Petri net based modelling and simulation of p16-Cdk4/6-Rb pathway

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Motivation

- p16 is important tumor suppressor gene
- p16 plays gatekeeper role at the G1/S checkpoint of the cell cycle
- p16 is responsible for replicative senescence
- defects in p16 result in uncontrolled cell division which leads to progression of malignancy in an organism

Tumor suppressor gene p16 plays important role in regulating cell grows and division at checkpoint G1/S [4] of the cell cycle. The p16 gene is major tumor suppressor gene that is responsible for replicative senescence. Cell division is not an infnitely continuous process as cells undergo a finite number of cumulative population doublings. Most human normal cells permanently stop dividing after a 50-75 cell divisions and enter a state termed cellular or replicative senescence [2]. Most tumors contain cells that appear to have bypassed this limit and evaded replicative senescence. Immortality, or even an extended replicative lifespan, greatly increases susceptibility to malignant progression because it permits the extensive cell divisions needed to acquire successive mutations. Thus, cellular senescence may act as a barrier to cancer and play an important role in tumor suppression [1]. Inactivation of tumor suppressor gene p16, which in fact keeps track of replicative senescence, results in uncontrolled cell division, which leads to cancer [1, 3].

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Biological context

During G1 phase, proteins Cdk4 and Cdk6 form complex with protein CycD, which in turn phosphorylates the Rb protein family. When Rb is phosphorylated by Cdk4/6 it loses its function and releases its target, the E2F family transcription factors, resulting in the initiation of DNA replication [1, 2]. Otherwise Rb inhibits transcription factor E2F [3]. E2F is a transcription factor which
initiates transcription of genes required for S phase [4]. In the case of malignant progression action of p16 inhibits binding of Cdk4/6 with CycD which leaves Rb, and other Rb related proteins [5, 6]. The p16 targets Cdk4 and Cdk6, rather than the CycD, and actually competes with CycD for Cdk binding. Binding of p16 results in changes in conformation of Cdk proteins so that they can no longer bind CycD [7]. The p16 may also deactivate preassembled Cdk4/6 CycD complex blocking their function [7].

The proteins and their complexes are involved in natural degradation. In addition, the CycD protein is also tightly regulated by ubiquitin-dependent degradation [8, 9, 10].

1. Stevaux O., Dyson N.J.: A revised picture of the E2F transcriptional network and RB function. Curr Opin Cell Biol 14, 684-691 (2002).
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3. Weinberg, R.A., The retinoblastoma protein and cell cycle control. Cell 81(3), 323-330 (1995).
4. Bartkova, J., Lukas, J., Guldberg, P., Alsner, J., Kirkin, A.F., Zeuthen, J., Bartek, J.: The p16-cyclin D/Cdk4-pRb pathway as a functional unit frequently altered in melanoma pathogenesis. Cancer Res, 56(23), 5475-5483 (1996).
5. Matheu, A., Maraver, A., Collado, M., Garcia-Cao, I., Canamero, M., Borras, C., Flores, J.M., Klatt, P., Vina, J., Serrano, M., Anti-aging activity of the Ink4/Arf locus. Aging Cell 8(2), 152-161 (2009).
6. Walkley, C.R., Orkin, S.H.: RB is dispensable for self-renewal and multilineage differentiation of adult hematopoietic stem cells. Proc Natl Acad Sci USA, 103(24), 9057-9062 (2006).
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8. Alao, J.P.: The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Molecular Cancer 6(24) (2007).
9. Germain, D., Russell, A., Thompson, A., Hendley, J.: Ubiquitination of free cyclin D1 is independent of phosphorylation on threonine 286. J Biol Chem 275(16), 12074-12079 (2000).
10. Lin, D.I., Barbash, O., Kumar, K.G., Weber, J.D., Harper, J.W., Klein- Szanto, A.J., Rustgi, A., Fuchs, S.Y., Diehl, J.A.: Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4-alphaB crystallin) complex. Mol Cell 24(3), 355-366 (2006).

Creating HFPN model

		G1-dysfunction: Yes	G1-dysfunction: No
Mutation	Yes	✓ p16 loses its inhibory function; ✓ Tumor cells bypass the limitation and evade replicative senescence; tumor cells gain immortality, or an extended replicative lifespan which leads to spreading out malignant progression in an orgasnism.	✓ Cells gain immortality which soon or late leads to progression of malignancies. ✓ p16 loses its inhibory function;
	No	✓ p16 inhibits binding of Cdk4/6 with CycD; ✓ Rb cannot be phosphorylated and consequently cell stops diving; ✓ Malignant progression cannot be spread out in an organism since cell has stoped diving.	✓ CycD binds to Cdk4/6 resulting in phosphorylation of Rb and consequently cell division; ✓ A cell permanently stop dividing after a 50-75 cell divisions and enter a state termed cellular or replicative senescence.

Creating HFPN model

- p16-Cdk4/6-Rb pathway: all in one
- p16-Cdk4/6-Rb pathway: 5 major fragments
- p16: transcription, translation, mutation, nuclear export, natural degradation
- Cdks: transcription, translation, nuclear export, natural degradation, binding
- CycD: transcription, translation, nuclear export, natural degradation, ubiquitination
- p16 inhibits binding of Cdk4/6 with CycD: binding, degradation, nuclear export
- Phosphorylation of Rb: phosphorylation, degradation

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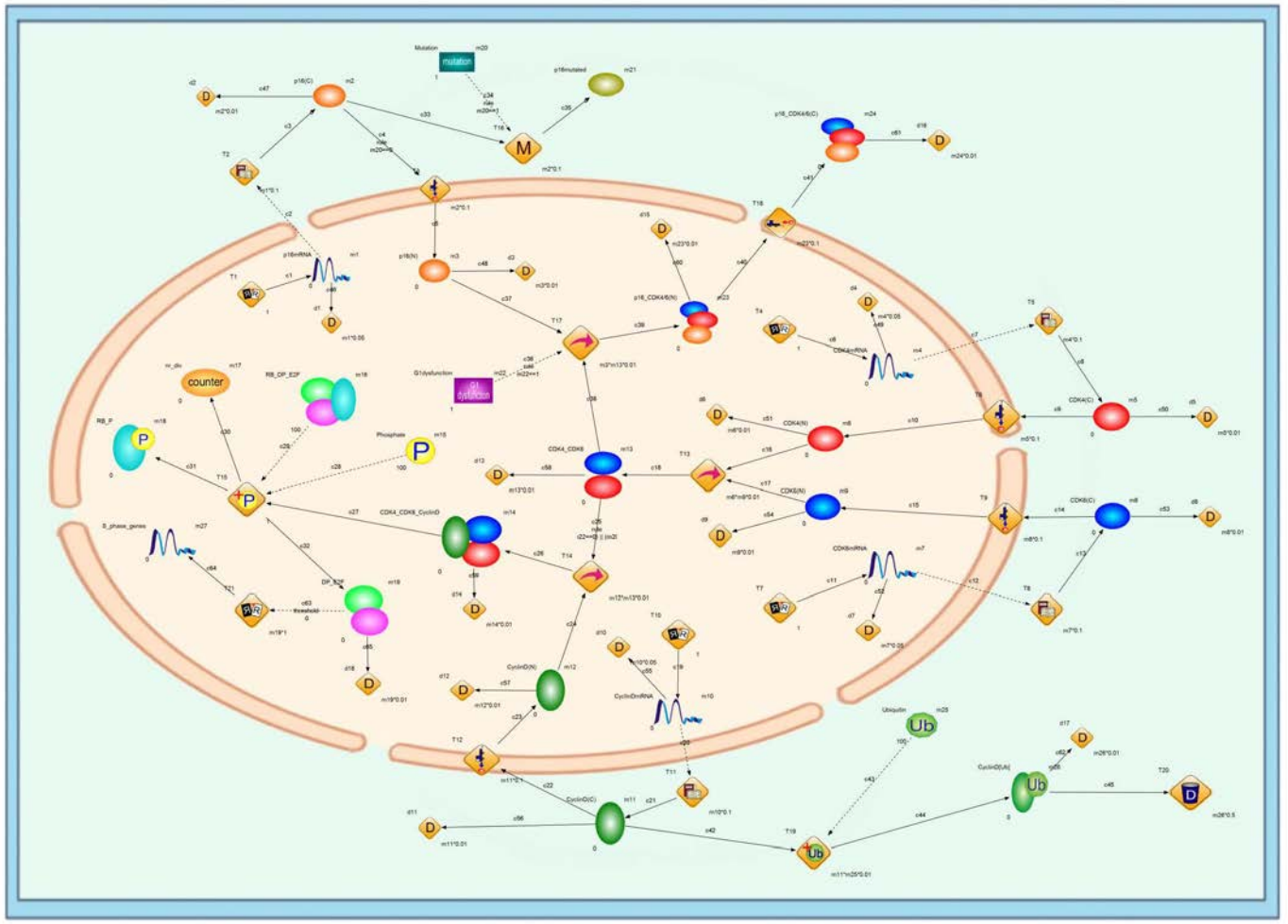
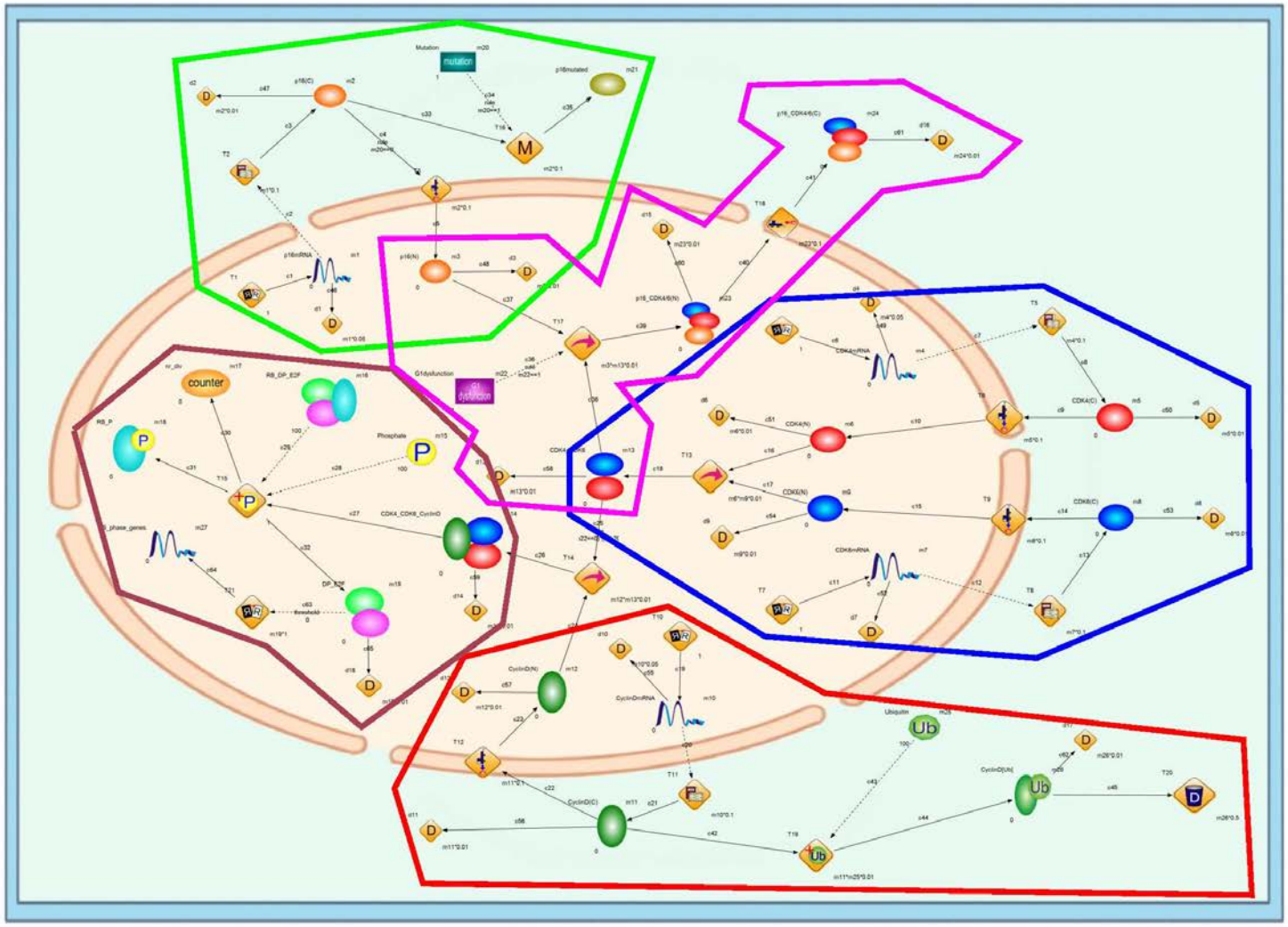
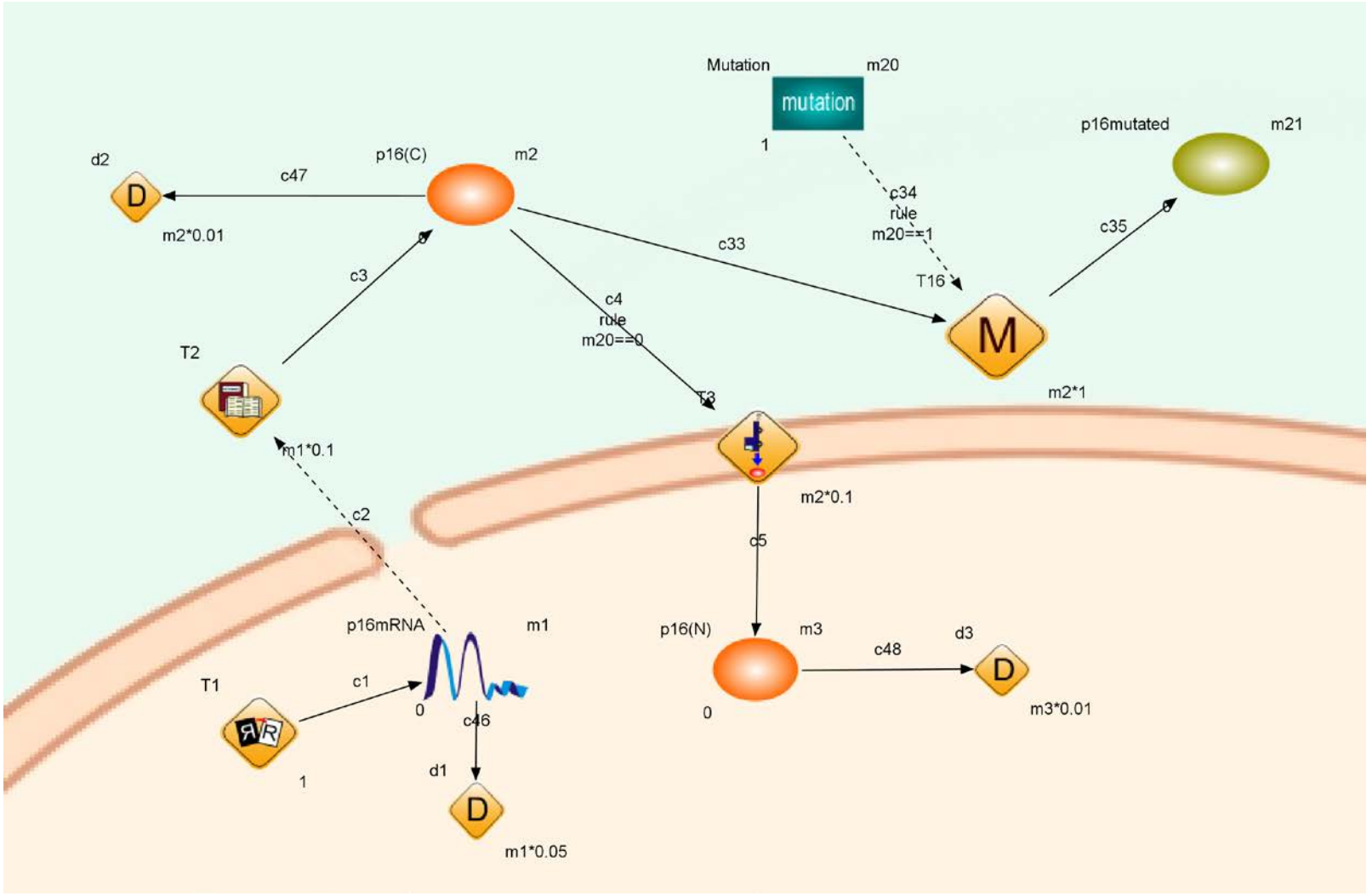
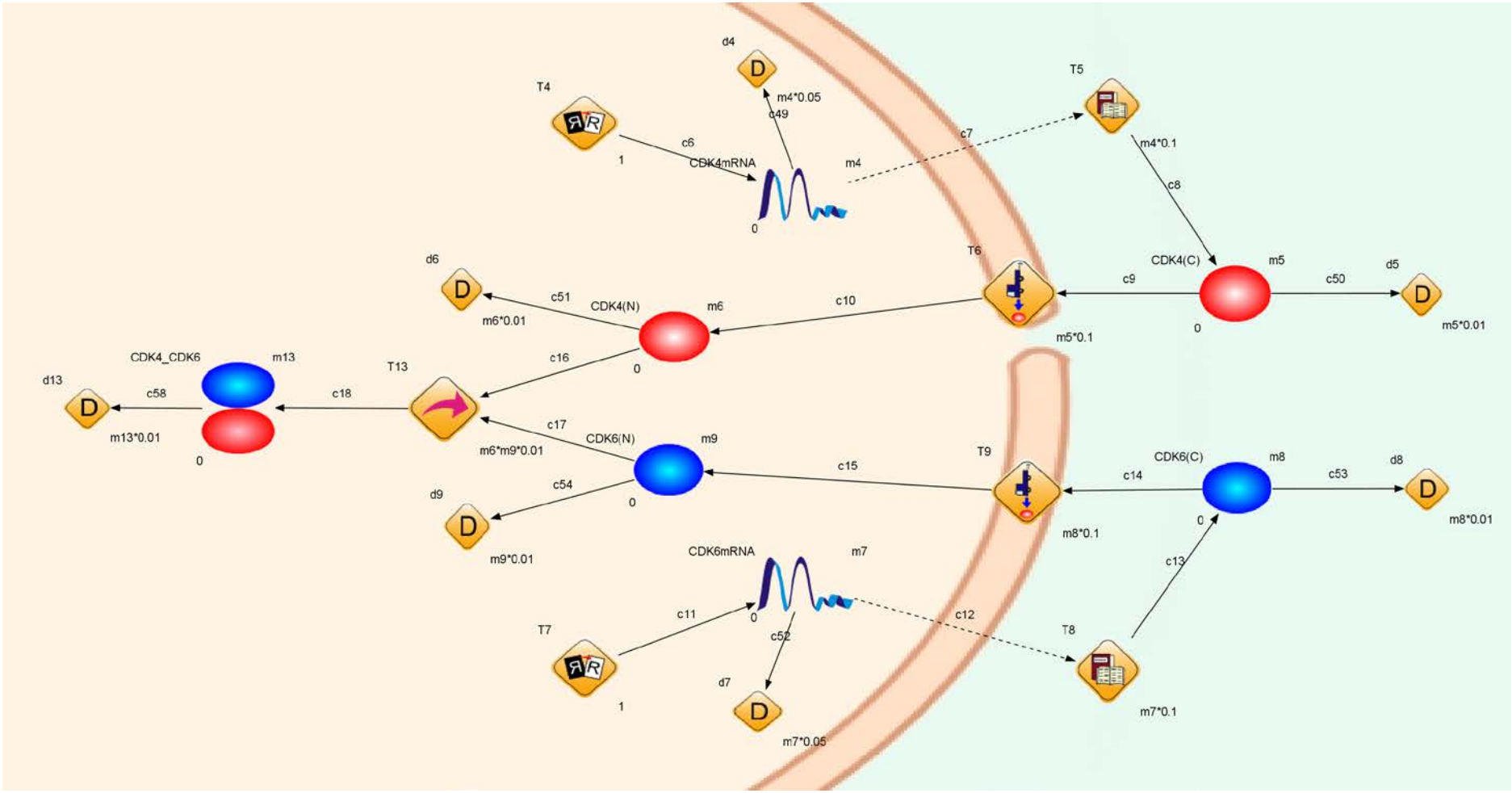
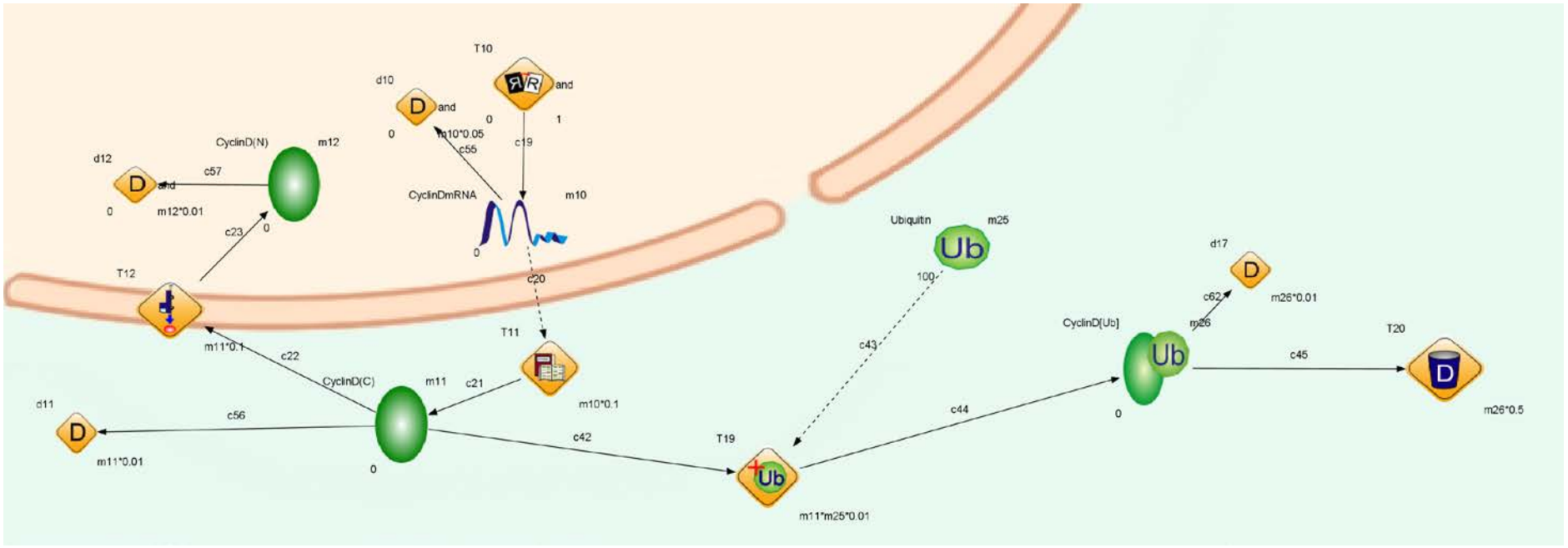
2. Doi, A., Fujita, S., Matsuno, H., Nagasaki, M., Miyano, S.: Constructing biological pathway models with Hybrid Functional Petri Nets. In Silico Biol 4(3), 271-291 (2004).

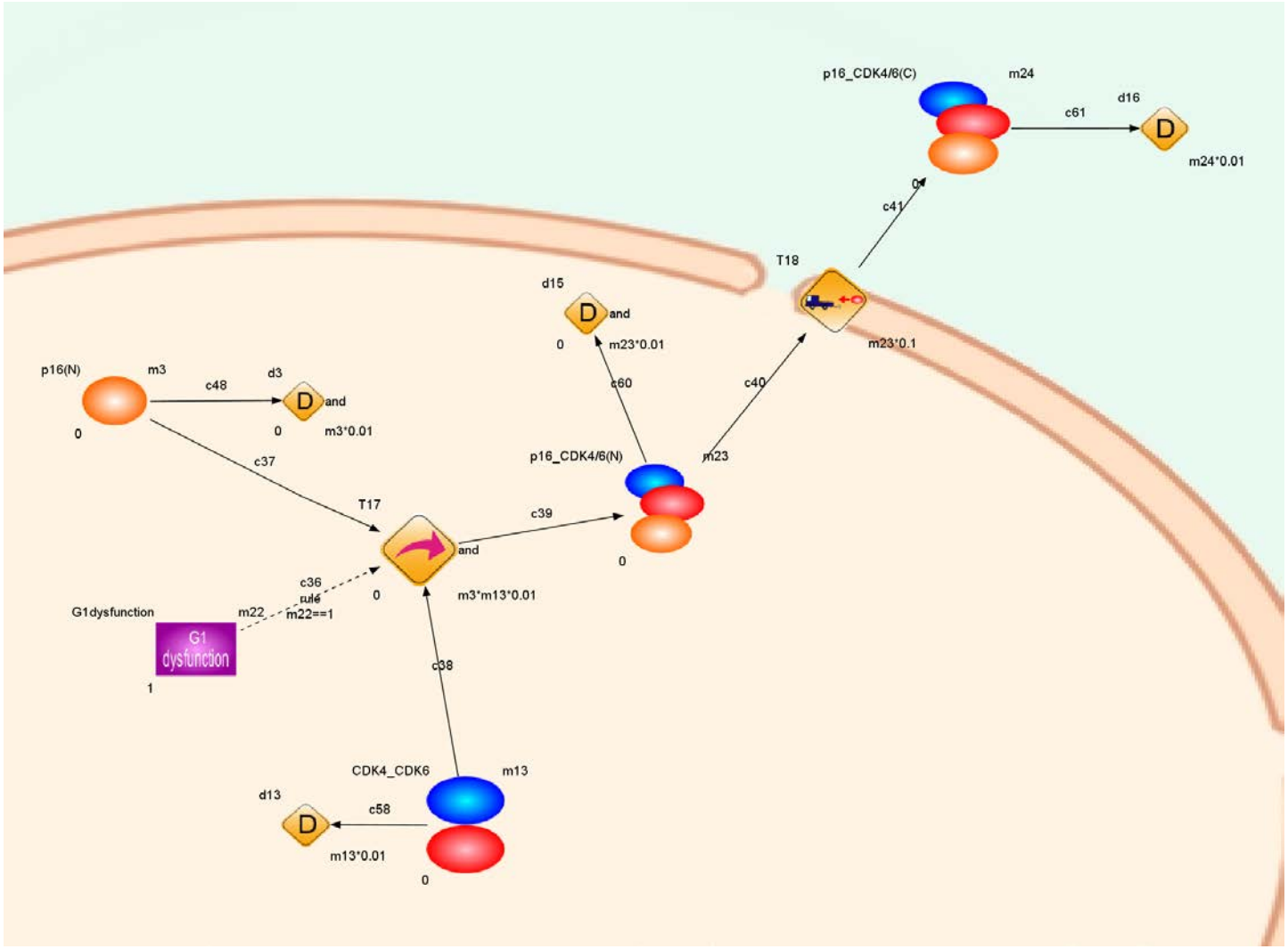

mutation
p16mutated
M
counter
G1dysfunction
Phosphate
Ubiquitin
Ub
CDK4_CDK6_CyclinD
CDK4_CDK6
CyclinD(N)
CyclinD(C)
CyclinDmRNA
CDK4(N)
CDK4(C)
CDK6(N)
CDK6(C)
CDK4mRNA
CDK6mRNA
p16mRNA
p16(C)
p16(N)
p16_CDK4/6(C)
p16_CDK4/6(N)
RB_P
RB_DP_E2F
DP_TDP
S_phase_genes

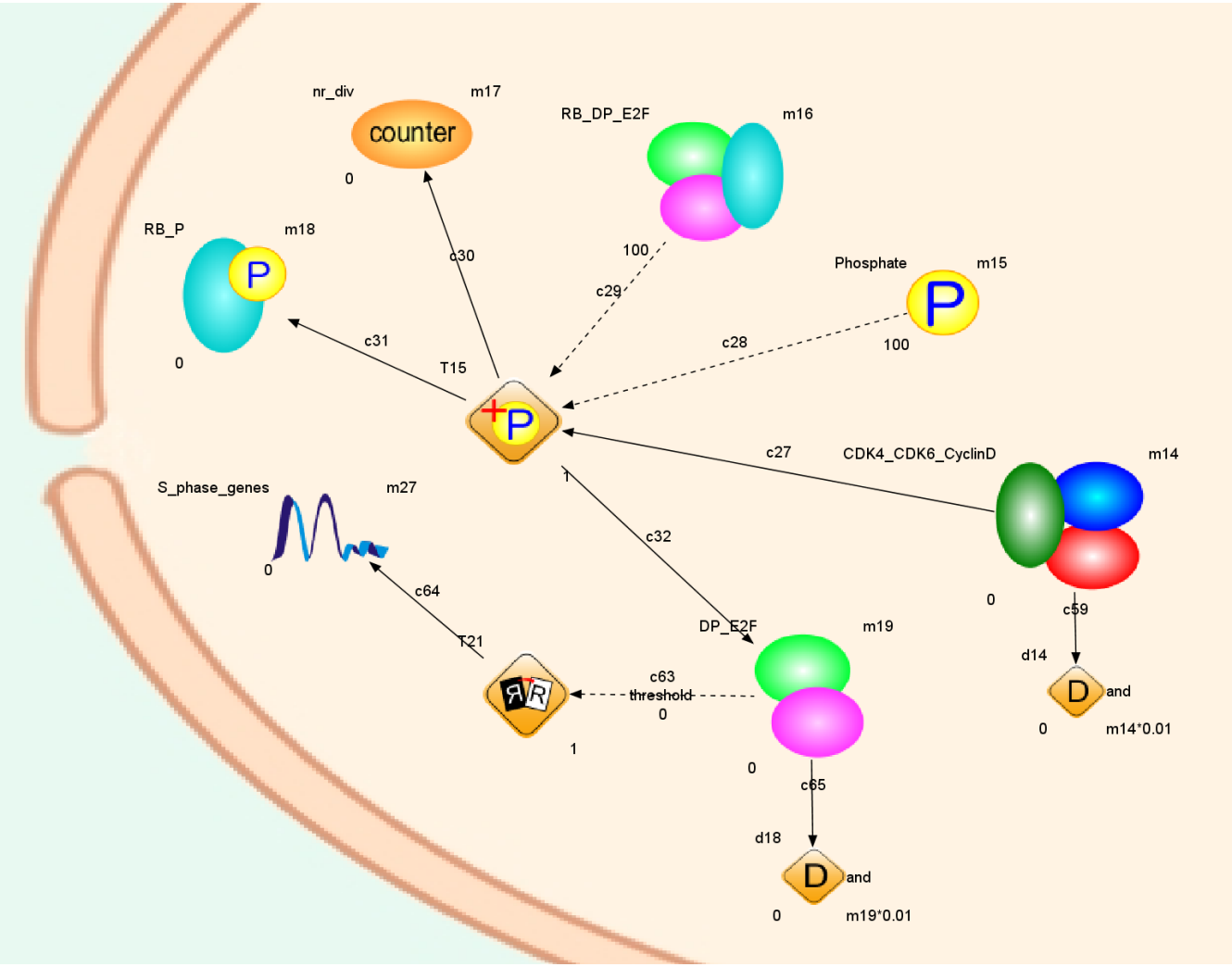

mutation
counter
G1dysfunction
Ubiquitin
Ub
Phosphate
CDK4_CDK6_CyclinD


Mutation
m20
mutation
1
p16mutated
m21
d2
p16(C)
m2
c47
c34
rule
m20==1
c35
D
m2*0.01
c33
c3
T16
c4
rule
m20==0
T2
M
m2*1
T3
m1*0.1
m2*0.1
c2
c5
p16mRNA
m1
p16(N)
m3
d3
c48
T1
c1
0
c46
D
m3*0.01
0
1
d1
D
m1*0.05


d4
D
m4*0.05
c49
T4
c6
1
CDK4mRNA
m4
0
c7
T5
m4*0.1
c8
d6
D
c51
CDK4(N)
m6
m6*0.01
c10
T6
CDK4(C)
m5
c9
c50
d5
D
m5*0.01
m5*0.1
0
CDK4_CDK6
m13
d13
c58
c18
T13
c16
0
D
m13*0.01
0
c17
m6*m9*0.01
CDK6(N)
m9
T9
c15
CDK6(C)
m8
c14
c53
d8
D
m8*0.01
m8*0.1
0
d9
c54
0
D
m9*0.01
CDK6mRNA
m7
c13
c11
0
c12
T7
c52
T8
d7
D
m7*0.05
1
m7*0.1


T10
d10
D
and
0
RR
and
1
m10*0.05
c55
c19
CyclinD(N)
m12
d12
D
and
c57
0
m12*0.01
c23
0
CyclinDmRNA
m10
0
c20
T12
T11
m11*0.1
c22
CyclinD(C)
m11
c21
m10*0.1
d11
D
c56
m11*0.01
0
c42
T19
Ub
m11*m25*0.01
Ubiquitin
m25
Ub
100
c43
d17
D
c62
m26*0.01
CyclinD[Ub]
m26
Ub
0
c44
c45
T20
D
m26*0.5


p16_CDK4/6(C)
m24
d16
c61
D
m24*0.01
0
c41
T18
d15
D
and
0
m23*0.01
m23*0.1
p16(N)
m3
c48
d3
D
and
0
m3*0.01
0
c37
c60
c40
p16_CDK4/6(N)
m23
T17
c39
and
0
c36
0
m3*m13*0.01
G1dysfunction
m22
m22==1
G1 dysfunction
1
c38
CDK4_CDK6
m13
d13
c58
D
m13*0.01
0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m17
counter
0
RB_DP_E2F
m16
100
RB_P
m18
P
0
c30
c29
Phosphate
m15
P
100
c31
c28
T15
P
c27
CDK4_CDK6_CyclinD
m14
1
S_phase_genes
m27
0
c32
0
c59
c64
DP_E2F
m19
d14
T21
c63
threshold
0
D
and
0
m14*0.01
1
0
c65
d18
D
and
0
m19*0.01

HFPN model components

Table 1. Correspondence between biological components and HFPN entities.

Entity name	Entity type	Variable	Initial value	Value type
p16mRNA	Continuous	m1	0	Double
p16(C)	Continuous	m2	0	Double
p16(N)	Continuous	m3	0	Double
CDK4mRNA	Continuous	m4	0	Double
CDK4(C)	Continuous	m5	0	Double
CDK4(N)	Continuous	m6	0	Double
CDK6mRNA	Continuous	m7	0	Double
CDK6(C)	Continuous	m8	0	Double
CDK6(N)	Continuous	m9	0	Double
CycDmRNA	Continuous	m10	0	Double
CycD(C)	Continuous	m11	0	Double
CycD(N)	Continuous	m12	0	Double
CDK4/6	Continuous	m13	0	Double
CDK4/6-CycD	Continuous	m14	0	Double
Phosphate	Continuous	m15	100	Double
Rb-DP-E2F	Continuous	m16	100	Double
nr div	Discrete	m17	0	Integer
RB P	Continuous	m18	0	Double
DP E2F	Continuous	m19	0	Double
Mutation	Generic	m20	true/false	Boolean
p16mutated	Continuous	m21	0	Double
G1-dysfunction	Generic	m22	true/false	Boolean
p16 CDK4/6(N)	Continuous	m23	0	Double
p16 CDK4/6(C)	Continuous	m24	0	Double
Ubiquitin	Continuous	m25	100	Double
CycD[Ub]	Continuous	m26	0	Double
S phase genes	Continuous	m27	0	Double

HFPN model components

Table 2. Correspondence between biological phenomena and HFPN processes.

Biological phenomenon	Process	Process type	Process rate
Transcription of p16mRNA	T1	Continuous	1
Translation of p16	T2	Continuous	m1*0.1
Nuclear import of p16	T3	Continuous	m2*0.1
Transcription of Cdk4mRNA	T4	Continuous	1
Translation of Cdk4	T5	Continuous	m4*0.1
Nuclear import of Cdk4	T6	Continuous	m5*0.1
Transcription of Cdk6mRNA	T7	Continuous	1
Translation of Cdk6	T8	Continuous	m7*0.1
Nuclear import of Cdk6	T9	Continuous	m8*0.1
Transcription of CycDmRNA	T10	Continuous	1
Translation of CycD	T11	Continuous	m10*0.1
Nuclear import of CycD	T12	Continuous	m11*0.1
Binding of Cdk4 and Cdk6	T13	Continuous	m6*m9*0.01
Binding of Cdk4/6 and CycD	T14	Continuous	m12*m13*0.01
Phosphorylation of Rb	T15	Continuous	m14*m15*m16*0.1
Mutation of p16	T16	Generic	m2*0.1
Binding of p16(N) and Cdk4/6	T17	Continuous	m3*m13*0.01
Nuclear export of p16-CDK4/6	T18	Continuous	m23*0.1
Ubiquitination of CycD	T19	Continuous	m11*m25*0.01
Degradation of CycD[Ub]	T20	Continuous	m26*0.5
Transcription of S phase genes	T21	Continuous	m19*1

HFPN model components

Table 3. Natural degradations in the HFPN model.

Biological phenomenon	Process	Process type	Process rate
Degradation of proteins	d2; d3; d5; d6; d8; d9; d11 - d18	Continuous	mi*0.01
Degradation of mRNAs	d1; d4; d7; d10	Continuous	mi*0.05

1. Doi, A., Fujita, S., Matsuno, H., Nagasaki, M., Miyano, S.: Constructing biological pathway models with Hybrid Functional Petri Nets. In Silico Biol 4(3), 271-291 (2004).
2. Doi, A., Nagasaki, M., Matsuno, H.,, Miyano, S.: Simulation-based validation of the p53 transcriptional activity with hybrid functional Petri net. In Silico Biology 6(1-2), 1-13 (2006).

HFPN model components

Table 4. Connectors in the HFPN model.

Connector	Firing style	Firing script	Connector type
c4	Rule	m20==1	Input process
c25	Rule	(m20==0&&m22==0)II (m20==1&&m22==0)II (m20==1 && m22==1)	Input process
c34	Rule	m22==1	Input association
c36	Rule	m20==0	Input process
c2,c7,c12,c20,c28,c29, c43,c63	threshold	0	Input association
c9,c14,c16,c17,c22,c24, c25,c27, c33,c37,c38,c40,c42,c45-c62,c65	threshold	0	Input process
c1,c3,c5,c6,c8,c10,c11,c13,c15,c18,c19,c21, c23,c26,c30,c31,c32,c35,c39,c41,c44,c64	threshold	0	Output process

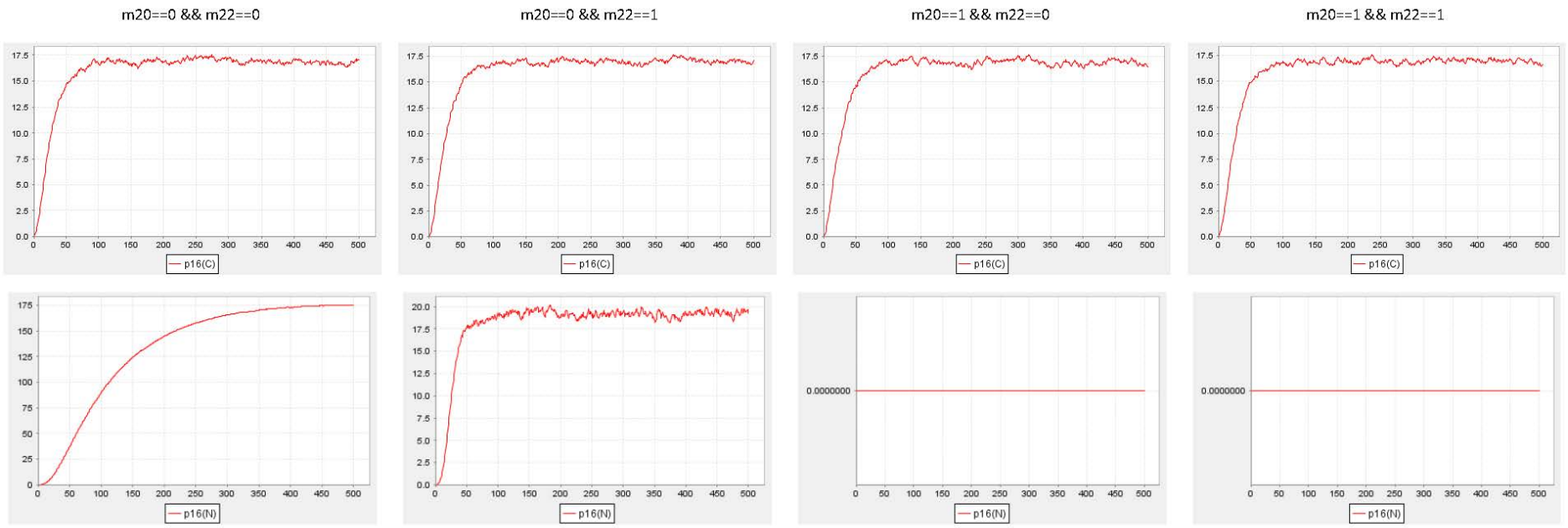
Simulation results

- Simulation results for p16
- Simulation results for Cdk4
- Simulation results for Cdk6
- Simulation results for p16-Cdk4/6

- The fact that in normal cells p16 protein is mainly accumulated in the nucleus but not in the cytoplasm [1] is confirmed by simulation results.
- The simulation results have shown that the p16-CDK4/6 protein complex is accumulated in cytoplasm rather than in nucleus. We were not able to find an experimental result to compare this finding with.
- The simulation results are in agreement with the fact that levels of Cdk proteins in cells vary little throughout the cell cycle [2].

1. Bartkova, J., Lukas, J., Guldberg, P., Alsner, J., Kirkin, A.F., Zeuthen, J., Bartek, J.: The p16-cyclin D/Cdk4-pRb pathway as a functional unit frequently altered in melanoma pathogenesis. Cancer Res, 56(23), 5475-5483 (1996).
2. Malumbres, M., BaRbacid, M.: Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer, 9(3), 153-166 (2009).

Simulation results



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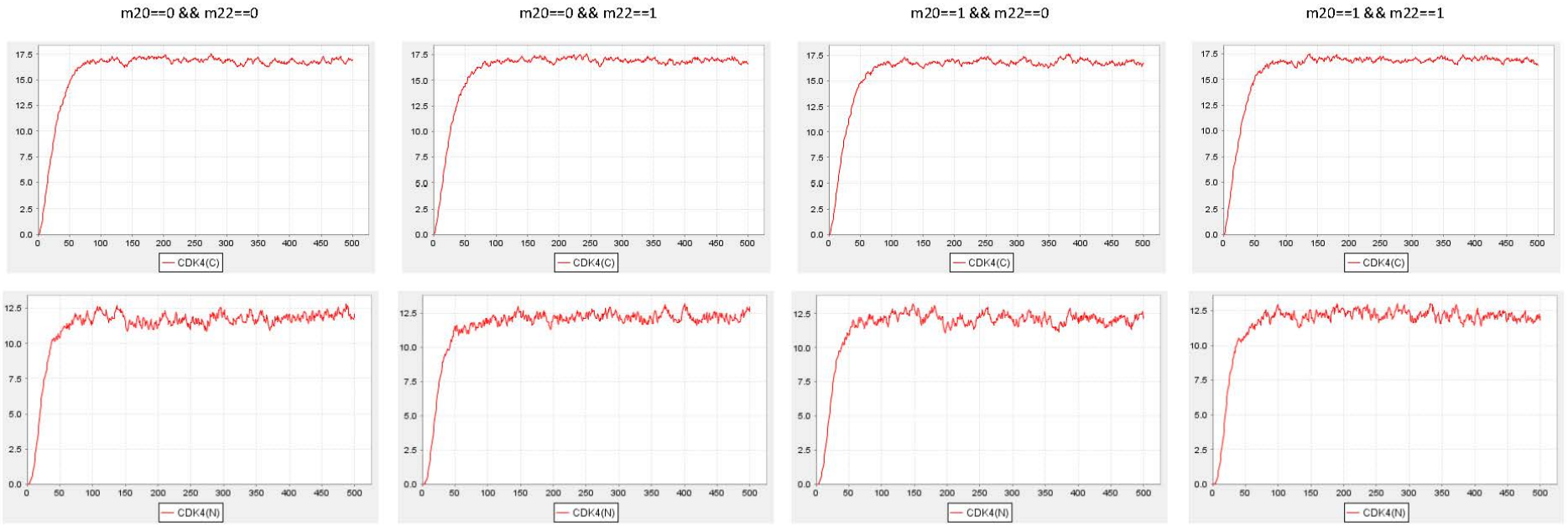

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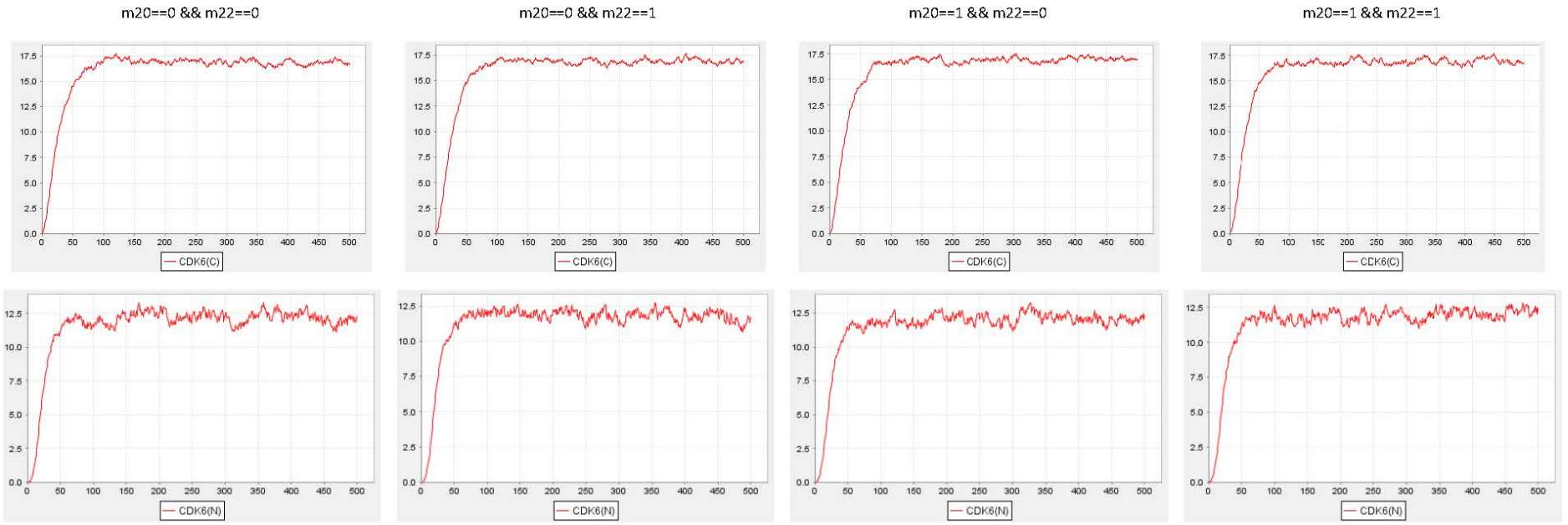

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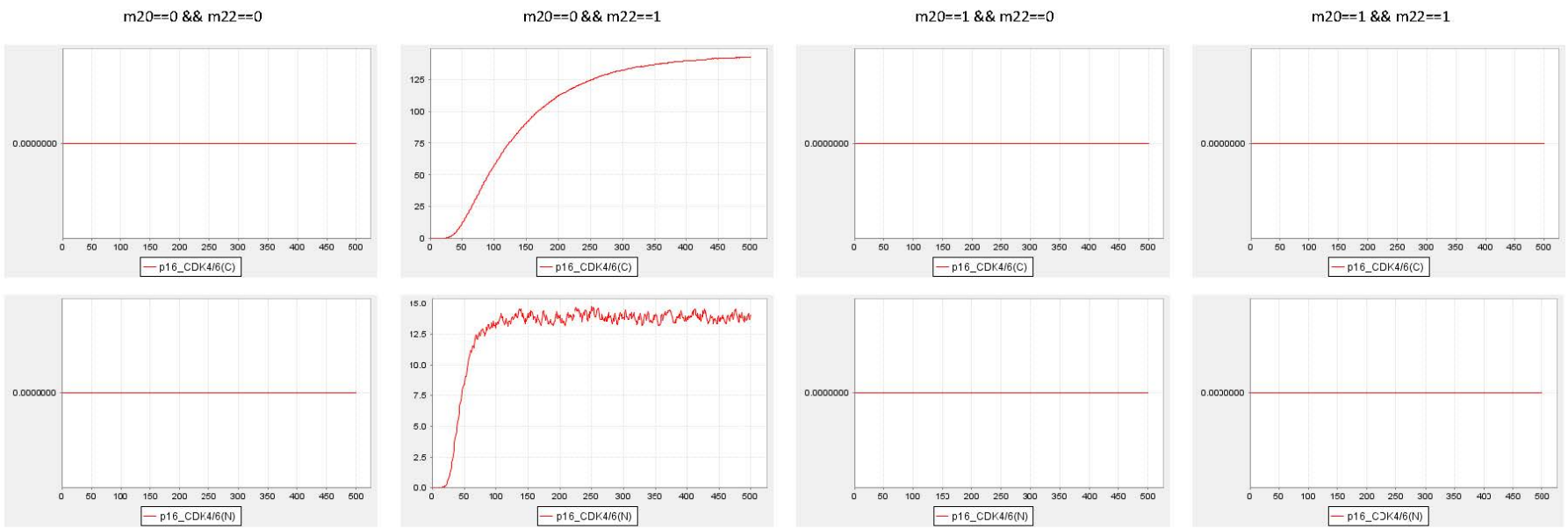

Time for questions