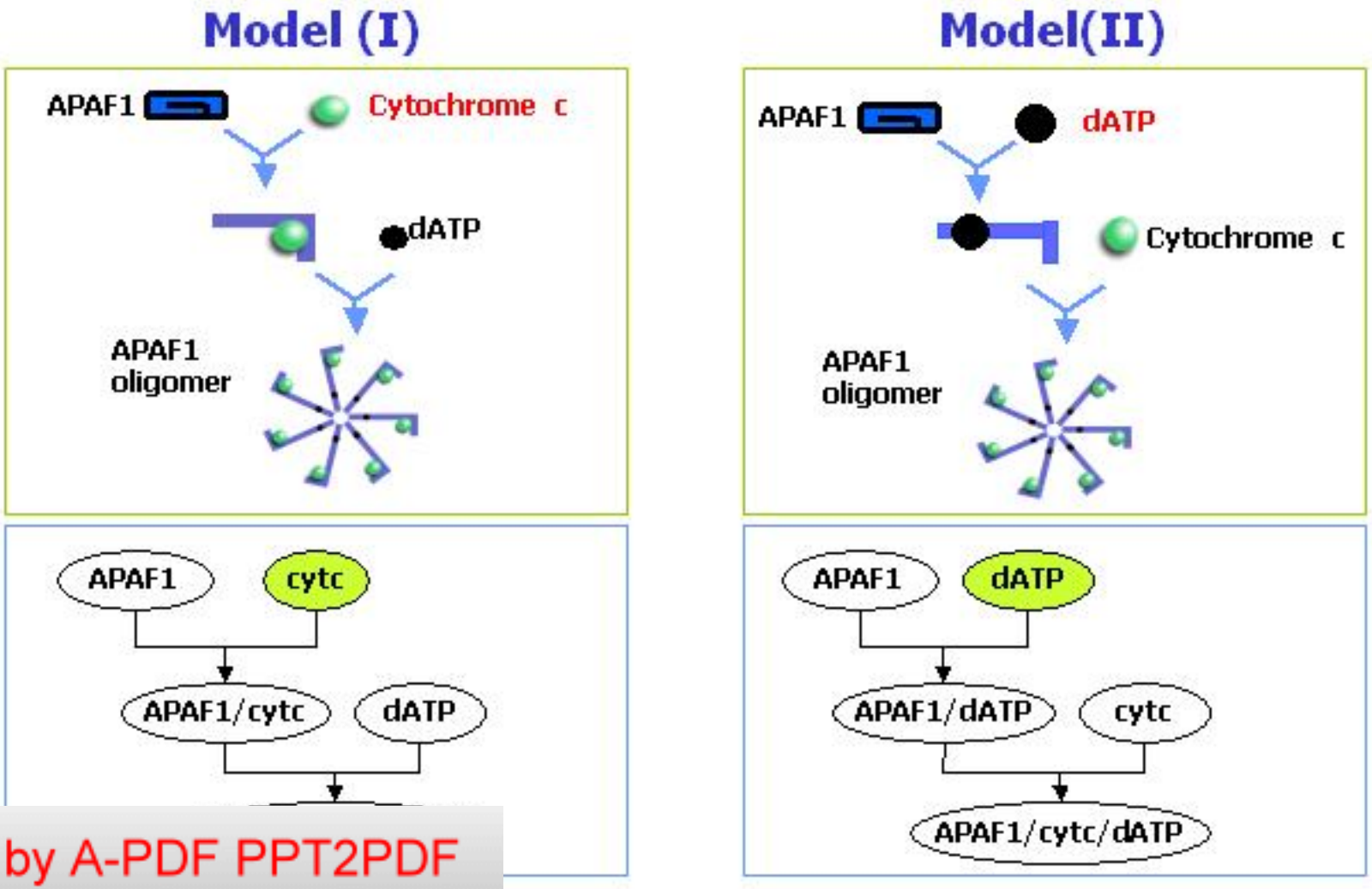
Which molecule initiates caspase-9 pathway?



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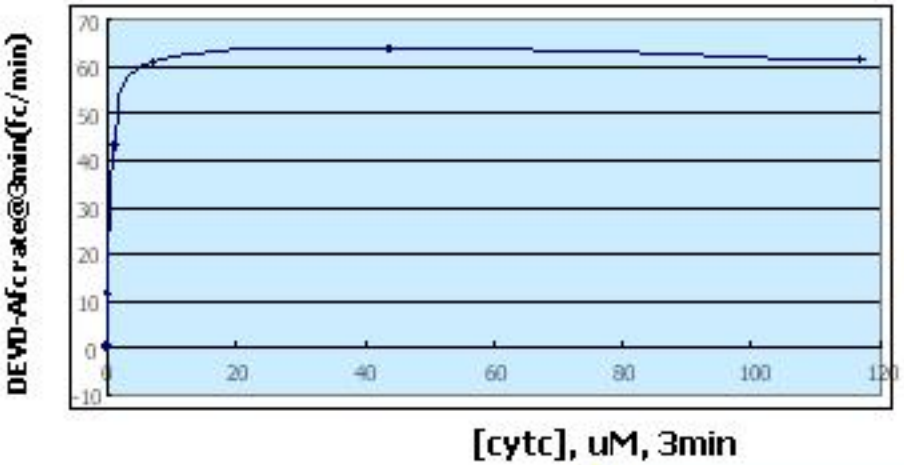
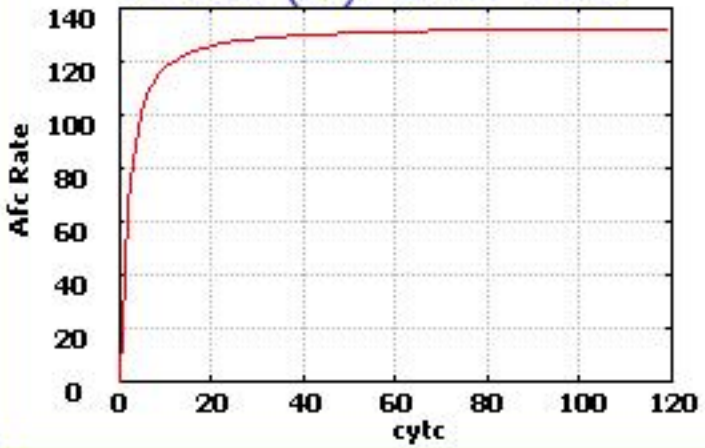
Recombinant System Can Validate a Model



Lazebnik lab (CSHL)

Model (I): CytC first

Model (II): dATP first



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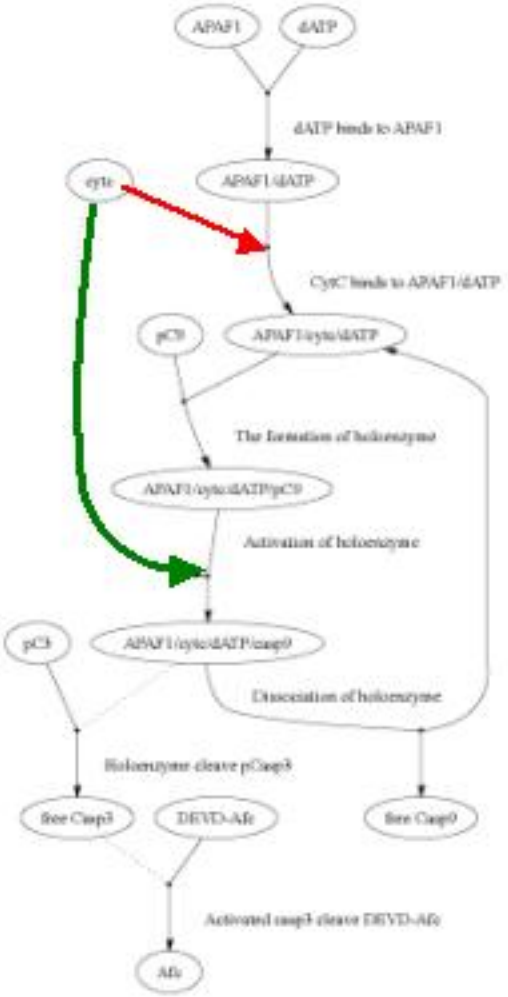
Are There Duals Roles for Cytochrome c?

- Determine from experimental data:
  - Cytochrome c is needed for APAF-1 multimerization
  - Cytochrome c stays in the holoenzyme complex after multimerization
  - Cytochrome c may have another role

(1) Facilitates APAF-1multimerization
→ model (I)

(2) Activate holoenzyme
→ model (II)



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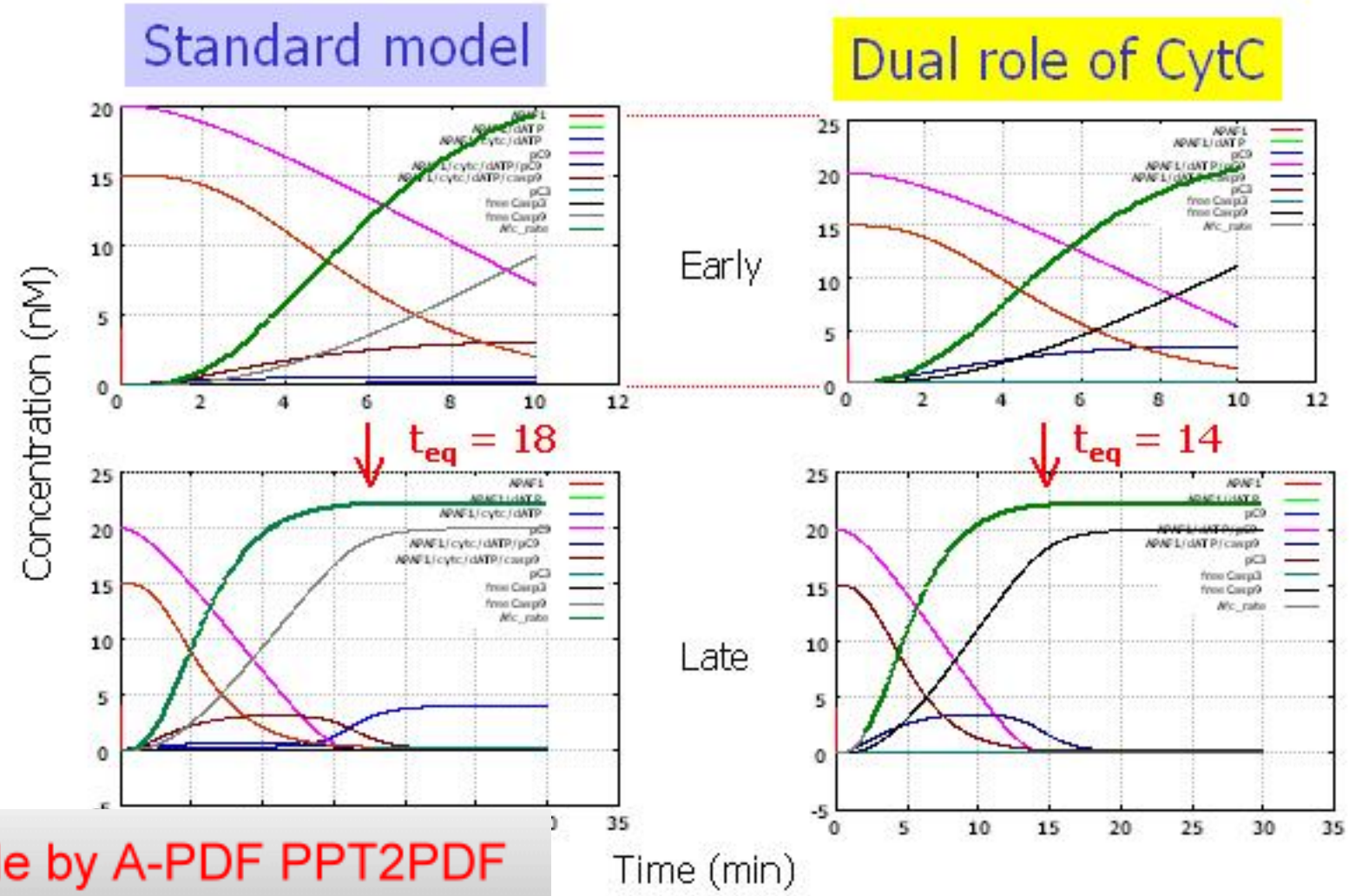
Results: Cytochrome c may NOT have a dual role



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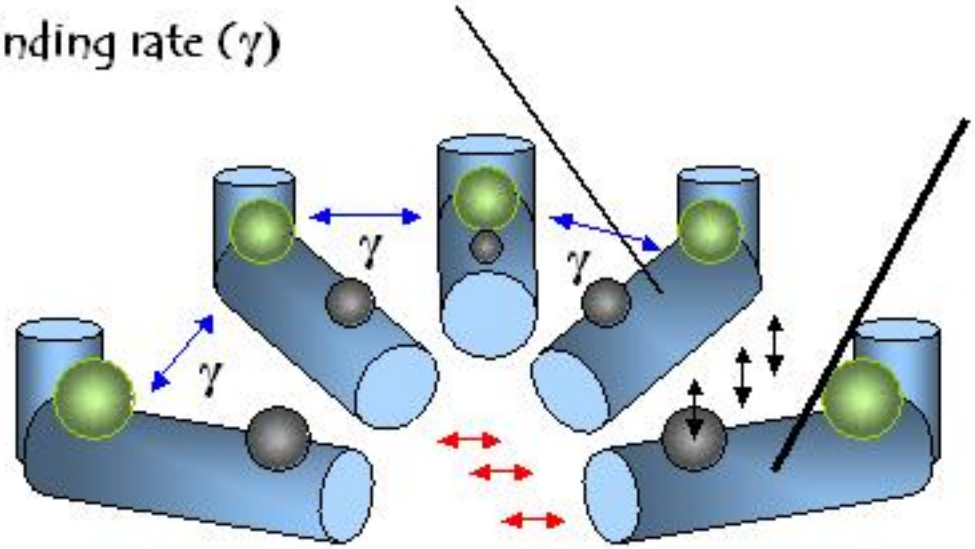
Is there cooperative binding during the formation of APAF-1 complex?

APAF-1 unit binding:

Binding rate (γ)



Next APAF-1 unit binding:

next binding rates

($\gamma_2 = \gamma_1$) or ($\gamma_2 >> \gamma_1$)

Multiple APAF-1 units promote the binding of the next APAF-1 unit.

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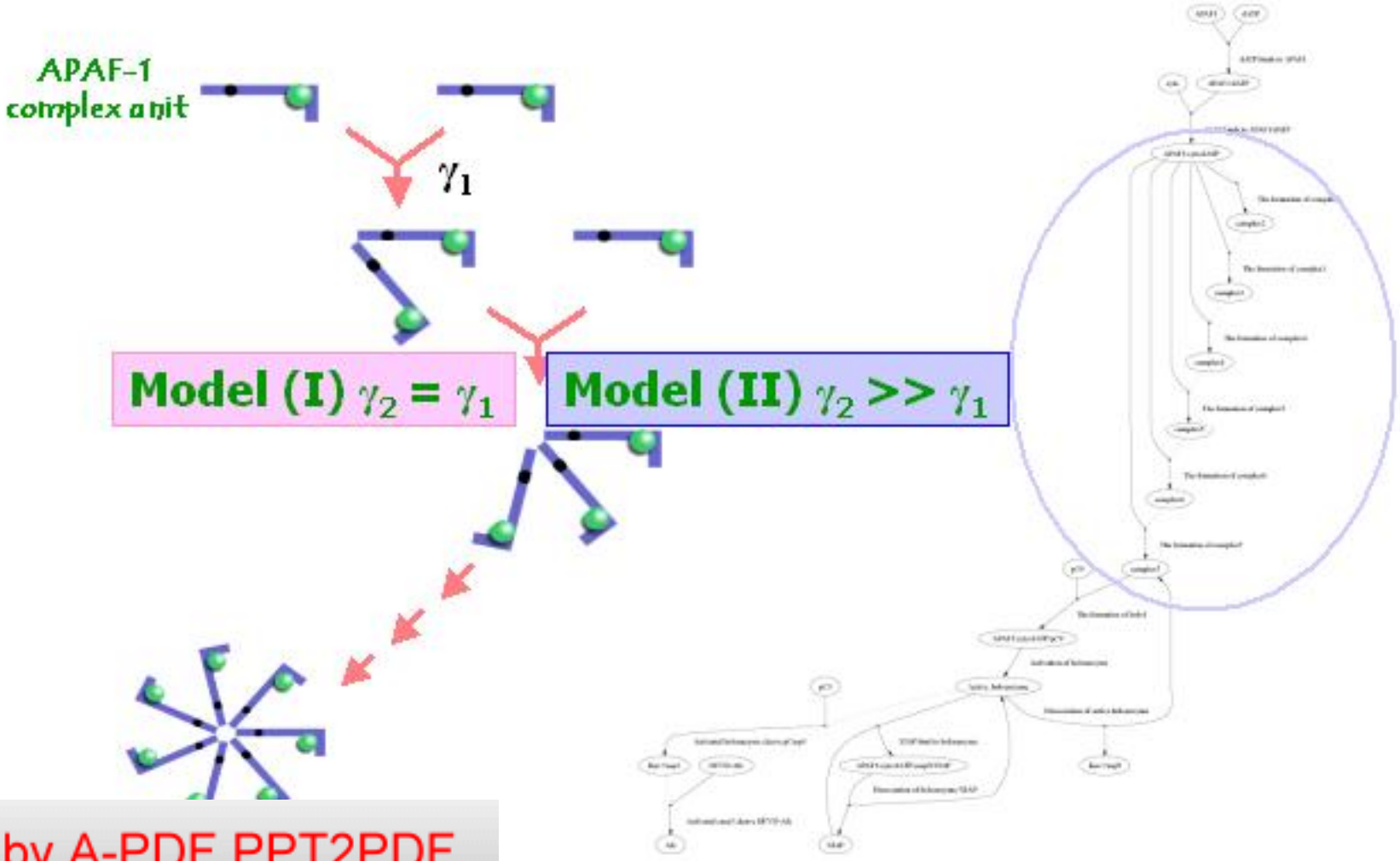
Modeling the formation of APAF-1 oligomer



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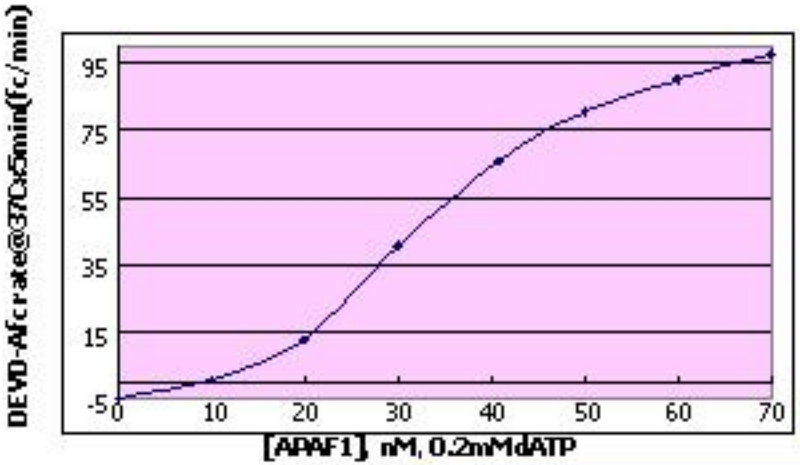
APAF-1 titration in recombinant system



Lazebnik lab (CSHL)

Model (I): $\gamma_2 = \gamma_1$

Model (II): $\gamma_2 >> \gamma_1$

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Is there cooperative binding during the holoenzyme formation?

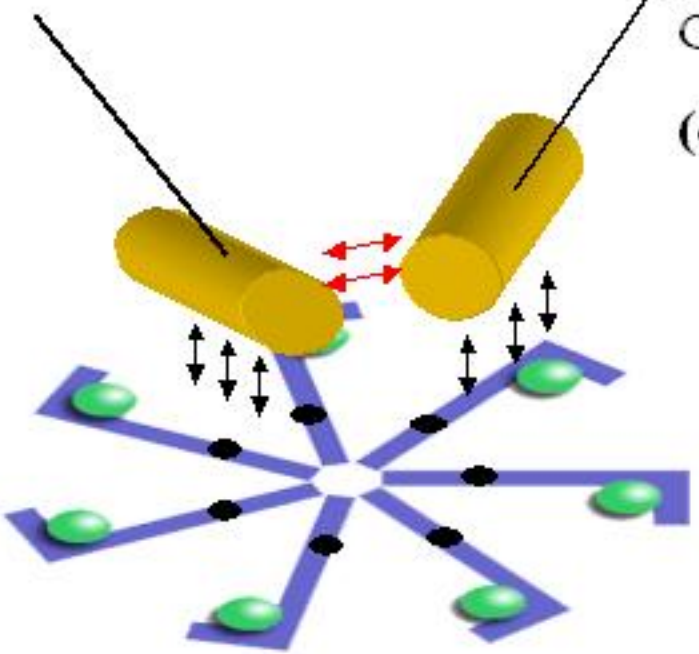
1st Procasp9 binding:

Only APAF1/casp9 interaction (δ_1)

2nd Procasp9 binding:

APAF1/ casp9 interaction + Casp9/casp9 interaction

($\delta_2 = \delta_1$) or ($\delta_2 >> \delta_1$)



Recruitment of 1st Procasp9 promotes the binding of the 2nd Procasp9.

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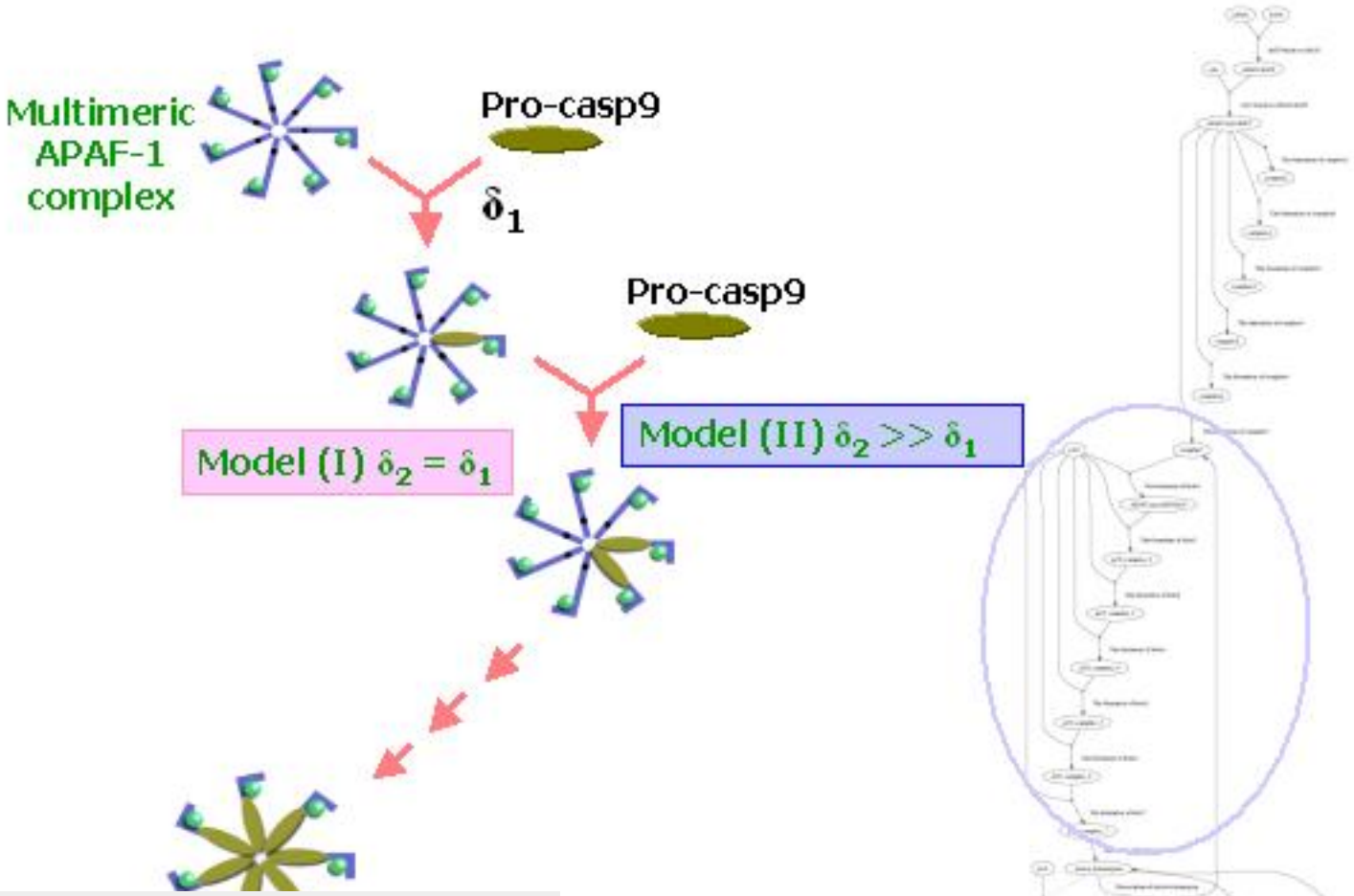
Modeling the formation of holoenzyme



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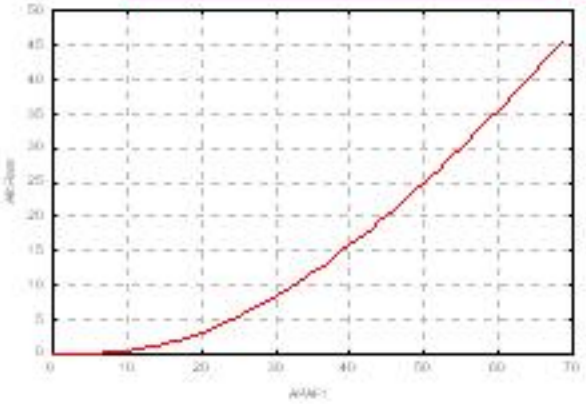
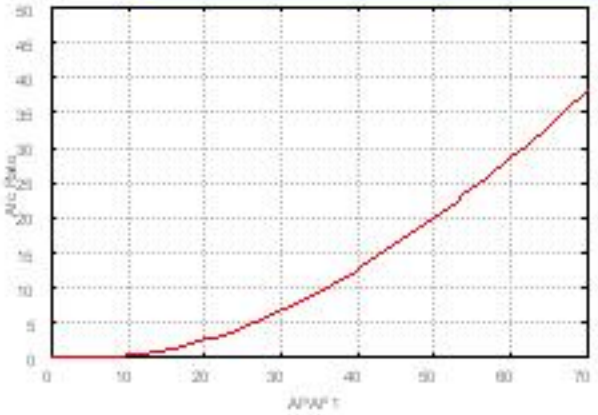
Cooperative behavior of holoenzyme is due to the binding of APAF-1 complex

Model (I) $\delta_2 = \delta_1$

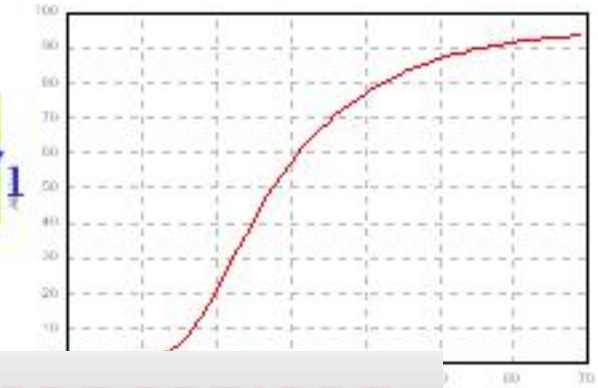
Model (II) $\delta_2 >> \delta_1$

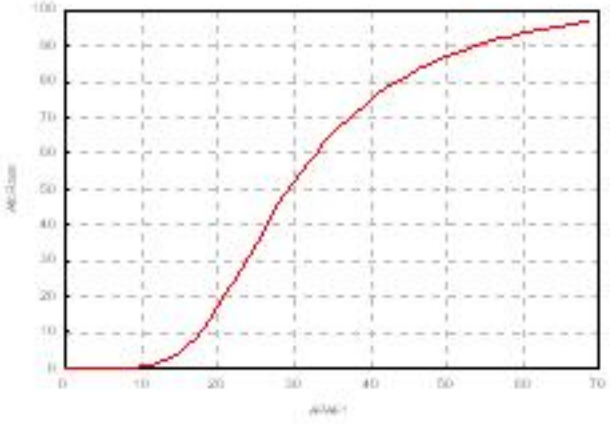
$\gamma_2 = \gamma_1$





$\gamma_2 >> \gamma_1$





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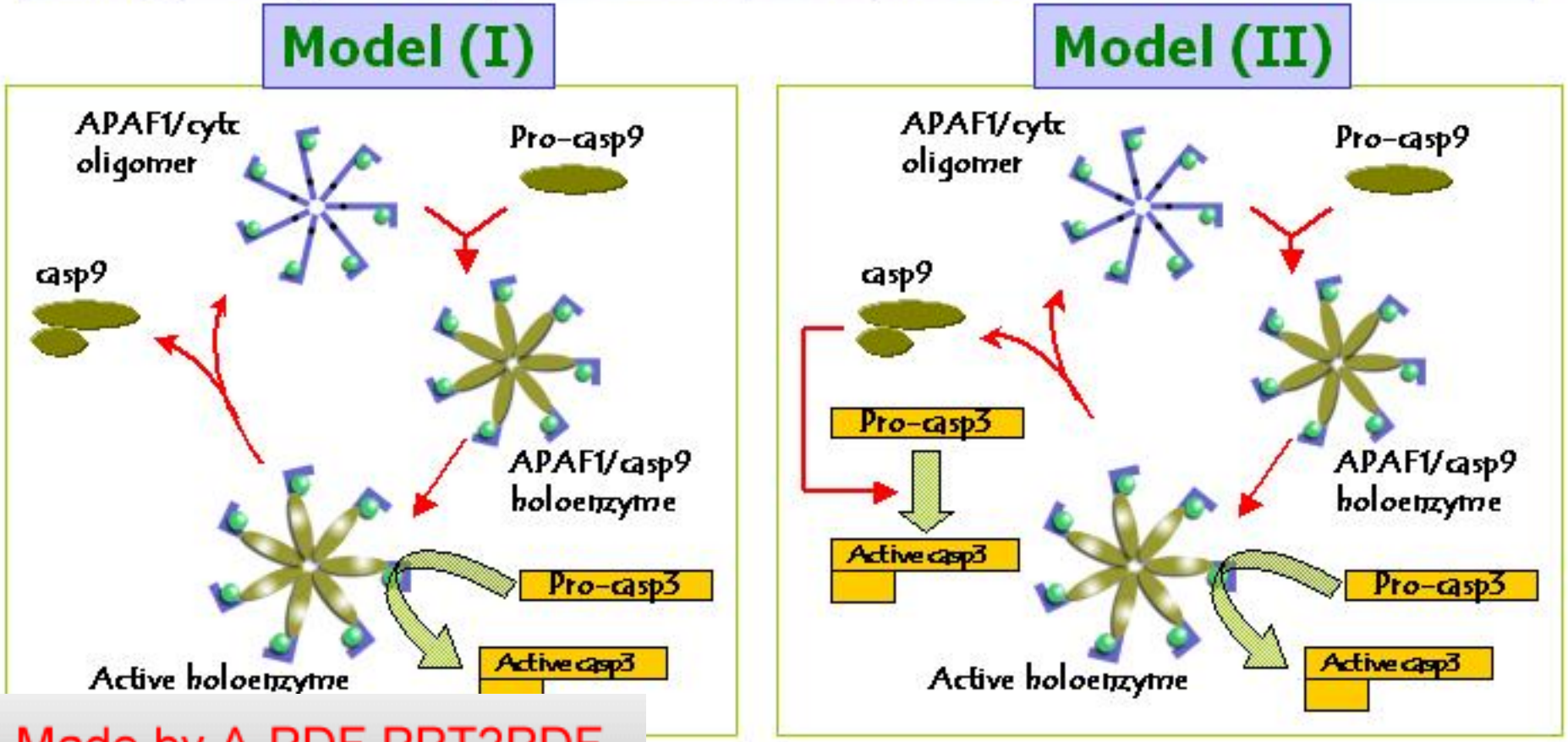
Free Caspase-9 Activity

Caspase-9 is much more active in holoenzyme, and free caspase-9 have little activity (Rodriguez et al., Genes & Dev., 1999). Is it valid?



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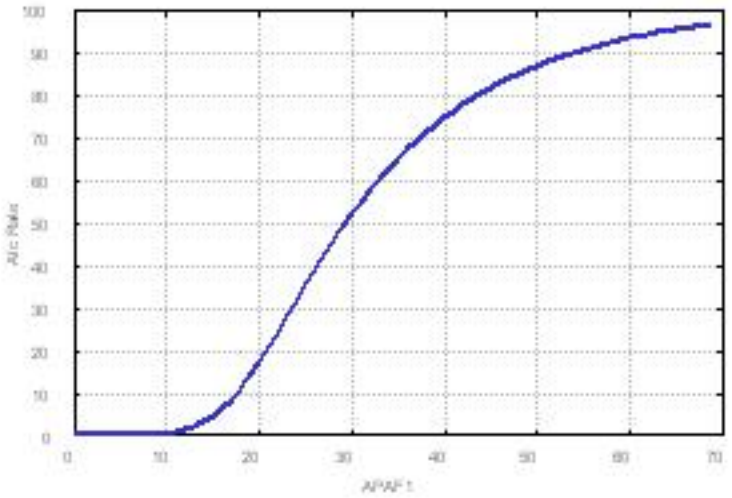
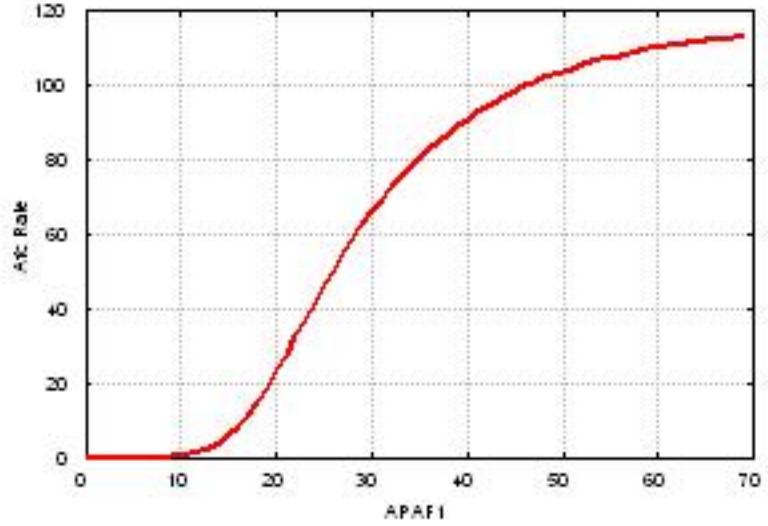
Caspase-3 Activity may not depend on Free Caspase-9

Model (I):
Only holoenzyme activates Casp3



Model (II):
Free casp9 also activates casp3



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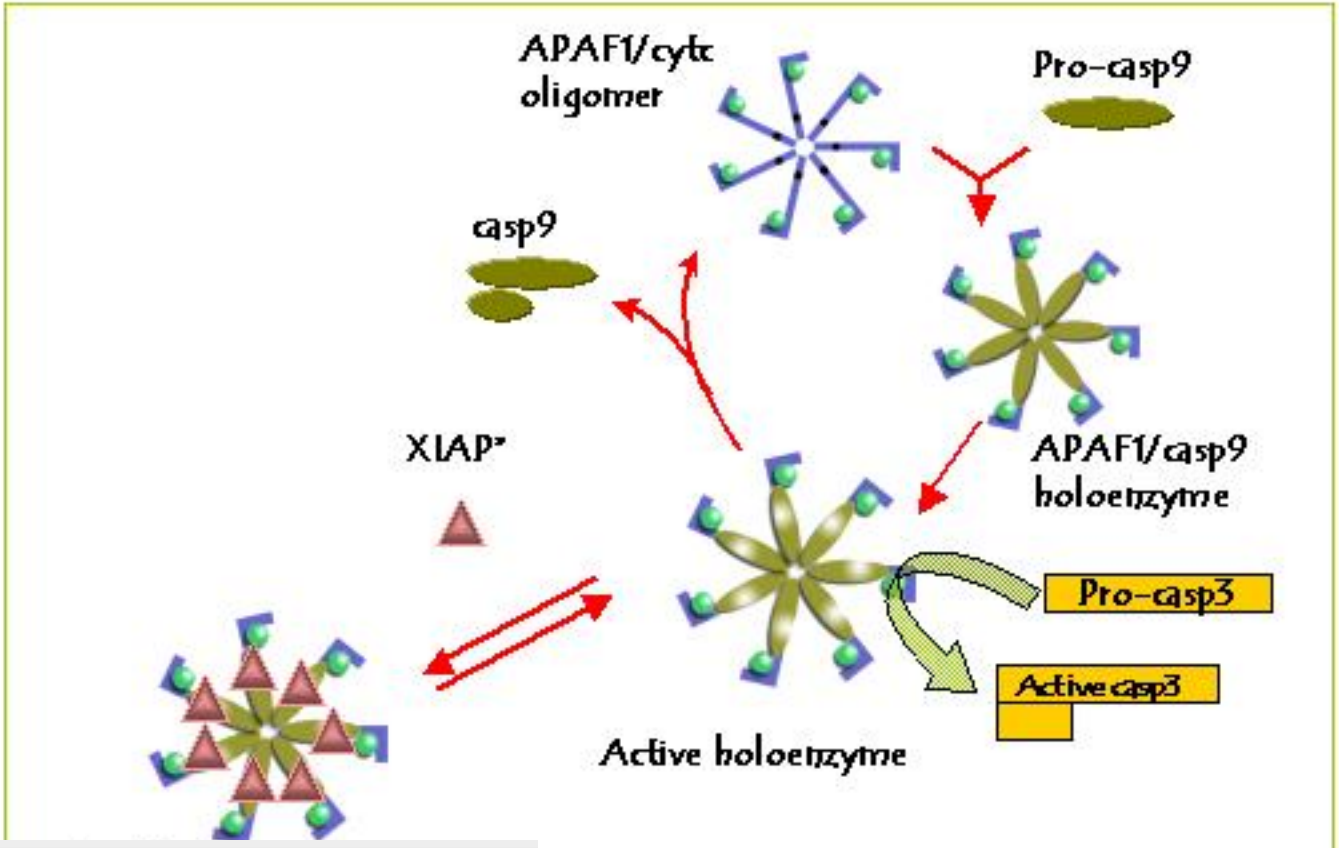
Caspase-9 inhibition

XIAP is thought to bind apoptosome via caspase9 and inhibit its activity. Does it exert a significant inhibition at that point?



*XIAP: human inhibitor of apoptosis

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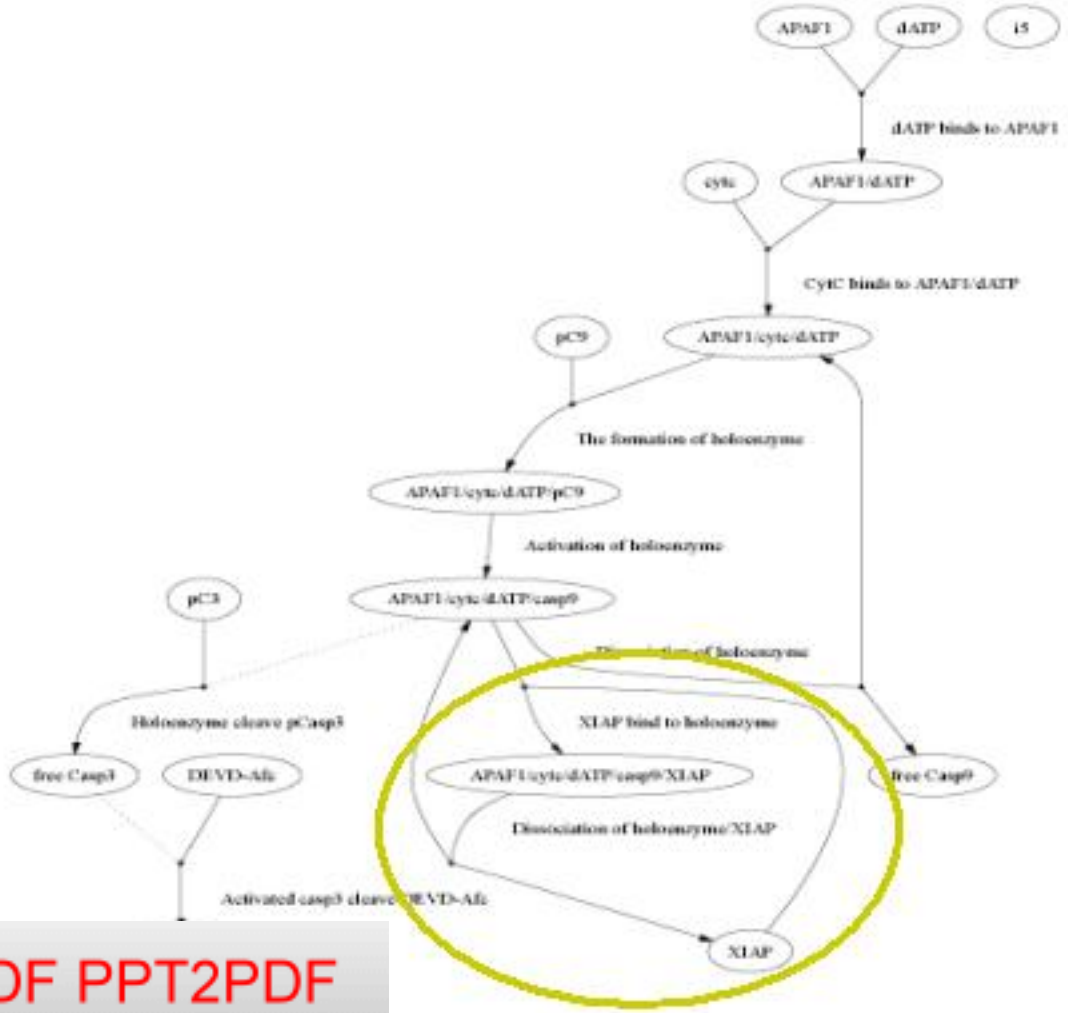
Simulate Caspase-9 inhibition



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XIAP Simulation with Simpathica

APAF1/CytC/dATP/casp9 + XIAP [50nM]

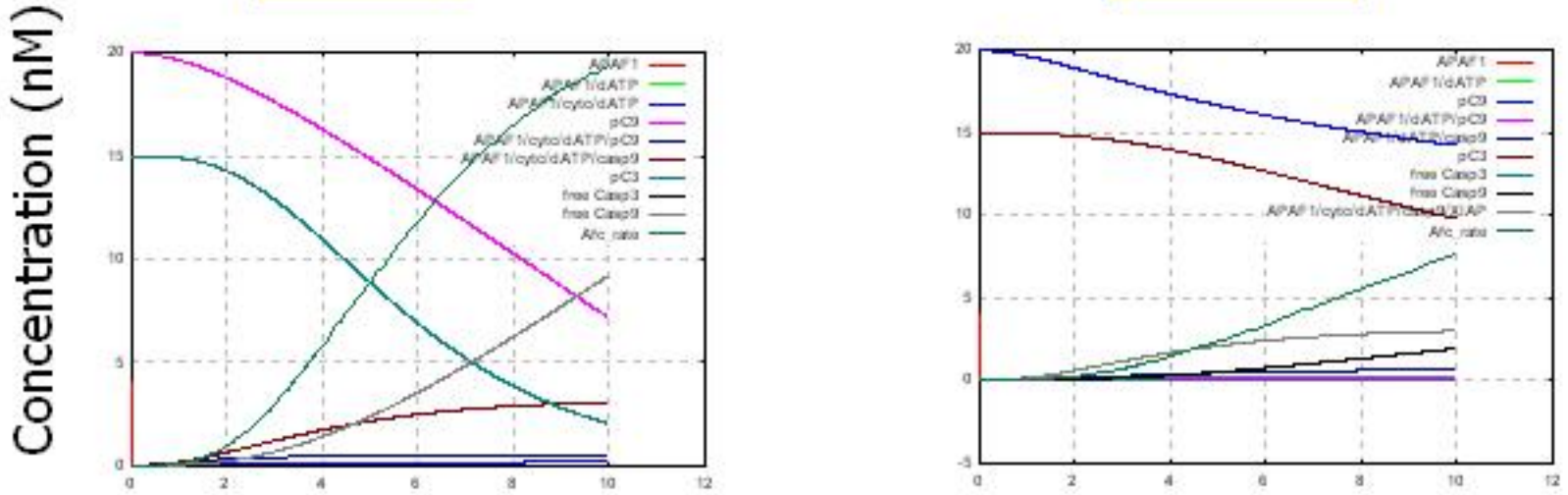
k-1 = 50

k1 = 0.5

APAF1/CytC/dATP/casp9/XIAP

No XIAP

With XIAP



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Summary

- Simpathica recapitulates
  - Formation of Caspase9/APAF1 holoenzyme.
  - dATP binds to APAF-1 and initiates the caspase-9 pathway.
  - Cytochrome c is not necessary to activate holoenzyme.
  - Non-linear interaction is due to cooperative binding of APAF-1 complex unit.
  - Free caspase-9 is not necessary to activate caspase-3.

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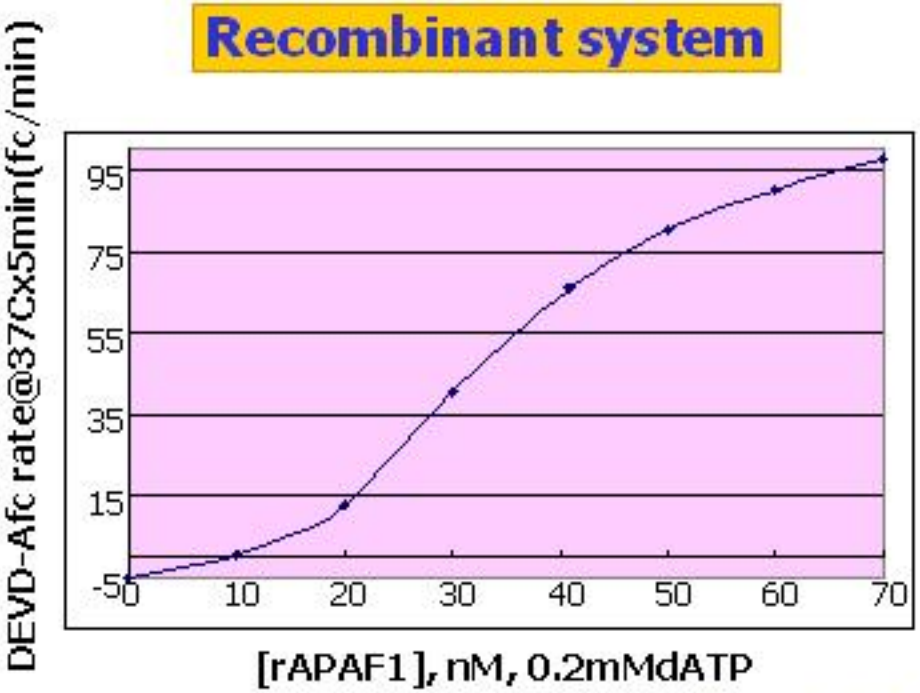
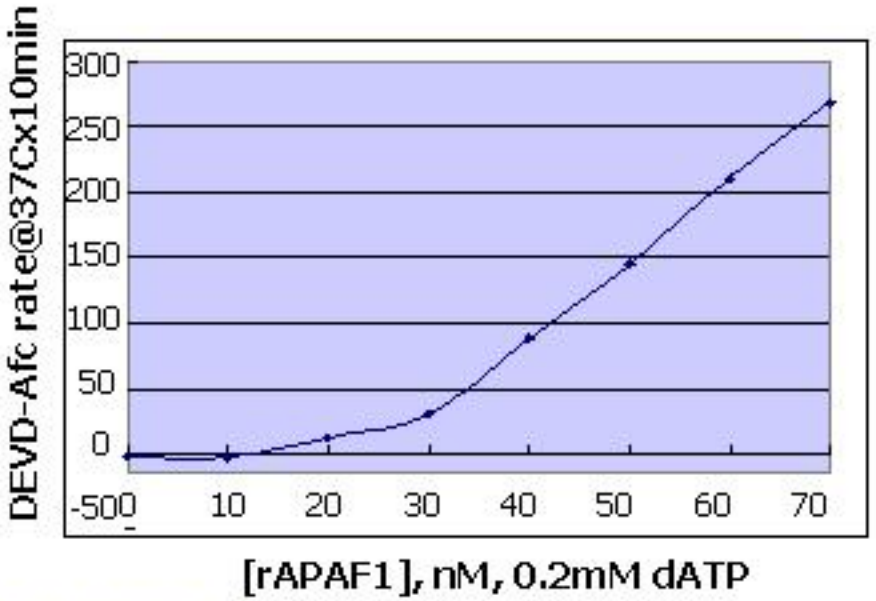
CONUNDRUM

- **Different outcomes from two experimental systems**

Recombinant system



In vitro system



Lazebnik lab (CSHL)

lators with simulation model

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Other Examples

- C. elegans (Gonad)
- Yeast and Mammalian Cell Cycle
- Wnt Signaling
- Host-pathogen Interactions
- RAS pathways…

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Some Biology

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Introduction to Biology

- Genome:
  - Hereditary information of an organism is encoded in its DNA and enclosed in a cell (unless it is a virus). All the information contained in the DNA of a single organism is its genome.
- DNA molecule
  - can be thought of as a very long sequence of nucleotides or bases:
    - $\Sigma = \{A, T, C, G\}$

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Complementarity

- DNA is a double-stranded polymer
  - should be thought of as a pair of sequences over Σ.
- A relation of **complementarity**
  - $A \Leftrightarrow T, C \Leftrightarrow G$
  - If there is an A (resp., T, C, G) on one sequence at a particular position then the other sequence must have a T (resp., A, G, C) at the same position.
- The sequence length
  - Is measured in terms of base pairs (bp): Human (H. sapiens) DNA is 3.3×10^9 bp, about 6 ft of DNA polymer completely stretched out!

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Genome Size

- **The genomes vary widely in size:**
  - Few thousand base pairs for viruses to 2 ~ 3×10^{11}bp for certain amphibian and flowering plants.
  - Coliphage MS2 (a virus) has the smallest genome: only 3.5×10^{3}bp.
  - Mycoplasmas (a unicellular organism) has the smallest cellular genome: 5×10^{5}bp.
  - *C. elegans* (nematode worm, a primitive multicellular organism) has a genome of size ~ 10^{8}bp.

Species	Haploid Genome Size	Chromosome Numer
E. Coli	4.64×10^{6}	1
S.cerevisae	1.205×10^{7}	16
C. elegans	10^{8}	11/12
D. melanogaster	1.7×10^{8}	4
M. musculus	3×10^{9}	20
H. sapiens	3×10^{9}	23
A. Cepa (Onion)	1.5×10^{10}	8

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DNA ⇒ Structure and Components

- Double helix
  - The usual configuration of DNA is in terms of a double helix consisting of two chains or strands coiling around each other with two alternating grooves of slightly different spacing.
  - The "backbone" in each strand is made of alternating sugar molecules (Deoxyribose residues: $C_5 O_4 H_{10}$) and phosphate ($(P O_4)^{-3}$) molecules.
- Each of the four bases, an almost planar nitrogenic organic compound, is connected to the sugar molecule.
  - The bases are:

    Adenine ⇒ A; Thymine ⇒ T; Cytosine ⇒ C; Guanine ⇒ G

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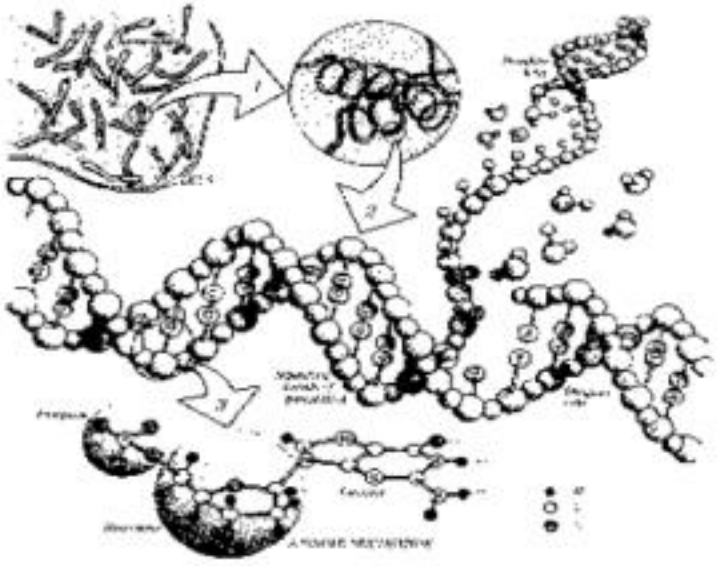
Genome in Detail



The Human Genome at Four Levels of Detail.

Apart from reproductive cells (gametes) and mature red blood cells, every cell in the human body contains 23 pairs of chromosomes, each a packet of compressed and entwined DNA (1, 2).

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DNA ⇒ Structure and Components

- Complementary base pairs
  - (A-T and C-G) are connected by hydrogen bonds and the base-pair forms a coplanar "rung"
    - Cytosine and thymine are smaller (lighter) molecules, called pyrimidines
    - Guanine and adenine are bigger (bulkier) molecules, called purines.
    - Adenine and thymine allow only for double hydrogen bonding, while cytosine and guanine allow for triple hydrogen bonding.

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DNA ⇒ Structure and Components

- Chemically inert and mechanically rigid and stable
  - Thus the chemical (through hydrogen bonding) and the mechanical (purine to pyrimidine) constraints on the pairing lead to the complementarity and makes the double stranded DNA both chemically inert and mechanically quite rigid and stable.
- Most uninteresting molecule:
  - "DNA, on its own, does nothing," smirked Natalie Angier recently. "It can't divide, it can't keep itself clean or sit up properly — proteins that surround it do all those tasks. Stripped of context within the body's cells ... DNA is helpless, speechless — DOA."

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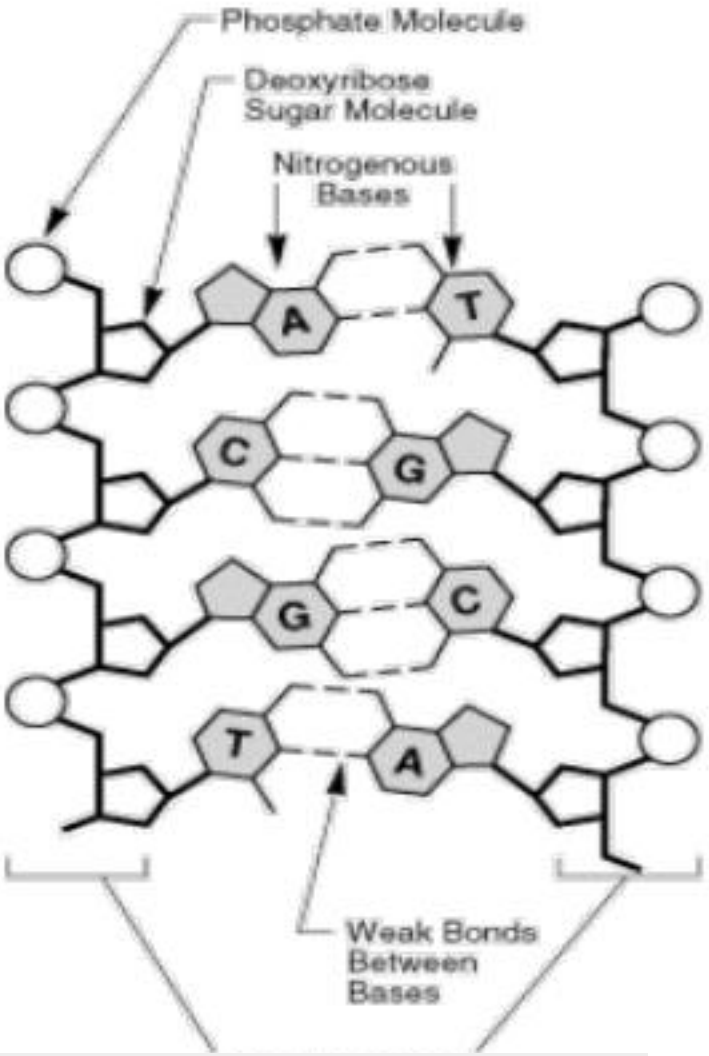
DNA Structure.



- The four nitrogenous bases of DNA are arranged along the sugar-phosphate backbone in a particular order (the DNA sequence), encoding all genetic instructions for an organism. Adenine (A) pairs with thymine (T), while cytosine (C) pairs with guanine (G). The two DNA strands are held together by weak bonds between the bases.

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DNA ⇒ Structure and Components

- The building blocks of the DNA molecule are four kinds of deoxyribonucleotides,
  - where each deoxyribonucleotide is made up of a sugar residue, a phosphate group and a base.
  - From these building blocks (or related, dNTPs deoxyribonucleoside triphosphates) one can synthesize a strand of DNA.

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DNA ⇒ Structure and Components

- The sugar molecule
  - in the strand is in the shape of a pentagon (4 carbons and 1 oxygen) in a plane parallel to the helix axis and with the 5th carbon (5′ C) sticking out.
- The phosphodiester bond (-O-P-O-)
  - between the sugars connects this 5′ C to a carbon in the pentagon (3′ C) and provides a directionality to each strand.
- The strands in a double-stranded DNA molecule are antiparallel.

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To be continued…

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