Supplemental Text

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The following tables contain an overview of all reactions and their parameterisation, together with additional explanatory text.

Table 1: Abbreviations for proteins and protein complexes used in the model reactions.

Entity Name	Description
PC3	Procaspase-3 (DIMER)
C3	Cleaved, unbound and active caspase-3 (DIMER)
PC9	Uncleaved, unbound and inactive procaspase-9 (monomer)
C9	Cleaved, unbound and inactive caspase-9 (monomer)
SMAC	Unbound SMAC
XIAP	Unbound XIAP
BIR12	BIR12 fragment of XIAP
BIR3	BIR3 fragment of XIAP, also including the RING domain
dATP/ATP	dATP/ATP
CytC_Mito	Mitochondrial cytochrome c fraction
CytC	cytochrome c released into cytosol
Apaf-1	Monomeric and inactive Apaf-1
[Apaf1~CytC]	Monomeric Apaf-1 with bound cytochrome c
[Apaf1~ATP]	Monomeric Apaf-1 with bound ATP
[Apaf1~CytC~ATP]	Monomeric Apaf-1 with bound cytochrome c and ATP. Active state of Apaf-1.
[Apaf1~CytC~ATP]_x [Apaf1~CytC~ATP]_y	Activated Apaf-1 homo-oligomer. x and y can be any number from 1-6, accordingly representing monomer, dimer, trimer, tetramer, pentamer and hexamer.
Apoptosome	Fully assembled heptameric Apaf-1 complex
Apoptosome_PC9_n_C9_m	Fully assembled heptameric Apaf-1 complex with bound procaspase-9 or/and caspase-9. n indicates the number of bound procaspase-9 monomers; m indicates the number for bound caspase-9 monomers. n and m can range from 0-7. Only a maximum of seven (n+m) pro-/caspase-9 monomers can bind to the heptameric Apaf-1 complex.
[Apoptosome_PC9_n_C9_m~XIAP]	The apoptosome_PC9_n_C9_m complex to which additionally XIAP is bound.
[XIAP~SMAC]	Complex consisting of XIAP and SMAC.
[XIAP~C3]	Complex consisting of XIAP and cleaved caspase-3.

Table 2: Reactions for the Apaf-1 activation process

Reaction 1 describes the release of mitochondrial cytochrome c into the cytosol. The activation Apaf-1 monomers is captured by reactions 2 to 5. Reactions 6 to 10 describe the degradation of the protein fractions. Reaction 11 describes the production of Apaf-1. The kinetics for these reactions can be found in table 3. Table 4 describes the parameterisation for the initial reactant concentrations.

Reaction No.	Reactant 1		Reactant 2		Product 1		Product 2	Comment
1	CytC_Mito			=>	CytC			
2	Apaf-1	+	CytC	<=>	[Apaf1~CytC]			
3	Apaf-1	+	dATP	<=>	[Apaf1~ATP]			
4	[Apaf1~CytC]	+	dATP	<=>	[Apaf1~CytC~ATP]			
5	[Apaf1~ATP]	+	CytC	<=>	[Apaf1~CytC~ATP]			
6	Apaf-1			=>				Degradation
7	CytC			=>				Degradation
8	[Apaf1~CytC]			=>				Degradation
9	[Apaf1~ATP]			=>				Degradation
10	[Apaf1~CytC~ATP]_x			=>				Degradation
11				=>	Apaf-1			Production

Table 3: Parameterisation of the reactions for the Apaf-1 activation process

Reaction No.	Forward reaction kinetics	Backward reaction kinetics	Reference
1	0.4621 $\mu M^{-1} min^{-1}$		(Rehm et al, 2003; Waterhouse et al, 2001)
2	0.24 $\mu M^{-1} min^{-1}$	0.006 min^{-1}	(Purring-Koch & McLendon, 2000; Purring et al, 1999)
3	0.1359 $\mu M^{-1} min^{-1}$	0.1155 min^{-1}	(Jiang & Wang, 2000; Reubold et al, 2009)
4	0.1359 $\mu M^{-1} min^{-1}$	0.1155 min^{-1}	(Jiang & Wang, 2000; Reubold et al, 2009)
5	0.24 $\mu M^{-1} min^{-1}$	0.006 min^{-1}	K_d (Purring-Koch & McLendon, 2000) K_{off} (Purring et al, 1999)
6	0.00048 min^{-1}		Half time 24h
7	0.000385 min^{-1}		(Ferraro et al, 2008)
8	0.0058 min^{-1}		(Eissing et al, 2004)
9	0.0058 min^{-1}		(Eissing et al, 2004)
10	0.0058 min^{-1}		(Eissing et al, 2004)
11	Degradation kinetic * initial concentration $\mu M*min^{-1}$		

Table 4: Parameterisation with protein concentrations to mimic HeLa cervical cancer cells

Protein	Concentration [µM]	Reference
Cytochrome C (CytC)	10.000	(Waterhouse et al, 2001)
dATP/ATP	920.000	(Mesner et al, 1999)
Procaspase-9 (PC9)	0.030	(Rehm et al, 2006)
Apaf-1	0.372	(Rehm et al, 2006)
Procaspase-3 (PC3)	0.120	(Rehm et al, 2006)
XIAP	0.063	(Rehm et al, 2006)
SMAC	0.126	(Rehm et al, 2006), assumed as 2x XIAP

Table 5: Reactions for Apaf-1 oligomerisation into the heptameric complex

Reaction 12 describes the oligomerisation of Apaf-1 for oligomer sizes ≤ 6. The variables x and y indicate the oligomeriation state and can range from 1 to 5. It has been taken into account that an oligomer can dissociate smaller oligomers or monomers, since reaction 12 is reversible. Reaction 13 describes the oligomerisation of Apaf-1 oligomers into the mature heptameric apoptosome. Here, the sum of x and y is 7. The degradation of the apoptosome is described in reaction 14. The corresponding kinetics for all reactions are provided in table 6.

No.	Reactant 1		Reactant 2		Product 1		Product 2	Comment
	For combinations where x+y<=6							
12	[Apaf1~CytC~ATP]_x	+	[Apaf1~CytC~ATP]_y	<=>	[Apaf1~CytC~ATP]_x+y			
	For combinations where x+y=7							
13	[Apaf1~CytC~ATP]_x	+	[Apaf1~CytC~ATP]_y	=>	Apoptosome			
14	Apoptosome			=>				Degradation

Table 6: Parameterisation of the reactions for Apaf-1 oligomerisation into the heptameric complex

No.	Forward reaction kinetics	Backward reaction kinetics	Reference
12	40 $\mu M^{-1} min^{-1}$	0.004 min^{-1}	(Cain et al, 2000)
13	40 $\mu M^{-1} min^{-1}$		(Cain et al, 2000)
14	0.0039 min^{-1}		(Eissing et al, 2004)

Table 7: Reactions for the inclusion of apoptosis execution reactions including (Pro-)caspase-9, XIAP, SMAC, (Pro-)caspase-3

Reaction 15 describes the cleavage of procaspase-9 at the apoptosome by caspase-3. Reaction 16 describes the cleavage of caspase-3 substrate by active caspase-3. Also free procasapse-9 can be cleaved by active caspase-3 (reaction 17). The reversible caspase inhibitor XIAP is able to bind to catalytically active and cleaved caspase-9 (reaction 18) as well as to free and active caspase-3 (reaction 19). SMAC can reverse caspase inhibition by XIAP (reactions 20, 22) and by binding to the unbound XIAP (reaction 21). Combinations of pro-/caspase-9 bound to the apoptosome and the cleavage of procaspase-3 are covered in Reactions 23-27. Reactions 28 and 29 are only integrated in the APOPTO-ALL model as they describe the allosteric activity of

either a procaspase-9 or a caspase-9 monomer on the apoptosome. Procaspase-9 auto-processing for APOPTO-ALL is implemented in Reaction 30. For APOPTO-DIM procaspase-9 auto-processing is described in Reactions 31 and 32. The binding of caspase-9 and procaspase-9 to the apoptosome is described in Reactions 33 to 35. Reaction 35 also implements the replacement of caspase-9 by procaspase-9 on the apoptosome. Reactions 36 to 47 describe the degradation of all proteins and protein complexes. Reactions 48 to 51 describe protein production for XIAP, Apaf-1, Procaspase-9 and Procaspase-3. The kinetics for all reactions are listed in Table 8.

No.	Reactant 1		Reactant 2		Product 1		Product 2	Comment
15	Apoptosome_PC9_n_C9_m	+	C3	=>	Apoptosome_PC9_ n-1_C9_m+1	+	C3	
16	C3	+	Substrate_C3	=>	C3	+	Cleaved_ substrate_c3	
17	C3	+	PC9	=>	C3	+	C9	
18	Apoptosome_PC9_n_C9_m	+	XIAP	<=>	[Apoptosome_PC9_ n_C9_m~XIAP]			
19	C3	+	XIAP	<=>	[XIAP~C3]			
20	[Apoptosome_PC9_n_C9_m~XIAP]	+	SMAC	<=>	Apoptosome_PC9_ n_C9_m	+	[XIAP~SMAC]	
21	XIAP	+	SMAC	<=>	[XIAP~SMAC]			
22	[XIAP~C3]	+	SMAC	<=>	C3	+	[XIAP~SMAC]	
23	If at least two caspase-9 (n>=2) proteins are bound: Apoptosome_PC9_0_C9_m	+	PC3	=>	Apoptosome_PC9_ 0_C9_m	+	C3	
24	If at least two procaspase-9 (n>=2) proteins are bound: Apoptosome_PC9_n_C9_0	+	PC3	=>	Apoptosome_PC9_ n_C9_0	+	C3	
25	If more procaspase-9 than caspase-9 proteins are bound (n>m, m>=1): Apoptosome_PC9_n_C9_m	+	PC3	=>	Apoptosome_PC9_ n_C9_m	+	C3	
26	If more caspase-9 than procaspase-9 proteins are bound (n<m, n>=1): Apoptosome_PC9_n_C9_m	+	PC3	=>	Apoptosome_PC9_ n_C9_m	+	C3	
27	If the number of procaspase-9 equals caspase-9 (n=m, n>=1, m>=1): Apoptosome_PC9_n_C9_m	+	PC3	=>	Apoptosome_PC9_ n_C9_m	+	C3	
28	*Reaction for APOPTO-ALL only* Apoptosome_PC9_1_C9_0	+	PC3	=>	Apoptosome_PC9_1_C9_0	+	C3	
29	*Reaction for APOPTO-ALL only* Apoptosome_PC9_0_C9_1	+	PC3	=>	Apoptosome_PC9_0_C9_1	+	C3	
	Reaction for APOPTO-ALL only, n>=1:							
30	Apoptosome_PC9_n_C9_m			=>	Apoptosome_PC9_n-1_C9_m+1			
	Reaction for APOPTO-DIM only If at least 1 procaspase-9 and one caspase-9 are bound (n>=1 & m>=1)							
31	Apoptosome_PC9_n_C9_m			=>	Apoptosome_PC9_n-1_C9_m+1			
	Reaction for APOPTO-DIM only If at least two procaspase-9 monomers are bound (n>=2)							
32	Apoptosome_PC9_n_C9_m			=>	Apoptosome_PC9_n-2_C9_m+2			
	n+m <=6							
33	Apoptosome_PC9_n_C9_m	+	PC9	<=>	Apoptosome_PC9_n+1_C9_m			
34	Apoptosome_PC9_n_C9_m	+	C9	<=>	Apoptosome_PC9_n_C9_m+1			
	n+m <=6 & C9 >=1							
35	[Apoptosome_PC9_n_C9_m]	+	PC9	=>	[Apoptosome_PC9_n+1_C9_ m-1]	+	C9	
36	[XIAP~SMAC]			=>				Degradation
37	[XIAP~C3]			=>				Degradation
38	PC3			=>				Degradation
39	PC9			=>				Degradation
40	XIAP			=>				Degradation
41	Caspase-3			=>				Degradation
42	Caspase-9			=>				Degradation

43	SMAC			=>				Degradation
44	BIR12			=>				Degradation
45	BIR3			=>				Degradation
46	[Apoptosome_PC9_n_C9_m]			=>				Degradation
47	[Apoptosome_PC9_n_C9_m~XIAP]			=>				Degradation
48				=>	XIAP			Production
49				=>	APAF-1			Production
50				=>	PC9			Production
51				=>	PC3			Production

Table 8: Parameterisation of the reactions which include (Pro-)caspase-9, XIAP, SMAC, (Pro-)caspase-3

No.	Forward reaction kinetics	Backward reaction kinetics	Reference
15	0.105 $\mu M^{-1} min^{-1}$		(Timmer et al, 2009)
16	12 $\mu M^{-1} min^{-1}$		(Stennicke et al, 2000)
17	0.105 $\mu M^{-1} min^{-1}$		(Timmer et al, 2009)
18	156 $\mu M^{-1} min^{-1}$	0.144 min^{-1}	(Riedl et al, 2001)
19	156 $\mu M^{-1} min^{-1}$	0.144 min^{-1}	(Riedl et al, 2001)
20	420 $\mu M^{-1} min^{-1}$	156 min^{-1}	(Huang et al, 2003)
21	420 $\mu M^{-1} min^{-1}$	0.133 min^{-1}	(Huang et al, 2003)
22	420 $\mu M^{-1} min^{-1}$	156 min^{-1}	(Huang et al, 2003)
23	*APOPTO-DIM & APOPTO-ALL:* 73.38 $\mu M^{-1} min^{-1}$ *Doubled for APOPTO-DIM fit2x:* (146.76 $\mu M^{-1} min^{-1}$)		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
24	*APOPTO-DIM & APOPTO-ALL:* 63.38 $\mu M^{-1} min^{-1}$ *Doubled for APOPTO-DIM fit2x:* (126.76 $\mu M^{-1} min^{-1}$)		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
25	*APOPTO-DIM & APOPTO-ALL:* 63.38 $\mu M^{-1} min^{-1}$ *Doubled for APOPTO-DIM fit2x:* (126.76 $\mu M^{-1} min^{-1}$)		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
26	*APOPTO-DIM & APOPTO-ALL:* 73.38 $\mu M^{-1} min^{-1}$ *Doubled for APOPTO-DIM fit2x:* (146.76 $\mu M^{-1} min^{-1}$)		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
27	*APOPTO-DIM & APOPTO-ALL:* 73.38 $\mu M^{-1} min^{-1}$ *Doubled for APOPTO-DIM fit2x:* (146.76 $\mu M^{-1} min^{-1}$)		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
28	*APOPTO- ALL:* 73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
29	*APOPTO- ALL:* 63.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
30	73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
31	73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
32	73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
33	APOPTO-ALL: 2.85 $\mu M^{-1} min^{-1}$ APOPTO-DIM (untrained): 2.85 $\mu M^{-1} min^{-1}$ $APOPTO\text{-}DIM_{Fit}$: 2.85 $\mu M^{-1} min^{-1}$ APOTPO-DIM (two catalytic sites) 1.42 $\mu M^{-1} min^{-1}$ $APOPTO\text{-}DIM_{Fit2x}$ (two catalytic sites): 5 $\mu M^{-1} min^{-1}$	APOPTO-ALL: 2 min^{-1} APOPTO-DIM (untrained): 2 min^{-1} $APOPTO\text{-}DIM_{Fit}$: 0.2 min^{-1} APOTPO-DIM (two catalytic sites) 1 min^{-1} $APOPTO\text{-}DIM_{Fit2x}$ (two catalytic sites): 1 min^{-1}	APOPTO-ALL: K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) APOPTO-DIM (untrained): K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) $APOPTO\text{-}DIM_{Fit}$: fitted APOTPO-DIM (two catalytic sites) K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) $APOPTO\text{-}DIM_{Fit2x}$ (two catalytic sites): fitted
34	APOPTO-ALL: 0.285 $\mu M^{-1} min^{-1}$	APOPTO-ALL: 2 min^{-1}	APOPTO-ALL: K_d (Malladi et al, 2009; Palacios-Rodriguez et al,

	APOPTO-DIM (untrained): 0.285 $\mu M^{-1} min^{-1}$ APOPTO-DIM_{Fit}: 0.285 $\mu M^{-1} min^{-1}$ APOTPO-DIM (two catalytic sites) 0.142 $\mu M^{-1} min^{-1}$ APOPTO-DIM_{Fit2x} (two catalytic sites): 0.5 $\mu M^{-1} min^{-1}$	APOPTO-DIM (untrained): 2 min^{-1} APOPTO-DIM_{Fit}: 0.2 min^{-1} APOTPO-DIM (two catalytic sites) 1 min^{-1} APOPTO-DIM_{Fit2x} (two catalytic sites): 1 min^{-1}	2011) APOPTO-DIM (untrained): K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) APOPTO-DIM_{Fit}: fitted APOTPO-DIM (two catalytic sites) K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) APOPTO-DIM_{Fit2x} (two catalytic sites): fitted
35	APOPTO-ALL: 2.85 $\mu M^{-1} min^{-1}$ APOPTO-DIM (untrained): 2.85 $\mu M^{-1} min^{-1}$ APOPTO-DIM_{Fit}: 2.85 $\mu M^{-1} min^{-1}$ APOTPO-DIM (two catalytic sites) 1.42 $\mu M^{-1} min^{-1}$ APOPTO-DIM_{Fit2x} (two catalytic sites): 5 $\mu M^{-1} min^{-1}$		APOPTO-ALL: K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) APOPTO-DIM (untrained): K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) APOPTO-DIM_{Fit}: fitted APOTPO-DIM (two catalytic sites) K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011) APOPTO-DIM_{Fit2x} (two catalytic sites): fitted
36	0.0347 min^{-1}		(Yoo et al, 2002)
37	0.0347 min^{-1}		(Yoo et al, 2002)
38	0.0039 min^{-1}		(Eissing et al, 2004)
39	0.0039 min^{-1}		(Eissing et al, 2004)
40	0.0116 min^{-1}		(Eissing et al, 2004)
41	0.0058 min^{-1}		(Eissing et al, 2004)
42	0.0058 min^{-1}		(Eissing et al, 2004)
43	0.000385 min^{-1}		(Ferraro et al, 2008)
44	0.0058 min^{-1}		(Eissing et al, 2004)
45	0.0347 min^{-1}		(Yoo et al, 2002)
46	0.0039 min^{-1}		(Eissing et al, 2004)
47	0.0039 min^{-1}		(Eissing et al, 2004)
48	Degradation kinetic * initial concentration $\mu M*min^{-1}$		
49	Degradation kinetic * initial concentration $\mu M*min^{-1}$		
50	Degradation kinetic * initial concentration $\mu M*min^{-1}$		
51	Degradation kinetic * initial concentration $\mu M*min^{-1}$		

Table 9: Reactions for (Pro-)caspase-9 binding to Apaf-1 oligomers

Reactions 52 and 53 (Table 9) describe the binding of procaspase-9 and caspase-9 towards Apaf-1 oligomers smaller than the fully assembled heptameric apoptosome. The number (x) of oligomerised Apaf-1 proteins sets the maximum binding capacity for pro-/caspase-9. Therefore Reactions 52 and 53 can only occur if at least one binding site is free. Reaction 54 describes the auto-processing of a procaspase-9 homo-dimer that is bound to an Apaf-1 oligomer. The auto-cleavage of a pro-/caspase-9 hetero-dimer is implemented in Reaction 55. Apaf-1 oligomers that contain bound pro-/caspase-9 can still reversibly oligomerise with other Apaf-1 monomers/oligomers into higher complexes that contain up to six Apaf-1 proteins (Reactions 56 and 57). Procaspase-9 proteins that are bound to Apaf-1 oligomers can be cleaved by caspase-3 (Reaction 58). If two pro-/caspase-9 proteins are bound to one Apaf-1 complex they possess catalytic activity to cleave procaspase-3 (Reaction 59). Reactions 60 and 61 describe the oligomerisation of Apaf-1 complexes with bound pro-/caspase-9

into the fully assembled apoptosome complex (seven Apaf-1 proteins). The active replacement of caspase-9 by procaspase-9 on Apaf-1 oligomers is implemented in Reaction 62. Reaction 63 describes the degradation of all Apaf-1 oligomers with bound pro-/caspase-9. The corresponding kinetics for the reactions in Table 9 can be found in Table 10.

No.	Reactant 1		Reactant 2		Product 1		Product 2	Comment
	Reaction requires that at least 1 Apaf-1 CARD binding site has to be free (n+m < x):							
52	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	PC9	<=>	[Apaf1~CytC~ATP] _x_PC9_n+1_C9_m			
	Reaction requires that at least 1 Apaf-1 CARD binding site has to be free (n+m < x):							
53	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	C9	<=>	[Apaf1~CytC~ATP] _x_PC9_n_C9_m+1			
	Reaction requires at least two procaspase-9 proteins (n>=2):							
54	[Apaf1~CytC~ATP]_x_PC9_n_C9_m			=>	Apaf1~CytC~ATP] _x_PC9_n-2_C9_m+2			
	Reaction requires at least 1 procaspase-9 and one caspase-9 protein (n=1 & m>=1):							
55	[Apaf1~CytC~ATP]_x_PC9_n_C9_m			=>	[Apaf1~CytC~ATP] _x_PC9_n-1_C9_m+1			
	Two Apaf-1 oligomers can oligomerise if they do not exceed a hexamer (x1+x2<7):							
56	[Apaf1~CytC~ATP] _x1_PC9_n1_C9_m1	+	[Apaf1~CytC~ATP] _x2_PC9_n2_C9_ m2	<=>	[Apaf1~CytC~ATP] _x1+x2_PC9_n1+n1_C9_m1+m 2			
	Two Apaf-1 oligomers can oligomerise if they do not exceed a hexamer (x+y<7):							
57	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	[Apaf1~ CytC~ATP]_y	<=>	[Apaf1~CytC~ATP] _x+y_PC9_n_C9_m			
	n>=1:							
58	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	C3	=>	[Apaf1~CytC~ATP] _x_PC9_n-1_C9_m+1	+	C3	
	n+m>=2							
59	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	PC3	=>	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	C3	
	Two Apaf-1 oligomers can oligomerise into the fully assembled apoptosome (x+y<7):							
60	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	[Apaf1~ CytC~ATP]_y	=>	Apoptosome_PC9_n_C9_m			
	Two Apaf-1 oligomers can oligomerise into the fully assembled apoptosome (x+y<7):							
61	[Apaf1~CytC~ATP] _x_PC9_n_C9_m	+	[Apaf1~CytC~ATP] _y_PC9_n_C9_m	=>	Apoptosome_PC9_n_C9_m			
62	[Apaf1~CytC~ATP]_x_PC9_n_C9_m	+	PC9	=>	[Apaf1~CytC~ATP]_x_PC9_n_ C9_m	+	C9	
63	[Apaf1~CytC~ATP_PC9_n_C9_m]			=>				Degradation

Table 10: Parameterisation of the reactions for (Pro-)caspase-9 binding to Apaf-1 oligomers

No.	Forward reaction kinetic	Backward reaction kinetic	Reference
52	2.85 $\mu M^{-1} min^{-1}$	2 min^{-1}	K_d (Palacios-Rodriguez et al, 2011)
53	0.285 $\mu M^{-1} min^{-1}$	2 min^{-1}	K_d (Palacios-Rodriguez et al, 2011)
54	73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
55	73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
56	40 $\mu M^{-1} min^{-1}$	0.004 min^{-1}	(Cain et al, 2000)
57	40 $\mu M^{-1} min^{-1}$	0.004 min^{-1}	(Cain et al, 2000)
58	0.105 $\mu M^{-1} min^{-1}$		(Timmer et al, 2009)
59	73.38 $\mu M^{-1} min^{-1}$		K_{cat} (Pop et al, 2006) K_m (Malladi et al, 2009)
60	40 $\mu M^{-1} min^{-1}$	0.004 min^{-1}	(Cain et al, 2000)
61	40 $\mu M^{-1} min^{-1}$	0.004 min^{-1}	(Cain et al, 2000)
62	2.85 $\mu M^{-1} min^{-1}$		K_d (Malladi et al, 2009; Palacios-Rodriguez et al, 2011)
63	0.0039 min^{-1}		(Eissing et al, 2004)

Table 11: Parameterisation with protein concentrations to mimic Molecular Timer experiments conducted by Malladi *et al.*

The concentrations listed below were used in in vitro settings by Malladi *et al.* and were used in simulations investigating the molecular timer function of the apoptosome.

Protein	Concentration [µM]	Reference
Cytochrome C (CytC)	10	(Malladi et al, 2009)
dATP/ATP	1000	(Malladi et al, 2009)
Procaspase-9 (PC9)	0.0125	(Malladi et al, 2009)
Apaf-1	0.3	(Malladi et al, 2009)
Procaspase-3 (PC3)	0.5	(Malladi et al, 2009)
Substrate Caspase-3	15	(Malladi et al, 2009)

References

Cain K, Bratton SB, Langlais C, Walker G, Brown DG, Sun XM, Cohen GM (2000) Apaf-1 oligomerizes into biologically active approximately 700-kDa and inactive approximately 1.4-MDa apoptosome complexes. *J Biol Chem* **275:** 6067-6070

Eissing T, Conzelmann H, Gilles ED, Allgower F, Bullinger E, Scheurich P (2004) Bistability Analyses of a Caspase Activation Model for Receptor-induced Apoptosis. *J Biol Chem* **279:** 36892-36897

Ferraro E, Pulicati A, Cencioni MT, Cozzolino M, Navoni F, di Martino S, Nardacci R, Carri MT, Cecconi F (2008) Apoptosome-deficient cells lose cytochrome c through proteasomal degradation but survive by autophagy-dependent glycolysis. *Molecular biology of the cell* **19:** 3576-3588

Huang Y, Rich RL, Myszka DG, Wu H (2003) Requirement of both the second and third BIR domains for the relief of X-linked inhibitor of apoptosis protein (XIAP)-mediated caspase inhibition by Smac. *J Biol Chem* **278:** 49517-49522

Jiang X, Wang X (2000) Cytochrome c promotes caspase-9 activation by inducing nucleotide binding to Apaf-1. *J Biol Chem* **275:** 31199-31203

Malladi S, Challa-Malladi M, Fearnhead HO, Bratton SB (2009) The Apaf-1*procaspase-9 apoptosome complex functions as a proteolytic-based molecular timer. *The EMBO journal* **28:** 1916-1925

Mesner PW, Jr., Bible KC, Martins LM, Kottke TJ, Srinivasula SM, Svingen PA, Chilcote TJ, Basi GS, Tung JS, Krajewski S, Reed JC, Alnemri ES, Earnshaw WC, Kaufmann SH (1999) Characterization of caspase processing and activation in HL-60 cell cytosol under cell-free conditions. Nucleotide requirement and inhibitor profile. *J Biol Chem* **274:** 22635-22645

Palacios-Rodriguez Y, Garcia-Lainez G, Sancho M, Gortat A, Orzaez M, Perez-Paya E (2011) Polypeptide modulators of caspase recruitment domain (CARD)-CARD-mediated protein-protein interactions. *J Biol Chem* **286:** 44457-44466

Pop C, Timmer J, Sperandio S, Salvesen GS (2006) The apoptosome activates caspase-9 by dimerization. *Molecular cell* **22:** 269-275

Purring-Koch C, McLendon G (2000) Cytochrome c binding to Apaf-1: the effects of dATP and ionic strength. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 11928-11931

Purring C, Zou H, Wang X, McLendon G (1999) Stoichiometry, Free Energy, and Kinetic Aspects of Cytochrome c: Apaf-1 Binding in Apoptosis. *Journal of the American Chemical Society* **121:** 7435-7436

Rehm M, Dussmann H, Prehn JH (2003) Real-time single cell analysis of Smac/DIABLO release during apoptosis. *The Journal of cell biology* **162:** 1031-1043

Rehm M, Huber HJ, Dussmann H, Prehn JH (2006) Systems analysis of effector caspase activation and its control by X-linked inhibitor of apoptosis protein. *The EMBO journal* **25:** 4338-4349

Reubold TF, Wohlgemuth S, Eschenburg S (2009) A new model for the transition of APAF-1 from inactive monomer to caspase-activating apoptosome. *J Biol Chem* **284:** 32717-32724

Riedl SJ, Renatus M, Schwarzenbacher R, Zhou Q, Sun C, Fesik SW, Liddington RC, Salvesen GS (2001) Structural basis for the inhibition of caspase-3 by XIAP. *Cell* **104:** 791-800

Stennicke HR, Renatus M, Meldal M, Salvesen GS (2000) Internally quenched fluorescent peptide substrates disclose the subsite preferences of human caspases 1, 3, 6, 7 and 8. *Biochem J* **350 Pt 2:** 563-568

Timmer JC, Zhu W, Pop C, Regan T, Snipas SJ, Eroshkin AM, Riedl SJ, Salvesen GS (2009) Structural and kinetic determinants of protease substrates. *Nature structural & molecular biology* **16:** 1101-1108

Waterhouse NJ, Goldstein JC, von Ahsen O, Schuler M, Newmeyer DD, Green DR (2001) Cytochrome c maintains mitochondrial transmembrane potential and ATP generation after outer mitochondrial membrane permeabilization during the apoptotic process. *The Journal of cell biology* **153:** 319-328

Yoo SJ, Huh JR, Muro I, Yu H, Wang L, Wang SL, Feldman RM, Clem RJ, Muller HA, Hay BA (2002) Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nature cell biology* **4:** 416-424