Two feedback loop stochastic model of p53 regulation

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Outline

- Introduction
- p53|Mdm2 regulatory module
- Existing models
- p53|Mdm2 model with feedback loop
- Model’s equations
- Results

Why p53|Mdm2 ?

- p53 regulates activity of hundreds of genes responsible among others for :

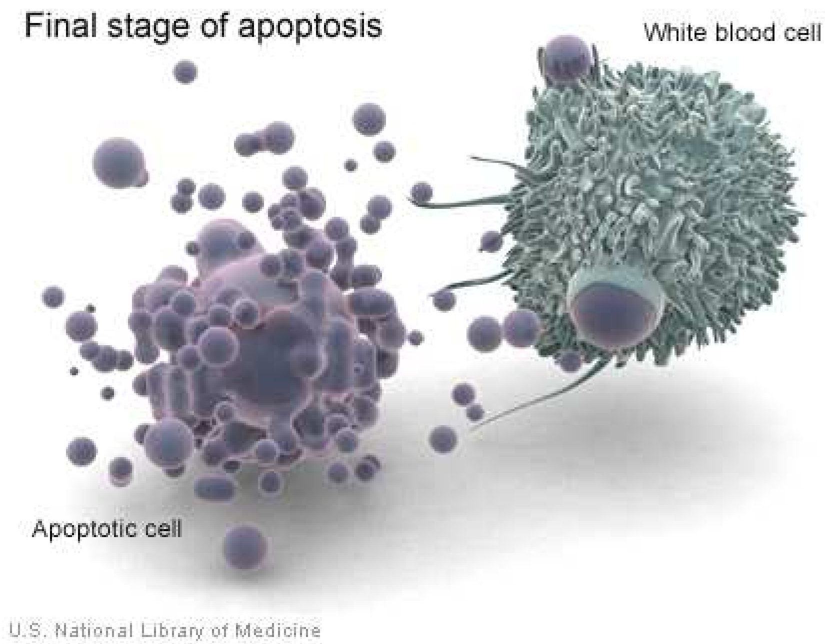
  - cell cycle arrest
  - DNA repair processes
  - apoptosis

- In 50% cancer cases p53 is mutated or not present. In remaining cases genes which are in it's regulatory module are mutated

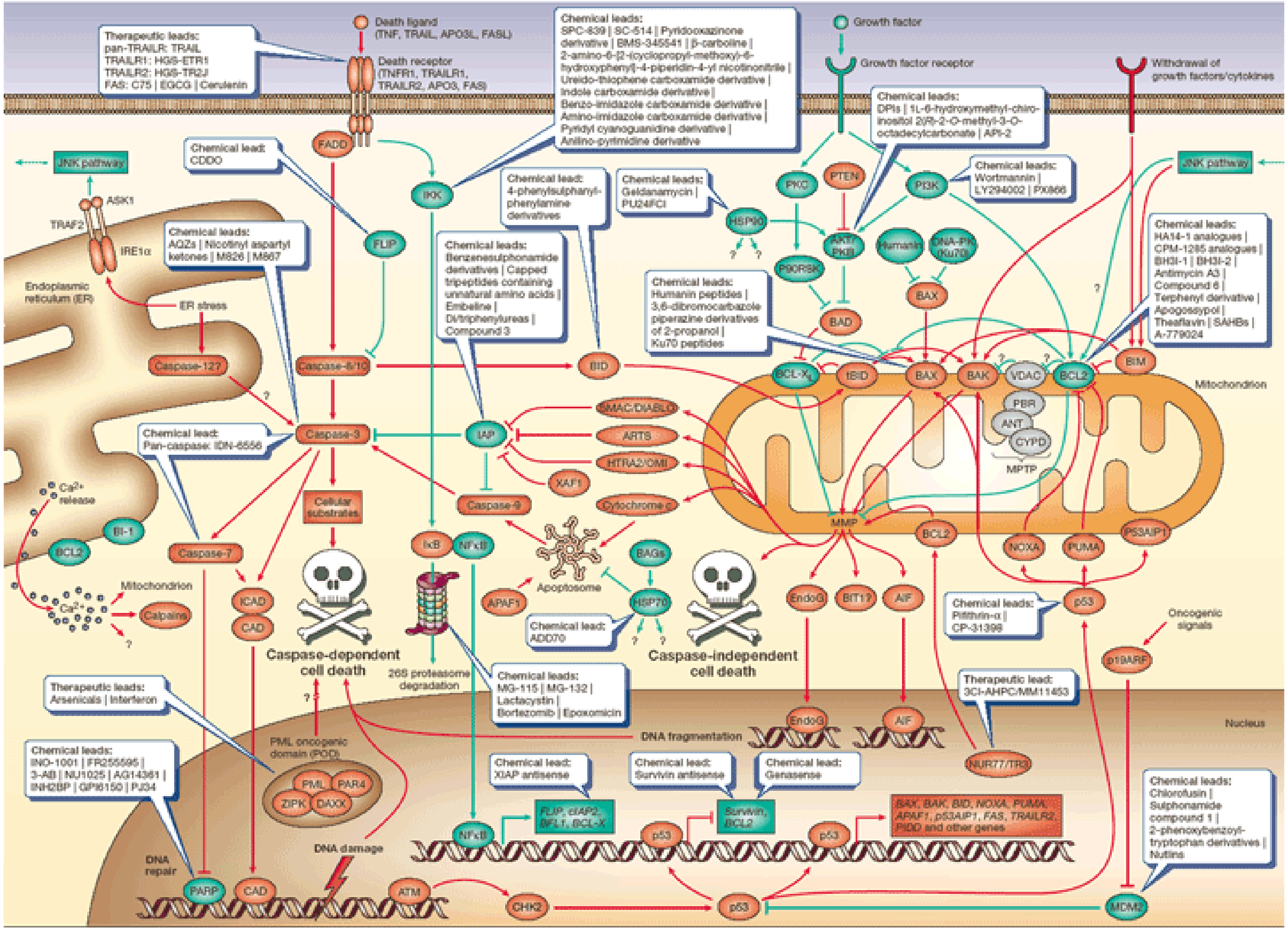
- There is over 50 000 experimental citations and only about 100 theoretical work.

Apoptosis

- Programmed cell death
- Characteristic cell morphology
- Safely cell's fragments removal
- About 50 - 70 billions of cells die every day due to apoptosis in average adult human

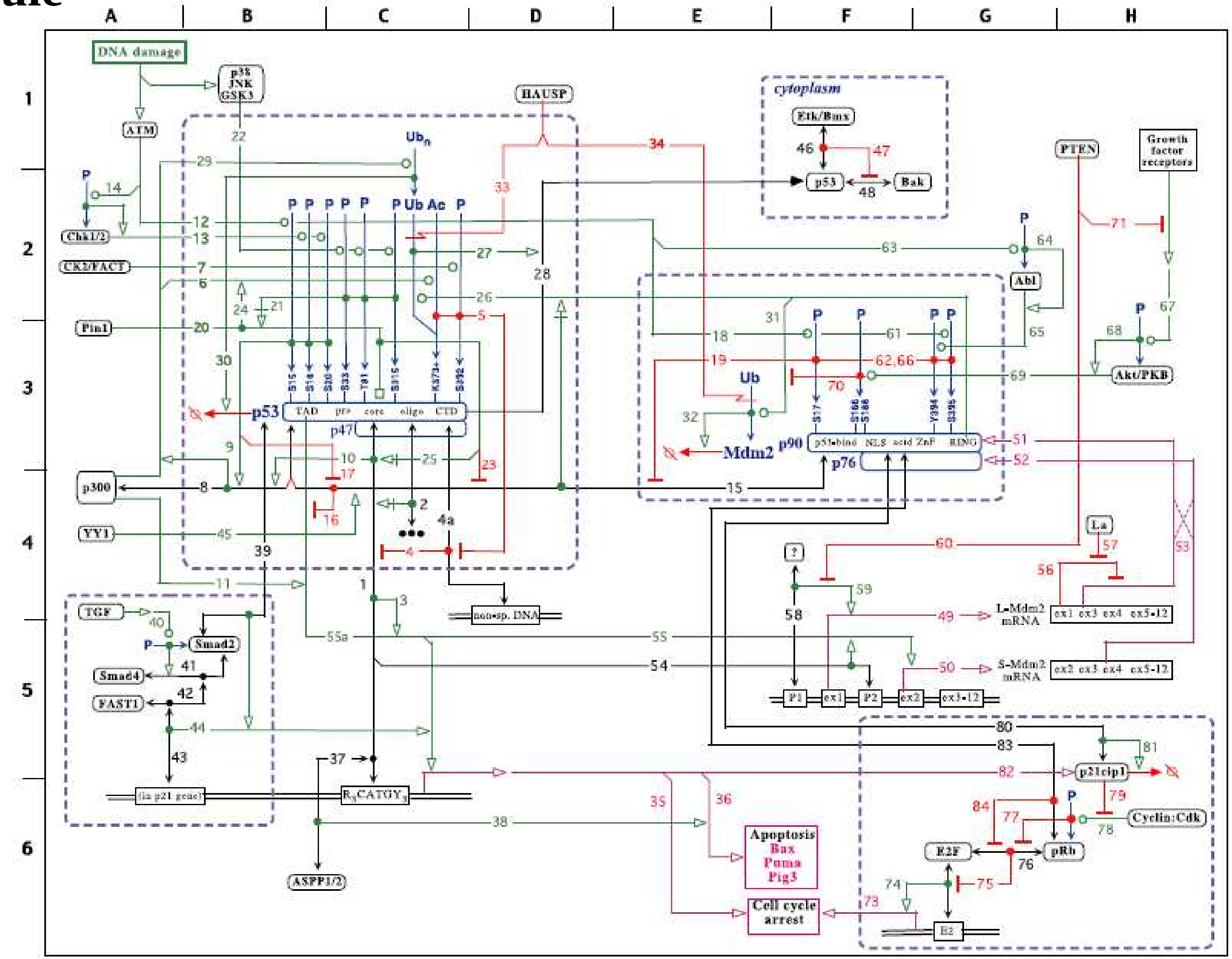


Apoptosis



Therapeutic leads:
pan-TRAILR: TRAIL
TRAILR1: HGS-ETR1
TRAILR2: HGS-TR2J
FAS: C75 | EGCG | Cerulenin
Death ligand
(TNF, TRAIL, APO3L, FASL)
Death receptor
(TNFR1, TRAILR1, TRAILR2, APO3, FAS)
Chemical leads:
SPC-839 | SC-514 | Pyridooxazinone derivative | BMS-345541 | β-carboline | 2-amino-6-[2-(cyclopropyl-methoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitrile | Ureido-thiophene carboxamide derivative | Indole carboxamide derivative | Benzo-imidazole carboxamide derivative | Amino-imidazole carboxamide derivative | Pyridyl cyanoguanidine derivative | Anilino-pyrimidine derivative
Growth factor
Growth factor receptor
Withdrawal of growth factors/cytokines
Chemical leads:
DPIs | 1L-6-hydroxymethyl-chiro-inositol 2(R)-2-O-methyl-3-O-octadecylcarbonate | API-2
FADD
JNK pathway
Chemical lead:
CDDO
ASK1
TRAF2
IRE1α
Endoplasmic reticulum (ER)
Chemical leads:
AQZs | Nicotinyl aspartyl ketones | M826 | M867
FLIP
IKK
Chemical lead:
4-phenylsulphanyl-phenylamine derivatives
Chemical leads:
Geldanamycin | PU24FCl
HSP90
PKC
PTEN
PI3K
Chemical leads:
Wortmannin | LY294002 | PX866
AKT/PKB
P90RSK
Humanin
DNA-PK (Ku70)
BAX
BAD
Chemical leads:
Benzenesulphonamide derivatives | Capped tripeptides containing unnatural amino acids | Embeline | Di/triphenylureas | Compound 3
Chemical leads:
Humanin peptides | 3,6-dibromocarbazole piperazine derivatives of 2-propanol | Ku70 peptides
Chemical leads:
HA14-1 analogues | CPM-1285 analogues | BH3I-1 | BH3I-2 | Antimycin A3 | Compound 6 | Terphenyl derivative | Apogossypol | Theaflavin | SAHBs | A-779024
ER stress
Caspase-12?
Caspase-8/10
BID
BCL-XL
tBID
BAX
BAK
VDAC
BCL2
BIM
Mitochondrion
PBR
ANT
CYPD
MPTP
Chemical lead:
Pan-caspase: IDN-6556
Caspase-3
IAP
SMAC/DIABLO
ARTS
HTRA2/OMI
XAF1
Cytochrome c
Caspase-9
Cellular substrates
Ca2+ release
BI-1
BCL2
Caspase-7
Mitochondrion
Ca2+
Calpains
ICAD
CAD
IκB
NFκB
Apoptosome
APAF1
BAGs
HSP70
Chemical lead:
ADD70
MMP
BCL2
NOXA
PUMA
P53AIP1
EndoG
BIT1?
AIF
Chemical leads:
Pifithrin-α | CP-31398
p53
Oncogenic signals
p19ARF
Caspase-dependent cell death
26S proteasome degradation
Caspase-independent cell death
Therapeutic leads:
Arsenicals | Interferon
Chemical leads:
MG-115 | MG-132 | Lactacystin | Bortezomib | Epoxomicin
Therapeutic lead:
3Cl-AHPC/MM11453
DNA fragmentation
EndoG
AIF
Nucleus
NUR77/TR3
PML oncogenic domain (POD)
PML
PAR4
ZIPK
DAXX
Chemical leads:
INO-1001 | FR255595 | 3-AB | NU1025 | AG14361 | INH2BP | GPI6150 | PJ34
Chemical lead:
XIAP antisense
Chemical lead:
Survivin antisense
Chemical lead:
Genasense
FLIP, cIAP2, BFL1, BCL-X
Survivin, BCL2
BAX, BAK, BID, NOXA, PUMA, APAF1, p53AIP1, FAS, TRAILR2, PIDD and other genes
Chemical leads:
Chlorofusin | Sulphonamide compound 1 | 2-phenoxybenzoyl-tryptophan derivatives | Nutlins
NFκB
p53
p53
DNA damage
DNA repair
PARP
CAD
ATM
CHK2
p53
MDM2

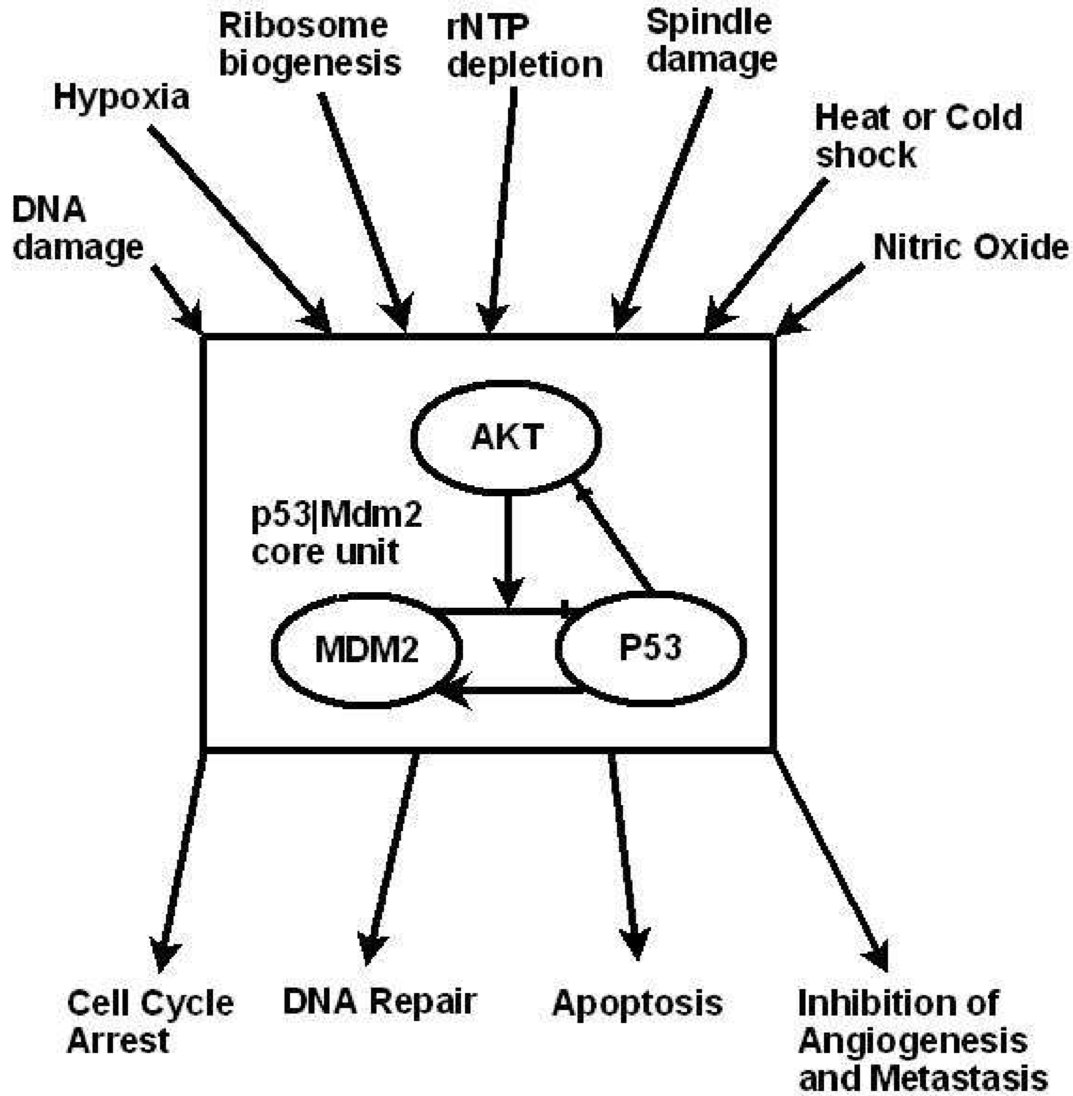
p53|Mdm2 module



10 or more feedbacks,
Over 100 components,
Stochastic noises

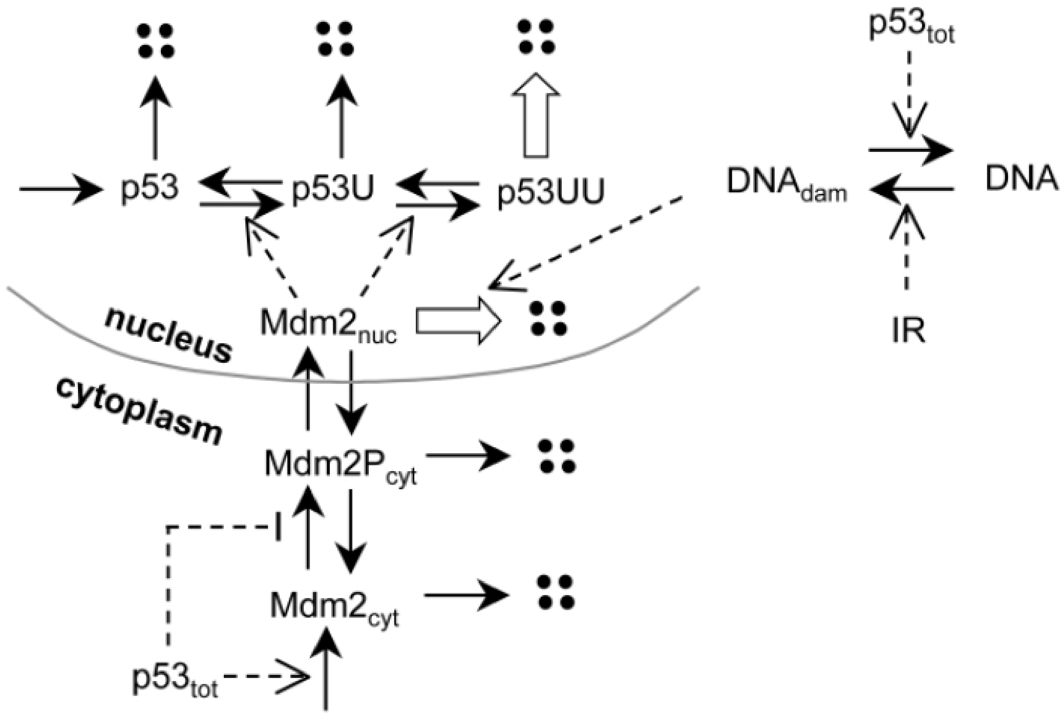
Kohn and Pommier 2005

Inputs and outputs of the p53|Mdm2 regulatory unit



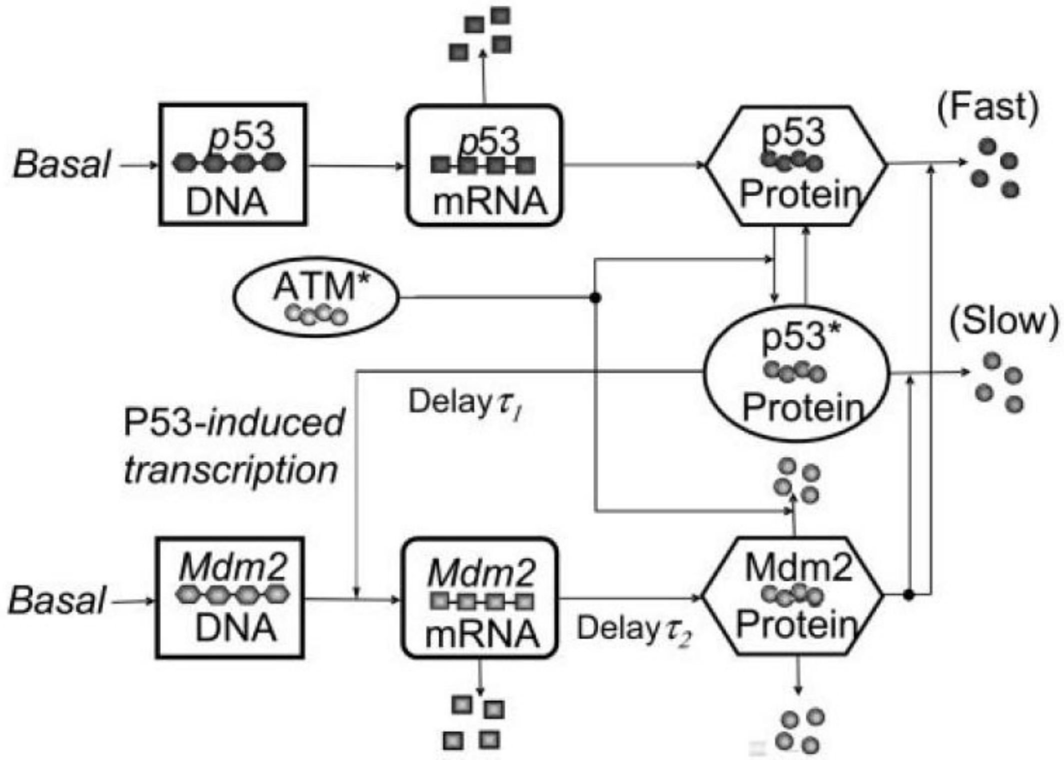
Cilliberto 2005

- Three forms of p53
- Positive feedback loop to simplified (PTEN, PIP3 i Akt proteins are absent)
- One stable state and limit cycle



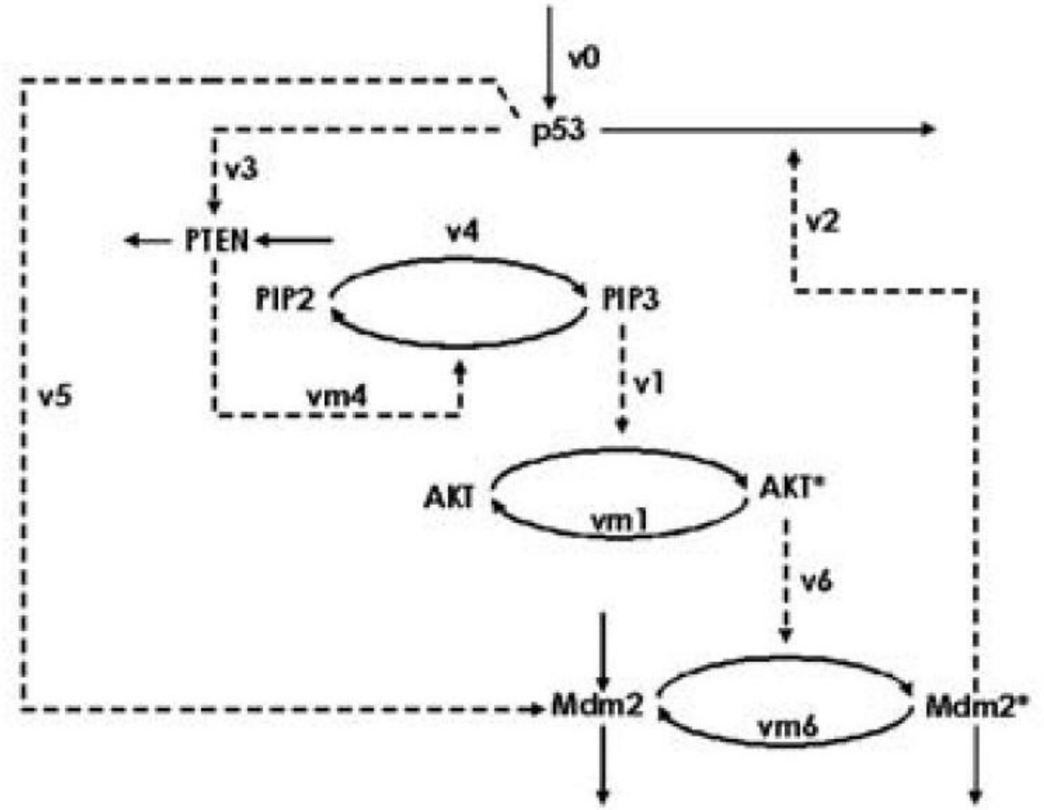
Ma 2005

- Two forms of p53
- The lack of the positive feedback
- One stable state and limit cycle



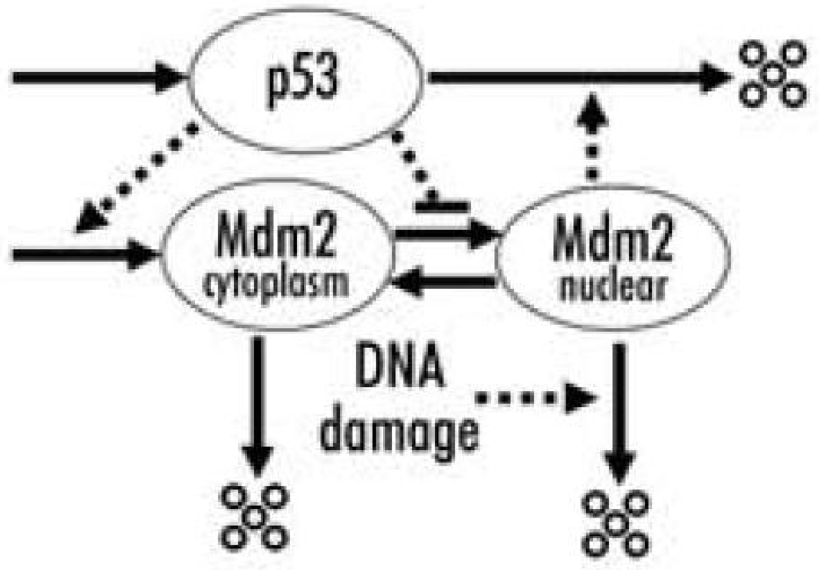
Wee 2006

- One form of p53
- Limit cycle

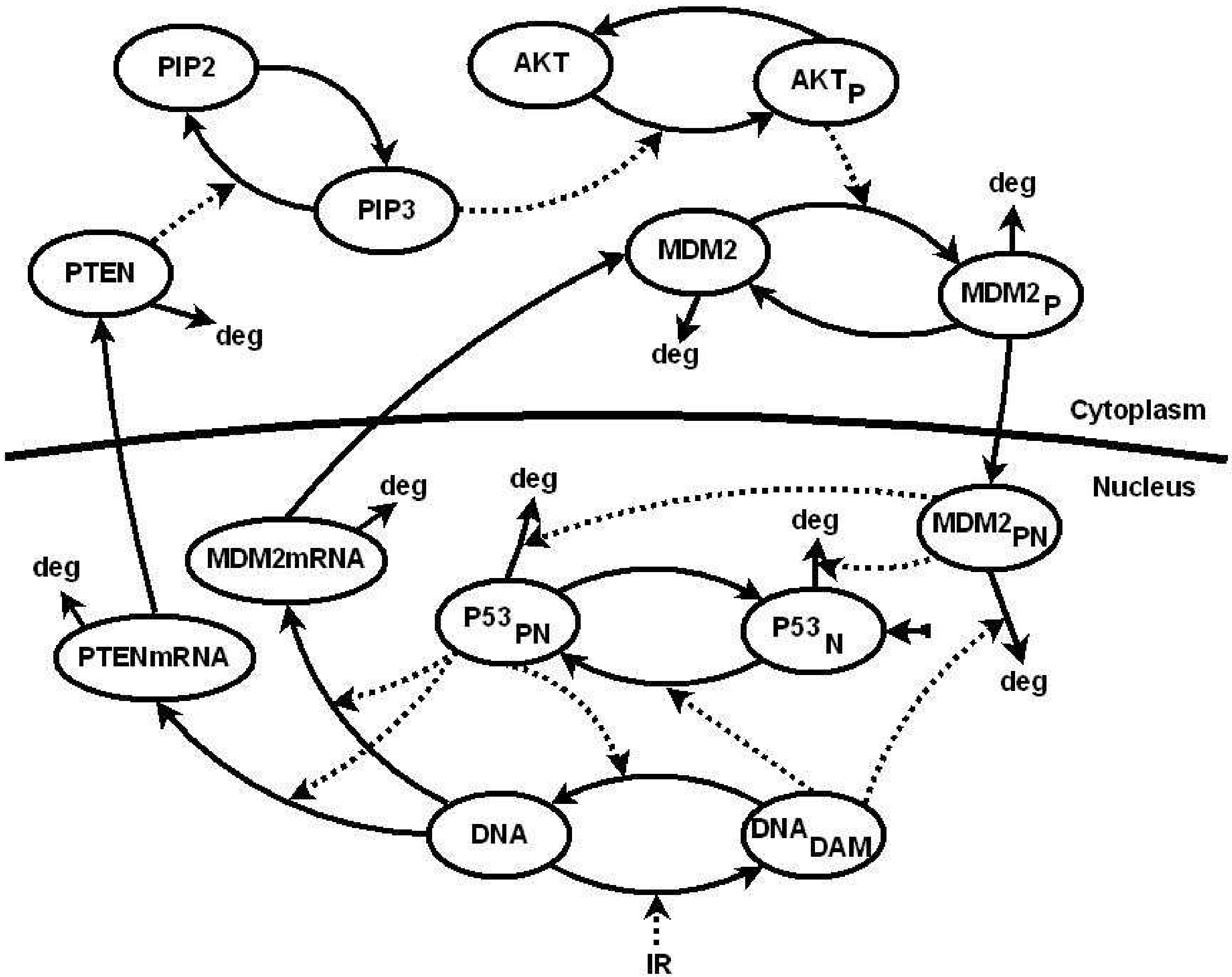


Zhang 2007

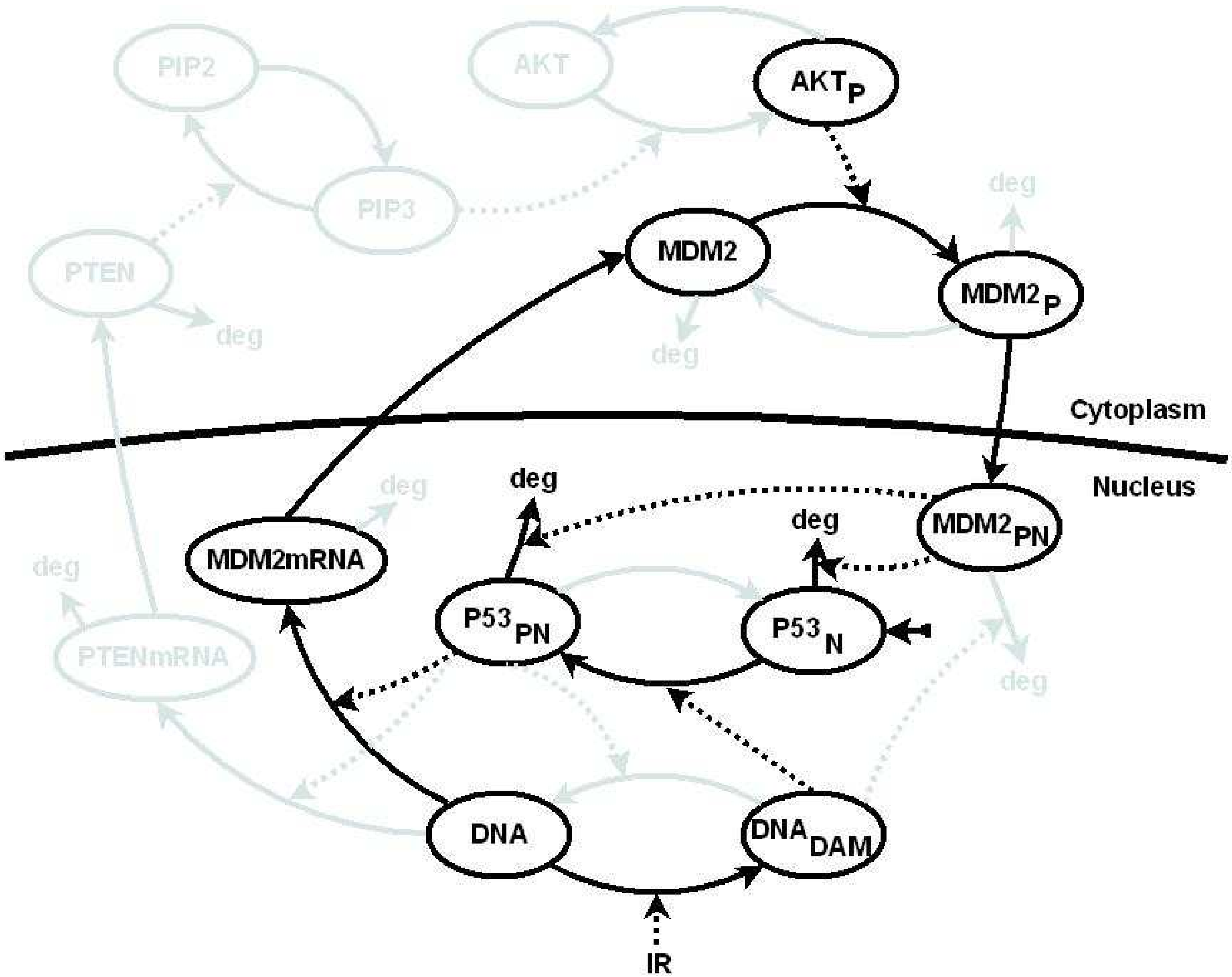
- One form of p53
- Neglected delays
- One stable state and limit cycle



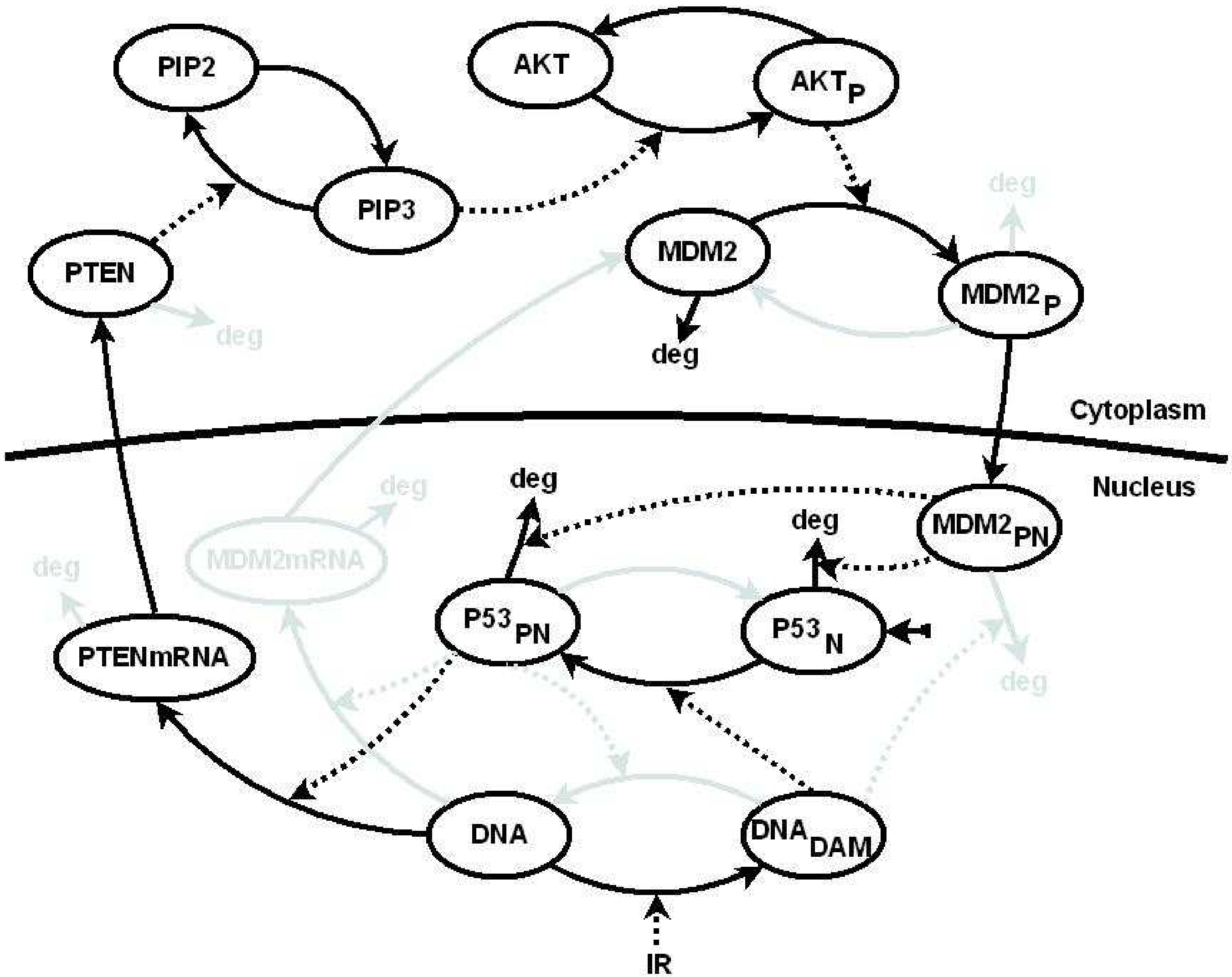
p53|Mdm2 model



Negative feedback loop

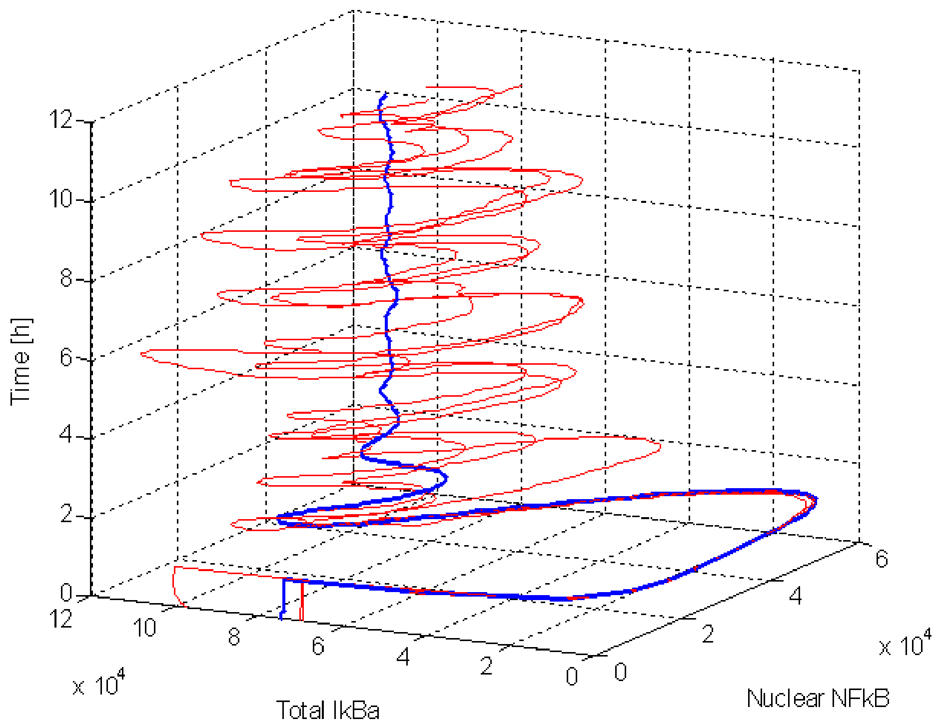


Positive feedback loop

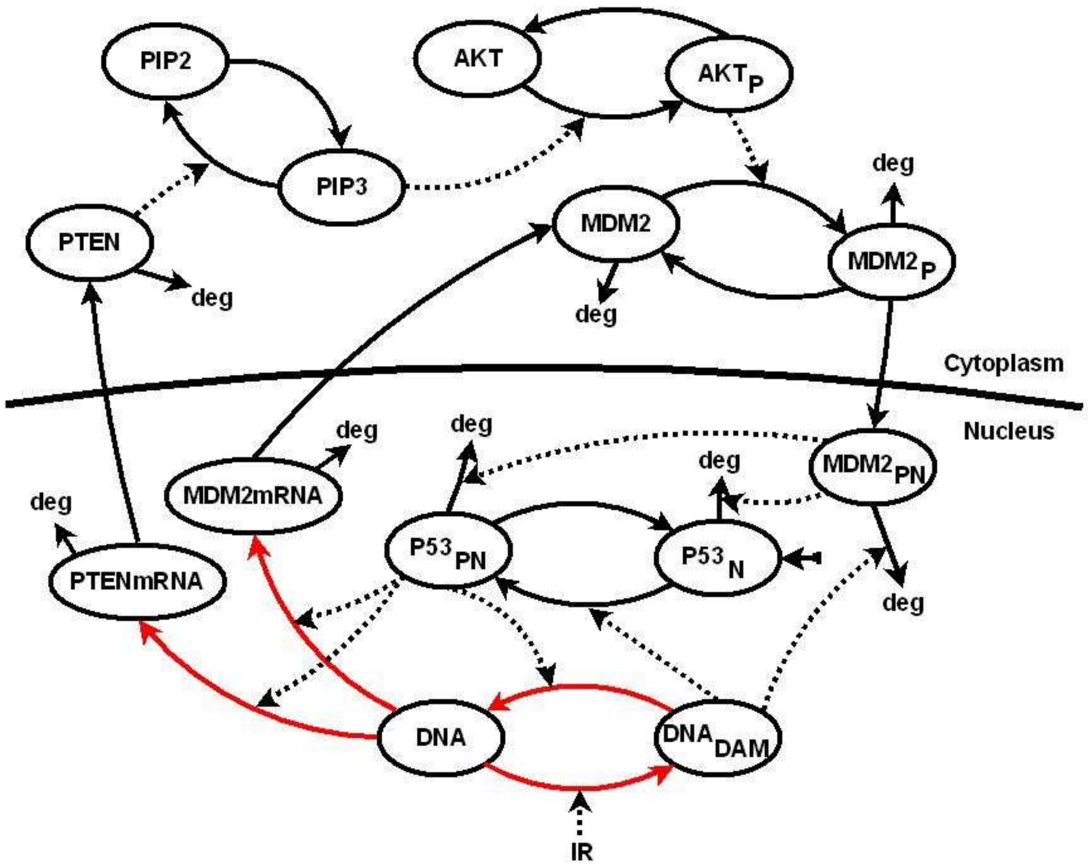


Modeling

- **Deterministic models based on ODE** – fast but can be used on population not the single cell level
- **Stochastic models based on Gillespie algorithm** – can work on the single cell level but are very slow
- **Haseltine and Rawlings approach – fast (modeled by using ODE) and slow (modeled stochastically) reaction channels** – fast and can work on the single cell level



Stochasticity in the p53|Mdm2 model



- IR dose increases the probability of DNA damage occurs
- Level of the p53pn protein increases the probability of the DNA repair and Mdm2 and PTEN gene activation

Equations

$$\frac{d}{dt}PTEN(t) = t_1\, PTEN_t(t) - d_2\, PTEN(t)$$

$$\frac{d}{dt}PIP_p(t) = a_2\, \left(PIP_{tot} - PIP_p(t)\right) - c_0\, PTEN(t)\, PIP_p(t)$$

$$\frac{d}{dt}AKT_p(t) = a_3\, \left(AKT_{tot} - AKT_p(t)\right)\, PIP_p(t) - c_1\, AKT_p(t)$$

$$\begin{aligned} \frac{d}{dt}MDM(t) &= t_0\, MDM_t(t) + c_2\, MDM_p(t) \\ &\quad - a_4\, MDM(t)\, AKT_p(t) - \left(d_0 + d_1\, \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\, MDM(t) \end{aligned}$$

$$\begin{aligned} \frac{d}{dt}MDM_p(t) &= a_4\, MDM(t)\, AKT_p(t) - c_2\, MDM_p(t) - i_0\, MDM_p(t) \\ &\quad + e_0\, MDM_{pn}(t) - \left(d_0 + d_1\, \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\, MDM_p(t) \end{aligned}$$

Equations

$$\frac{d}{dt}MDM_{pn}(t) = i_0\,MDM_p(t) - e_0\,MDM_{pn}(t) - \left(d_0 + d_1\,\frac{N^2(t)}{h_0^2 + N^2(t)}\right)\,MDM_{pn}(t)$$

$$\begin{aligned}\frac{d}{dt}P53_n(t) \quad &= \quad p_0 - \left(a_0 + a_1\,\frac{N^2(t)}{h_0^2 + N^2(t)}\right)\,P53_n(t) + c_3\,P53_{pn}(t)\\ &\quad - \left(d_3 + d_4\,MDM_{pn}^2(t)\right)\,P53_n(t)\end{aligned}$$

$$\begin{aligned}\frac{d}{dt}P53_{pn}(t) \quad &= \quad \left(a_0 + a_1\,\frac{N^2(t)}{h_0^2 + N^2(t)}\right)\,P53_n(t) - c_3\,P53_{pn}(t)\\ &\quad - \left(d_5 + d_6\,MDM_{pn}^2(t)\right)\,P53_{pn}(t)\end{aligned}$$

$$\frac{d}{dt}MDM_t(t) = s_0\,(G_{M1} + G_{M2}) - d_7\,MDM_t(t)$$

$$\frac{d}{dt}PTEN_t(t) = s_1\,(G_{P1} + G_{P2}) - d_8\,PTEN_t(t)$$

$$\frac{d}{dt}A(t) = p_1\,\frac{q_3\,P53_{np}^2(t)}{q_4 + q_3\,P53_{np}^2(t)} - d_9 A(t)$$

Proapoptotic factors

Stochasticity in model

probability of gene copy activation:

$$P^b(t,\Delta t) = \Delta t \times (q_0 + q_1 \times P53^2_{np}(t))$$

probability of gene copy deactivation:

$$P^d(t,\Delta t) = \Delta t \times q_2$$

transcriptional efficiency of p53
(probability that the gene copy is active if $p53_{np}(t)$=const):

$$P_A(t) = \frac{q_0 + q_1 \times P53^2_{np}(t)}{q_2 + q_0 + q_1 \times P53^2_{np}(t)}$$

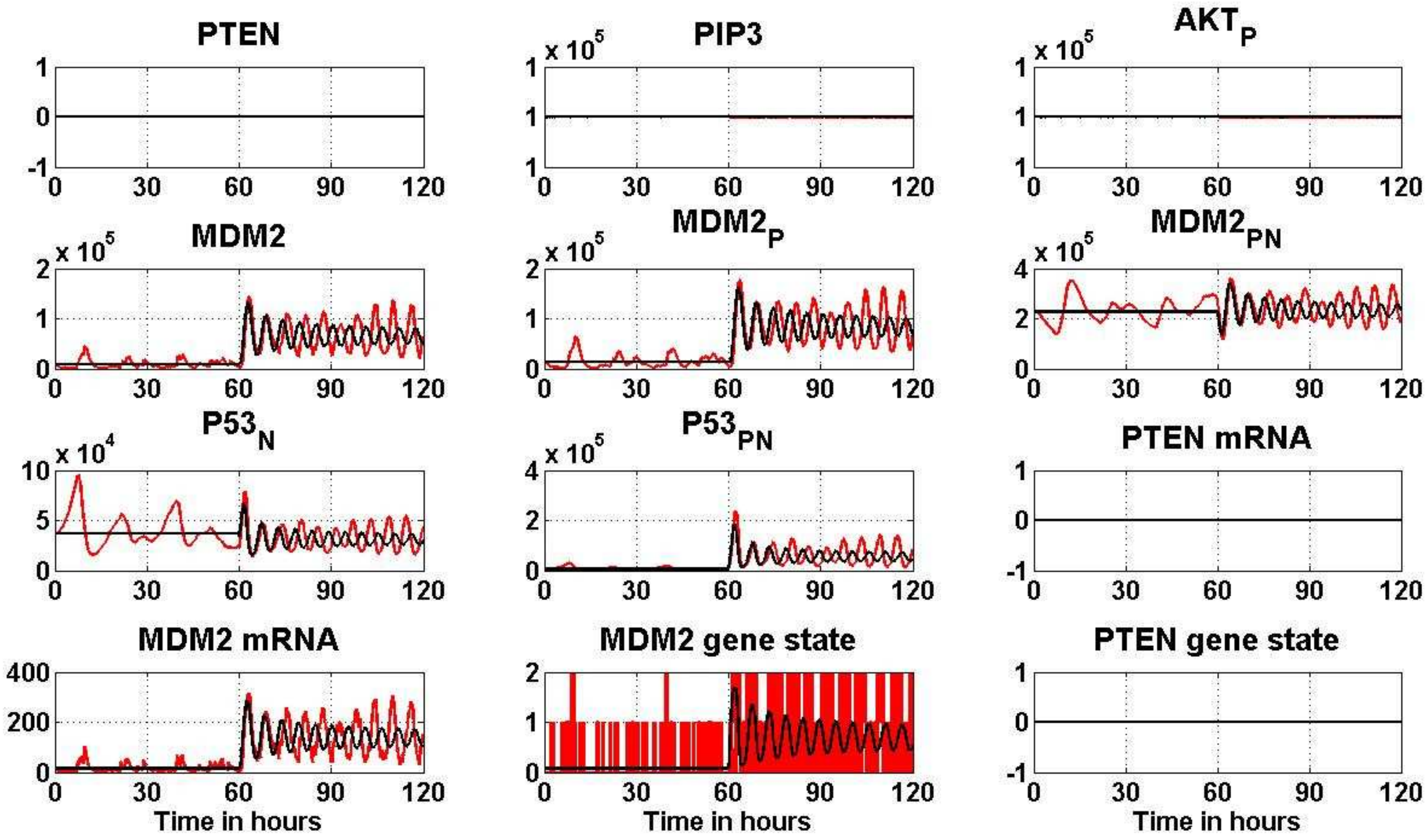
probability that new DSB appears:

$$P^{DAM}(t,\Delta t) = \Delta t \times d_{DAM} \times R + \Delta t \times a_6 \left(\frac{A(t)}{A_{\max}}\right)^4$$

propability that the number of DSB decreases by one:

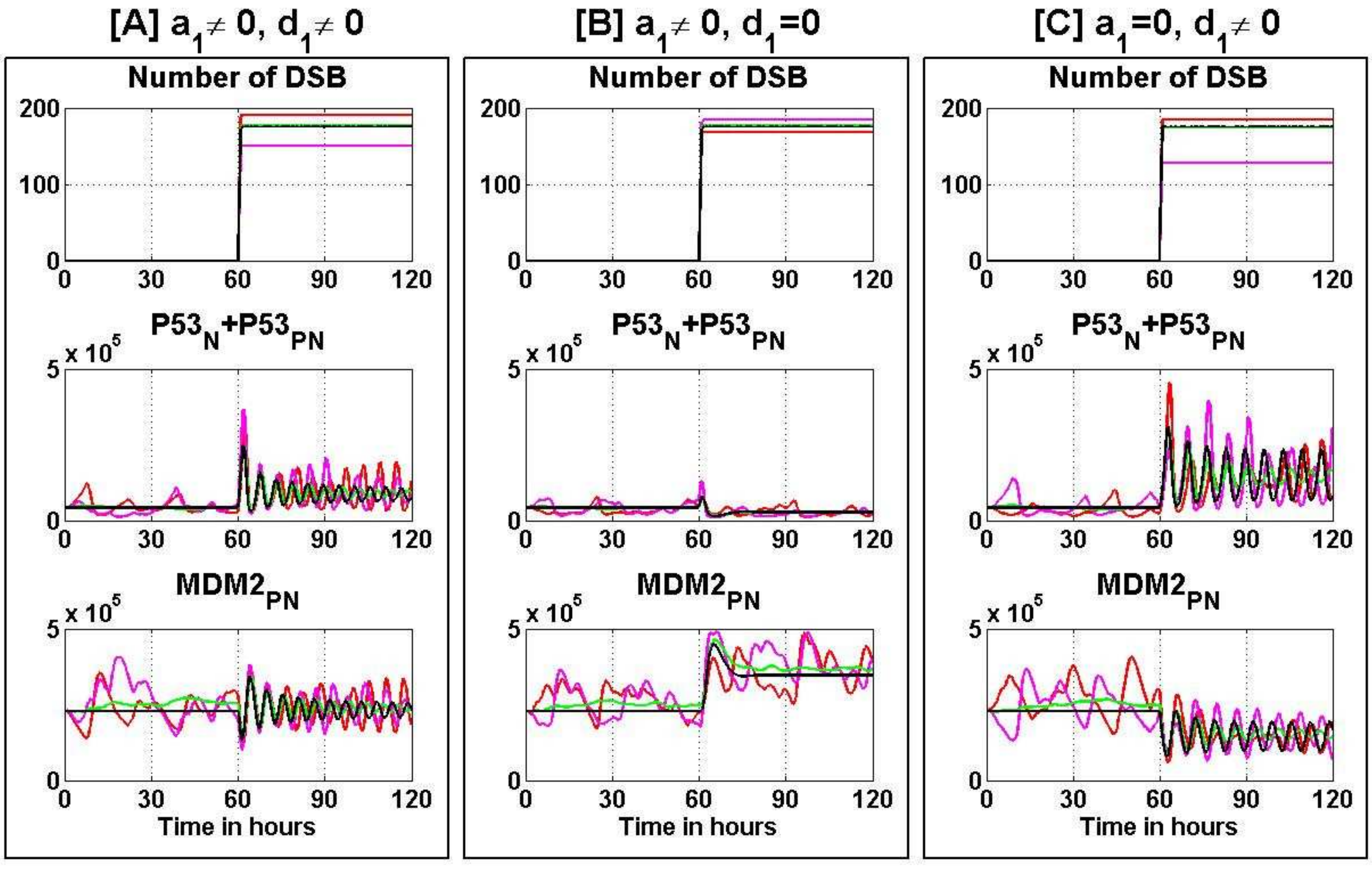
$$P^{REP}(t,\Delta t) = N(t)\frac{\Delta t \times d_{REP} \times P_A(t)}{N(t) + N_{SAT} \times P_A(t)}$$

PTEN off, DNA repair off, dose 5Gy (oscillations)

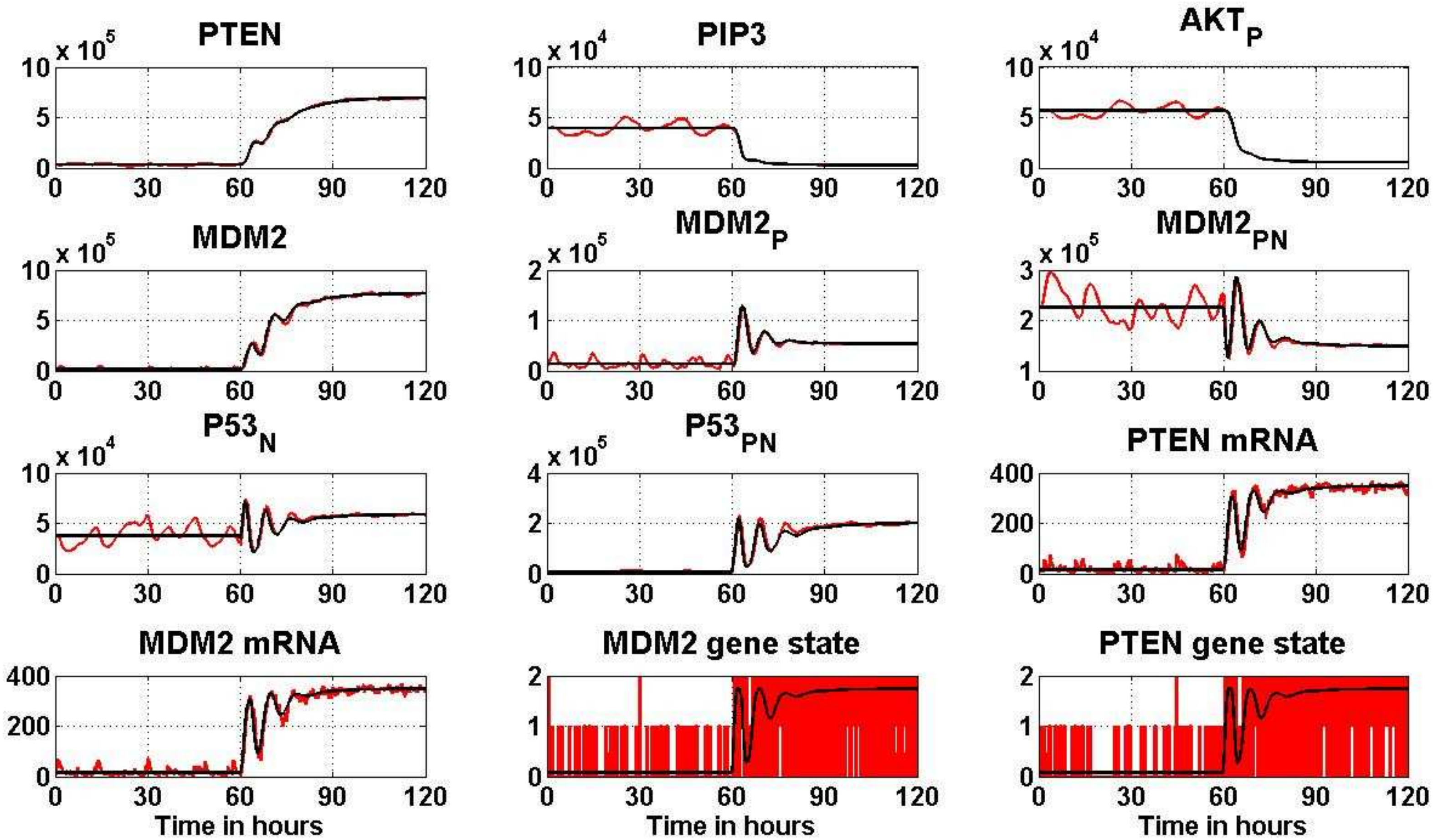


PTEN off, DNA repair off, dose 5Gy

(a_1 – p53 activation, d_1 – Mdm2 degradation)

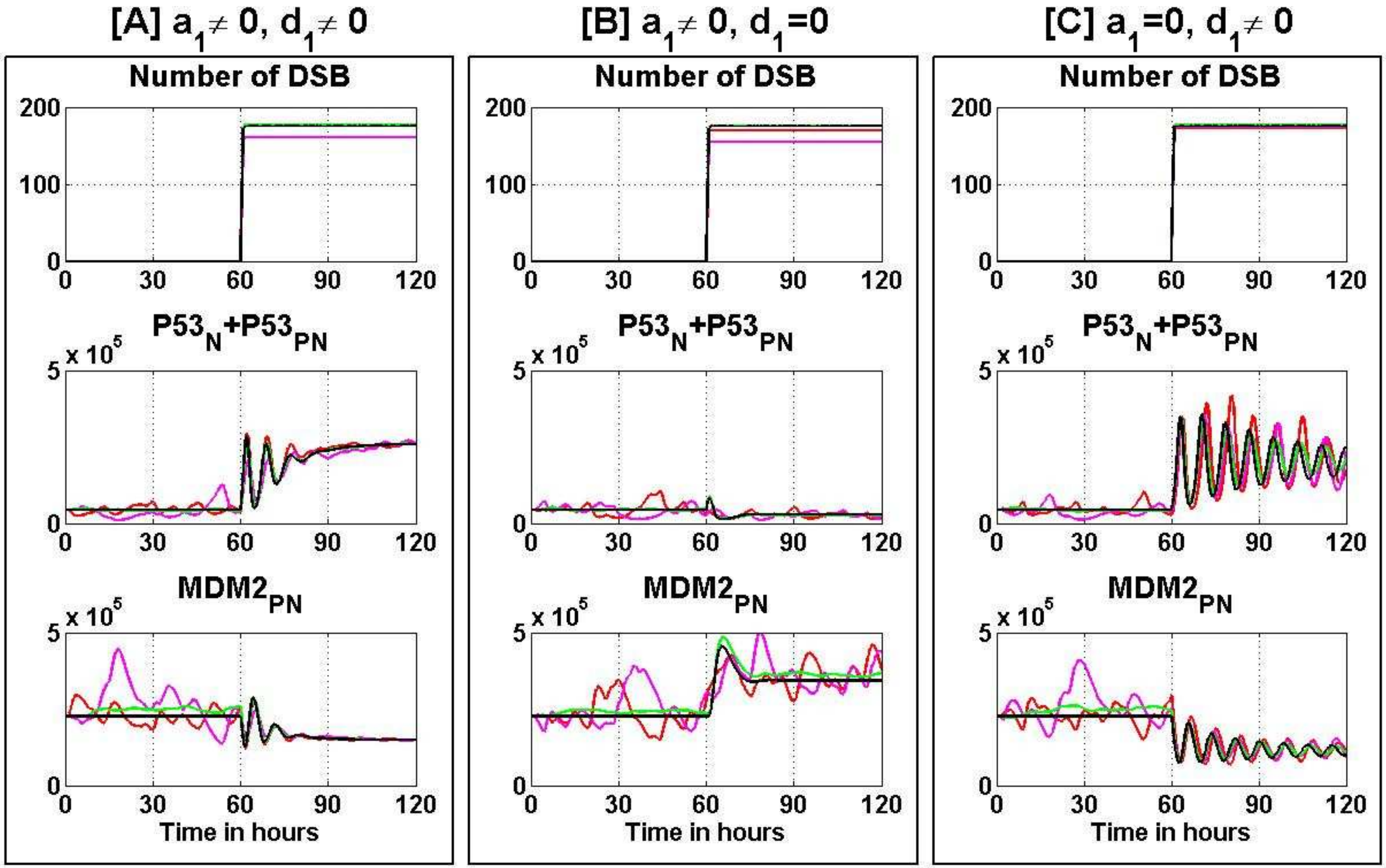


PTEN on, DNA repair off, dose 5Gy (apoptosis)

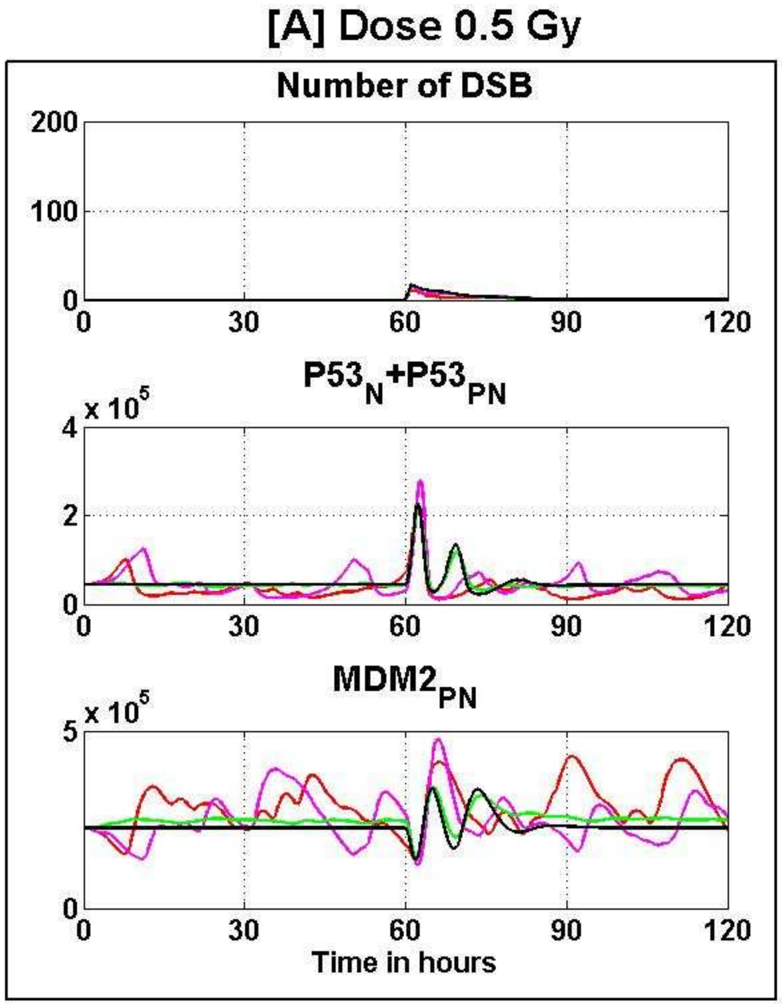


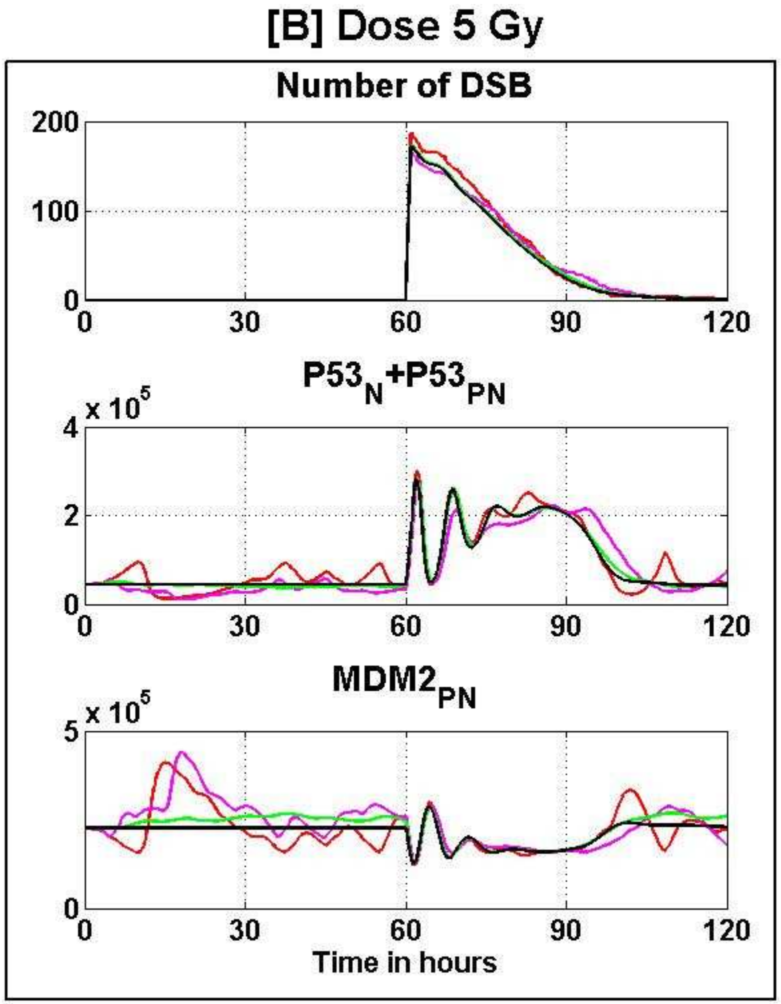
PTEN on, DNA repair off, doses 5Gy

(a_1 – p53 activation, d_1 – Mdm2 degradation)

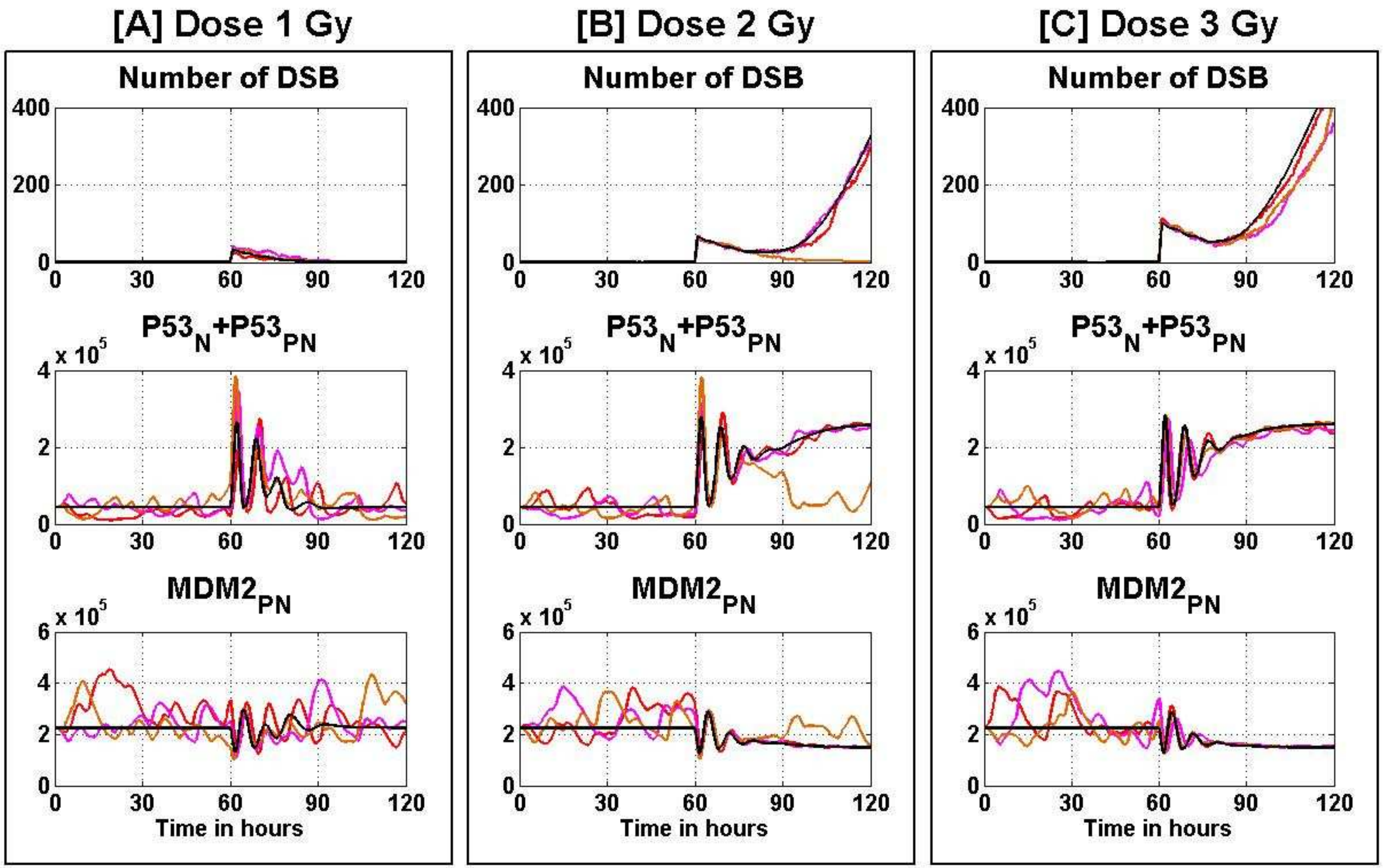


PTEN on, DNA repair on (competition)

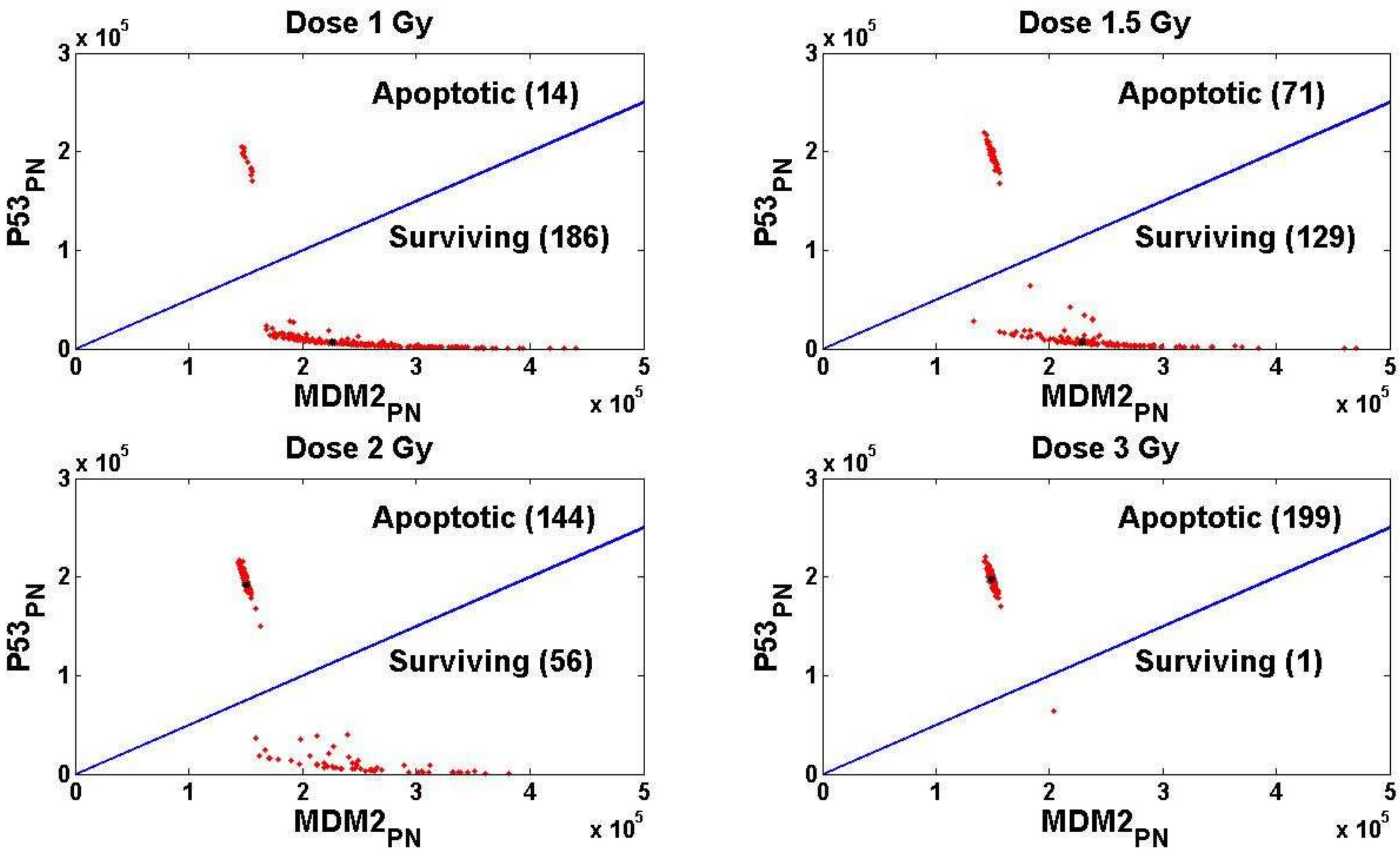




PTEN on, DNA on + proapoptotic factors (competition)

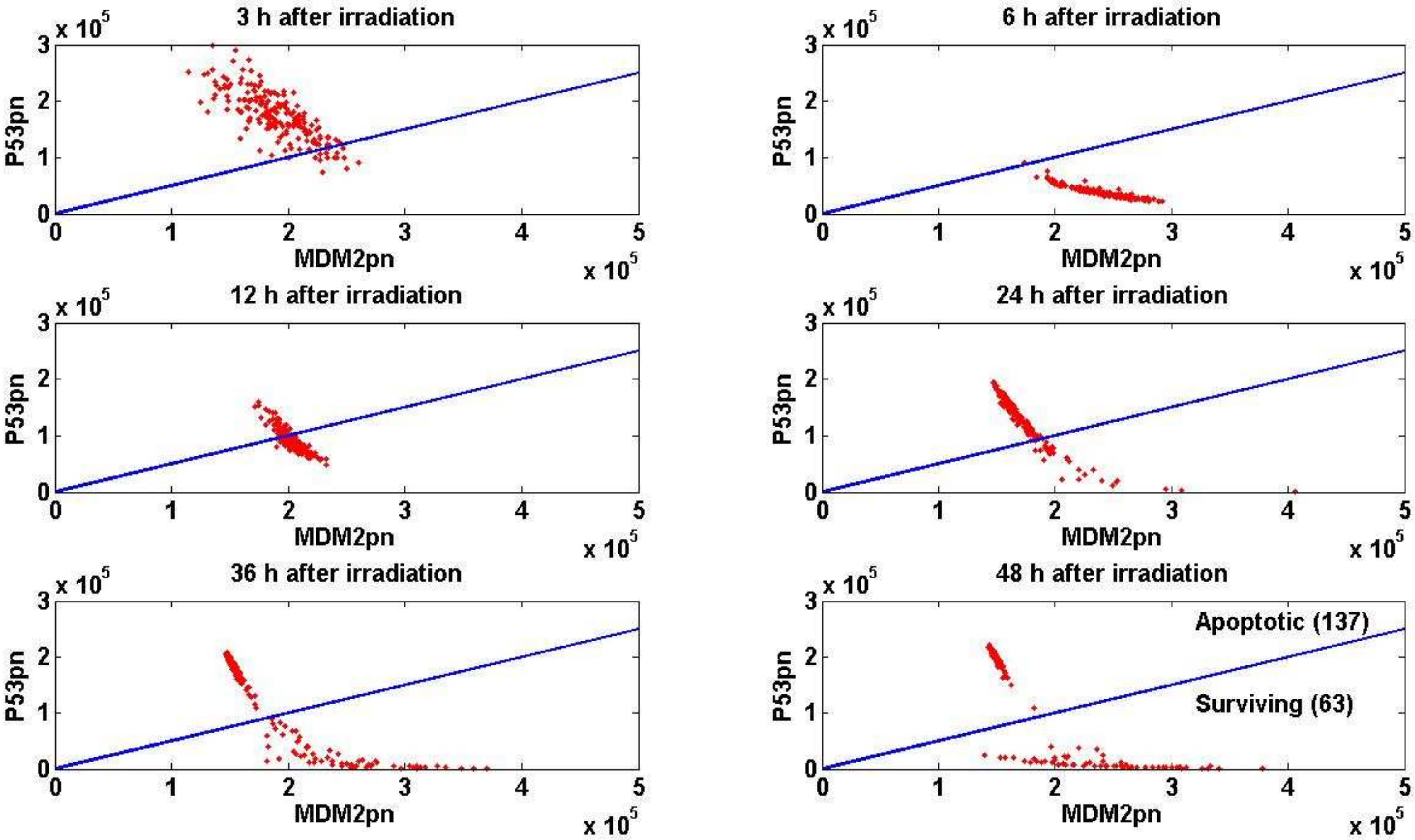


Cell fate

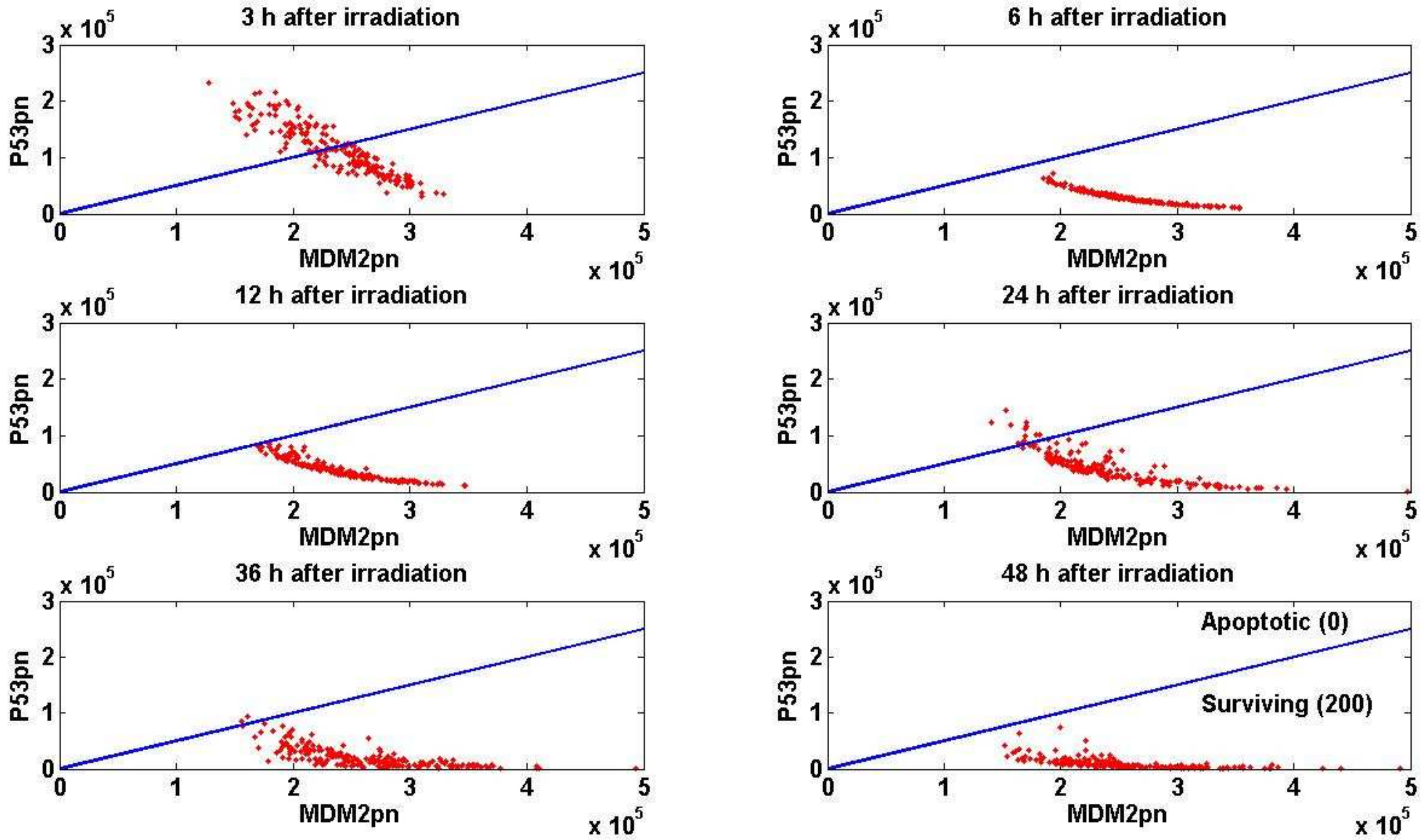


Dose 1 Gy
Apoptotic (14)
Surviving (186)
Dose 1.5 Gy
Apoptotic (71)
Surviving (129)
Dose 2 Gy
Apoptotic (144)
Surviving (56)
Dose 3 Gy
Apoptotic (199)
Surviving (1)
P53PN
MDM2PN
x 10^5

Cell fate - PTEN on



Cell fate – PTEN off



Thank you

Parameters

Parameter	Description	Value	Range
a_0	spontaneous $P53_n$ phosphorylation rate	$1 \times 10^{-4}/s$	$(0.2 - 5) \times 10^{-4}$
a_1	DSB-induced $P53_n$ phosphorylation rate	$1 \times 10^{-3}/s$	$(0.3 - 3) \times 10^{-3}$
a_2	PIP activation rate	$5 \times 10^{-5}/s$	$(1 - 10) \times 10^{-5}/s$
a_3	AKT activation rate	$2 \times 10^{-9}/s$	$(0.4 - 4) \times 10^{-9}/s$
a_4	MDM phosphorylation rate	$1 \times 10^{-8}/s$	$(0.2 - 2) \times 10^{-8}/s$
c_0	PIP_p dephosphorylation rate (by $PTEN$)	$2.5 \times 10^{-9}/s$	$(1.25 - 12.5) \times 10^{-9}/s$
c_1	AKT_p inactivation rate	$2 \times 10^{-4}/s$	$(1.3 - 10) \times 10^{-4}/s$
c_2	MDM_p dephosphorylation rate	$1 \times 10^{-4}/s$	$(0.2 - 5) \times 10^{-4}/s$
c_3	spontaneous $P53_{pn}$ dephosphorylation rate	0	$(0 - 2) \times 10^{-4}/s$
d_0	Mdm2 spontaneous deg. rate (all Mdm2 forms)	$3 \times 10^{-5}/s$	$(0.6 - 6) \times 10^{-5}/s$
d_1	DSB-induced Mdm2 deg. rate (all Mdm2 forms)	$1.5 \times 10^{-4}/s$	$(0.75 - 2.25) \times 10^{-4}/s$
d_2	$PTEN$ degradation rate	$5 \times 10^{-5}/s$	$(1 - 10) \times 10^{-5}/s$
d_3	spontaneous $P53_n$ degradation rate	$1 \times 10^{-4}/s$	$(0.2 - 5) \times 10^{-4}/s$
d_4	MDM_{pn}-induced $P53_n$ degradation rate	$1 \times 10^{-13}/s$	$(0.5 - 4) \times 10^{-13}/s$
d_5	spontaneous $P53_{pn}$ degradation rate	$1 \times 10^{-4}/s$	$(0.2 - 3) \times 10^{-4}/s$
d_6	MDM_{pn}-induced $P53_{pn}$ degradation rate	$1 \times 10^{-14}/s$	$(0.2 - 5) \times 10^{-14}/s$
d_7	MDM_t degradation rate	$3 \times 10^{-4}/s$	$(2 - 4.5) \times 10^{-4}/s$
d_8	$PTEN_t$ degradation rate	$3 \times 10^{-4}/s$	$(0.6 - 6) \times 10^{-4}/s$
e_0	MDM_{pn} nuclear export	0	$(0 - 2) \times 10^{-4}/s$
i_0	MDM_p nuclear import	$5 \times 10^{-4}/s$	$(1.6 - 25) \times 10^{-4}/s$

Parameters

Parameter	Description	Value	Range
p_0	$P53_n$ production rate	$2 \times 10^2/s$	$(0.4 - 6) \times 10^2/s$
s_0	MDM_t transcription rate	$6 \times 10^{-2}/s$	$(4 - 9) \times 10^{-2}/s$
s_1	$PTEN_t$ transcription rate	$6 \times 10^{-2}/s$	$(3 - 30) \times 10^{-2}/s$
t_0	MDM translation rate	$5 \times 10^{-1}/s$	$(3 - 7.5) \times 10^{-1}/s$
t_1	$PTEN$ translation rate	$1 \times 10^{-1}/s$	$(0.5 - 5) \times 10^{-1}/s$
h_0	Michaelis const. for DSB-induced $P53_n$ activation and for DSB-induced Mdm2 deg. (all Mdm2 forms)	7	$(1.4 - 35)$
q_0	spontaneous activation of Mdm2 and PTEN genes	$1 \times 10^{-4}/s$	$(0.2 - 2.2) \times 10^{-4}/s$
q_1	$P53_{pn}$-depended activation of Mdm2 and PTEN genes	$5 \times 10^{-13}/s$	$(1 - 26) \times 10^{-13}/s$
q_2	Mdm2 and PTEN genes inactivation rate	$3 \times 10^{-3}/s$	$(1.7 - 16) \times 10^{-3}/s$
N_{SAT}	saturation coefficient in DNA repair	50	$(10 - 250)$
d_{DAM}	DNA damage rate	35/Gy	
d_{REP}	DNA repair rate	$3 \times 10^{-3}/s$	$(0.6 - 15) \times 10^{-3}/s$
AKT_{tot}	total number of Akt molecules $(AKT + AKT_p)$	2×10^5	$(0.4 - 10) \times 10^5/s$
PIP_{tot}	total number of PIP molecules $(PIP + PIP_p)$	1×10^5	$(0.2 - 5) \times 10^5/s$
a_6	max DNA damage rate (induced by the apoptotic factor)	$1 \times 10^{-1}/s$	$- - -$
d_9	apoptotic factors degradation rate	$1 \times 10^{-4}/s$	$- - -$
p_1	max synthesis rate of apoptotic factor	$1 \times 10^2/s$	$- - -$
q_3	coefficient governing apoptotic factor synthesis	$8 \times 10^{-14}/s$	$- - -$
q_4	Michaelis const. for apoptotic factor synthesis	$3 \times 10^{-3}/s$	$- - -$