International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 4, Issue 1, pp: (36-44), Month: April 2016 - September 2016, Available at: www.researchpublish.com

In *Silico* Drug Discovery for Cancer Therapeutics Using Dynamical Modeling

Diego Bonilla[1,2*], Yurany Moreno[3], Jorge Petro[2], Eduardo Ramírez[4], Jose Molina[5], Adis Ayala[1]

[1] Grupo de Investigación en Bioquímica y Biología Molecular, Universidad Distrital Francisco José de Caldas, Bogotá D.C., Colombia.
[2] Grupo de Investigación en Ciencias de la Actividad Física, el Deporte y la Salud, Universidad de Córdoba, Montería, Colombia.
[3] Cancer Research Academic Unit, University of Southampton, Southampton SO17 1BJ, UK.
[4] Área Biomédica, Facultad de Educación Física, Recreación y Deporte, Politécnico Jaime Isaza Cadavid, Medellín, Colombia.
[5] Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica.
* Corresponding author: dabonilla@g-se.com / +57 317 7555184

Abstract: **Drug discovery is a complex multistep process. A central question in drug discovery is the potency and efficacy of the compound being tested. For cancer, which is at its core a proliferative disorder, inhibiting cell proliferation is an effective therapeutic strategy. Recent studies of plant derived compounds have shown that they are selective inhibitors of key components of cell cycle network (cyclin-dependent kinases and Cdc25). Our goal is to use dynamical modelling to determine which compounds are the best candidates for future chemical modifications to make them into potential drugs. We use the ordinary differential equation (ODE) models in MATLAB to determine the ranking of hypothetical compounds as potential drugs by testing the effect of different concentrations on inhibition of the eukaryotic cell cycle (minimal CDK network 2015), assuming that greater inhibition of the cell cycle at lower concentration of the drug will mean a better drug. Our results rank the hypothetical drugs in order of their efficacy, based on ATP competitive inhibition, and give hints about effectiveness of big sets of compounds with potential anti-cancer activity. Hence, we present a preliminary *In Silico* drug-discovery method which strengthens the mathematical modelling as a cost-effective first step and powerful approach for investigating complex cell signalling networks.**

Keywords: **Drug-discovery, Cancer Prevention and Treatment, Cell Cycle Model, Physical Activity, Anticancer.**

I. INTRODUCTION

Cell cycle has been an actively-studied cellular process in molecular biology since more than 30 years, especially due to its implications on cancer progression which is seen as a proliferative cell disorder. The purpose of cell cycle is to ensure life of all biological organisms in earth, by means of conservation of integrity, reparation and evolution of DNA through each cycle from mother to daughter cells, so that it is considered as a crucial biological process. The cell cycle starts when a quiescent cell, also known as G0 cells, receives a mitogenic stimulus that activates the cell division machineries. The stimulated cell reaches the G1 stage, characterized by containing the most critical restriction point based on the regulated noteworthy proteins known as cyclins. These proteins subsequently activate in a specific-manner cyclin-dependent kinases (CDKs) by dimerization, which play an essential role during cell cycle progression. After overcome this checkpoint, S phase takes place in order to replicate DNA, which allows the cell to duplicate its chromosomic material and grow in size. Finally, the cell enters in the preparatory stage for M phase (mitosis), or G2 phase, in which cytoplasm division begins and a markedly structure modification takes place [1].

As all important biochemical processes, cell cycle is controlled by many entities (kinases, phosphatases, protein complexes, etc.) organized within network motifs, which allow to determine checkpoints and hubs throughout cell division [2]. The major checkpoints are between G1 and S-phase and G2 and M-phase, highlighting the fact that CDKs are very important regulatory points, with high redundancy in their functions [3] necessary to guarantee the progression of cell division. Furthermore, other proteins such as Wee1, Cdc25, Rum1 among others, have several implications across M-phase progression either by promoting or inhibiting CDK/cyclin complexes formation. In recent years, scientists have postulated some of the proteins described before as therapeutic targets to control and arrest cell cycle during cancer [4],[5]. The most studied molecules that have become a validated therapeutic target are CDKs, several of these kinase inhibitors are under Phase III of clinical evaluations. Also, Cdc25 inhibitors have shown good results to arrest the cell cycle [6]. Some of these drugs are natural products extracted from plants (e.g. staurosporine, falvopiridol, SV37, etc.) while others are purely synthetic (e.g. dinaciclib, LEE-011, etc.). A high proportion of CDK inhibitors are ATP-competitive inhibitors, where the drug binds to the active site on the CDK, albeit development of ATP non-competitive and allosteric inhibitors has been a recent tactic [6],[7],[8]. As Krystof & Uldrijan [9] stated "*poor therapeutic outcomes and serious side effects, together with acquired resistance to multiple drugs, are common problems of current cancer therapies. Therefore, there is an urgent need for new cancer-targeted drugs…*" However, drug-discovery methods are quite expensive and time demanding, with no mention of high lab requirements. In this way, dynamical modeling emerges as an effective alternative to solve some points of this issue because it provides with systematic properties, reproducibility and future refining of the model according to molecular advances. Furthermore, dynamical models allow saving time and predicting system behavior after determined perturbations. Therefore, this strategy complements the pipeline for drug-discovery methods as a previous analysis to guide future experimental designs. Hence, we aim to develop a preliminary *In Silico* drug-discovery method for cancer therapeutics based on a minimal CDK network of eukaryotic cell cycle from Gérard et al. 2015 [10], where cells progress through S and M in perfectly wild type fashion by using a fusion protein cdc13-L-cdc2 with both SPF (S-phase promoting factor) and MPF (M-phase promoting factor) activities and other molecular inferences (See [10] for details).

II. COMPUTATIONAL APPROACH

The mathematical model is based on the minimal CDK network for cell cycle control in eukaryotes described by Gérard et al. (2015) [10]. In the paper they used both stochastic and deterministic methods to model the cell cycle, we focus on the deterministic model though. Although this model does not represent fully a real mammalian cell cycle, it is a good framework to construct a simple model. Please focus on Fig. 1 (adapted from Gerard et al. [10]) where a Cdk (cyclin-dependent kinase) which is called Cdc2 and a phosphatase called Cdc25 controls the yeast cell cycle. We assume that the extent of avoiding mitosis is directly proportional to extent of inhibition of proliferation of cancer cells. Thus, Cdc2 and Cdc25 were selected as therapeutic targets in our model. In brief, we used the Ordinary Differential Equations (See supplemental material ODEs of the model) from Gérard's cell cycle to establish a set of inhibitory approaches on either Cdc2 or Cdc25 for five hypothetical plant-derived compounds, PDC (See Appendix 1), in MATLAB R2015b version 8.6. Finally, concentration effect of different PDCs on cell cycle arrest was analyzed, in order to rank compounds and establish the *In Silico* drug discovery (See supplemental code in MATLAB for PDC1 and PDC5).

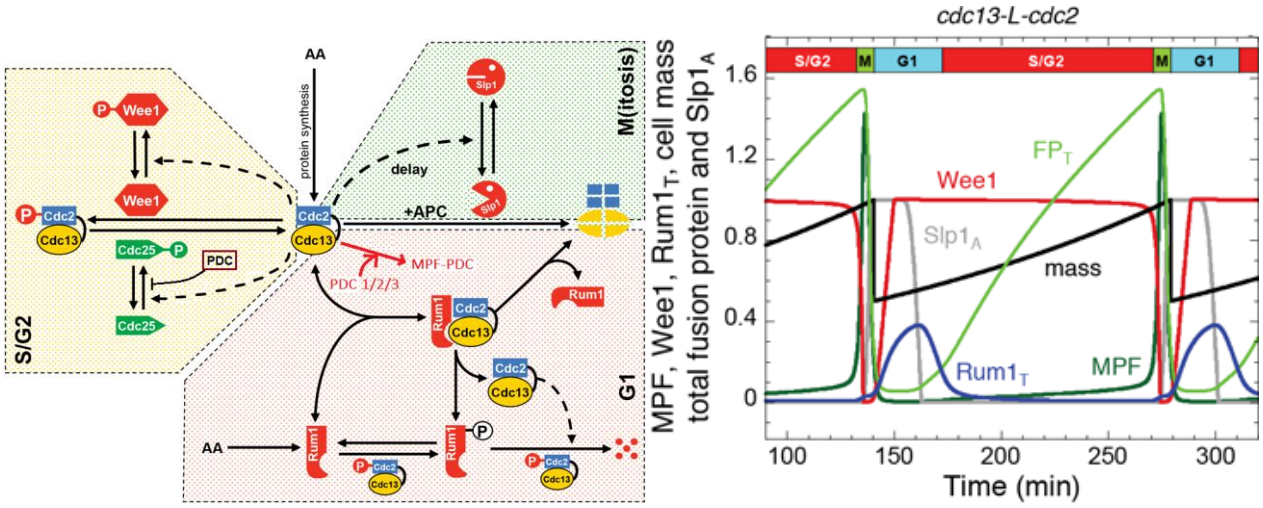

Figure 1. MCN and Dynamical behavior of cell cycle model by Gérard et al. [4]. Up. Adapted reaction scheme for the minimal Cdk network driving the cell cycle in fission yeast. Solid lines represent biochemical reactions, while dashed lines define catalytic effects. Only one Cdk:cyclin complex (the fusion protein Cdc13-L-Cdc2, referred to as MPF) controls the successive progression through DNA replication and mitosis. Inhibition PDCs is included on their respective targets. **Down.** MCN strain (cdc13-L-cdc2 Δcdc13 Δcdc2 ΔCCP). Time evolution of total fusion protein (light green, FP_T), active MPF (dark green), active Wee1 (red), total Rum1 (blue), active APC:Slp1 (grey) and cell mass (black). Total Rum1 is defined by $Rum1_T$ = Rum1 + Rum1P + MPF:Rum1. Descriptions and adapted figures taken from: Gérard C, Tyson JJ, Coudreuse D, Novák B. Cell Cycle Control by a Minimal Cdk Network. *PLoS Computational Biology*. 2015. **11** (2): e1004056.

III. RESULTS

In the minimal strain model proposed by Gérard et al. [10], the MPF activity relies on complex Cdc2-L-Cdc13 (where Cdc2 is a CDK, L is a linker or fusion protein, and Cdc13 is a cyclin), especially on fusion protein because there are not Cdc2 and Cdc13 monomers. The minimal cell network (MCN) and dynamical behavior for this cell cycle model is represented in Fig. 1. According to above, cell cycle inhibition by PDC01, PDC02 and PDC03 will be described in terms of MPF inhibition instead of net Cdc2 inhibition.

In general terms, the PDCs effects on their respective targets are the increase in cell size, longer G1-phase time and block on S/G2. Particularly, the introduction of MPF inhibitors (PDC01, PDC02 and PDC03) leads to a notorious increase in cell mass (Fig. 2).

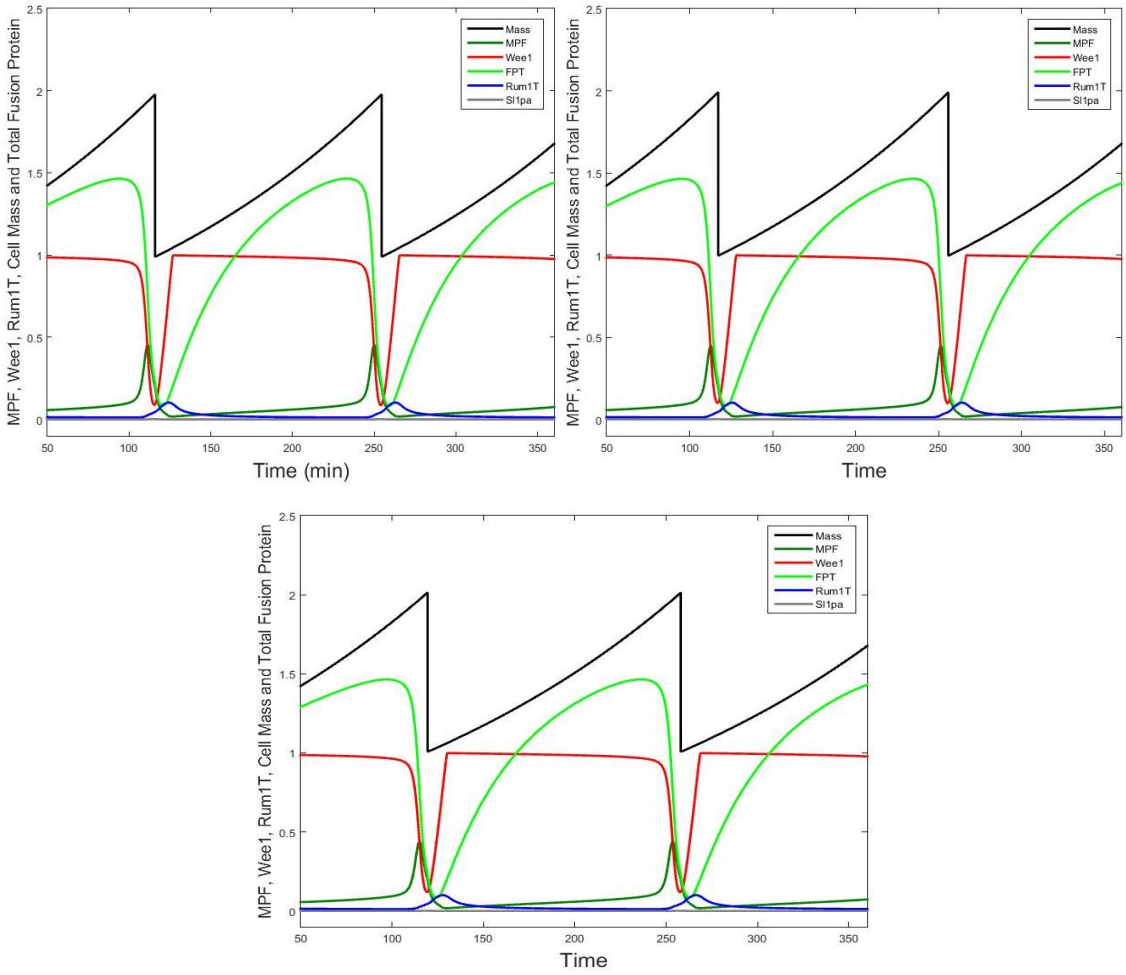

Fig. 2. MPF inhibition on cell cycle dynamics. Dynamics of different cell cycle components after MPF inhibition by PDC01 (Upper Left), PDC02 (Upper Right) and PDC03 (Down) addition. Concentrations of PDCs are listed on Table 1. Note that three plant derived compounds inhibit effectively MPF (active state) and increase cell mass with regard to

normal cell cycle (Figure 1), which can produce mitotic catastrophe and cell death. Moreover, there is a small increase in G1-phase time is depicted.

On the other hand, Cdc25 inhibitors (PDC04 and PDC05) not only augment the cell size after cell division but also produce an S/G2 block and a subsequently longer G1/S time (Fig. 3), which delay cell cycle.

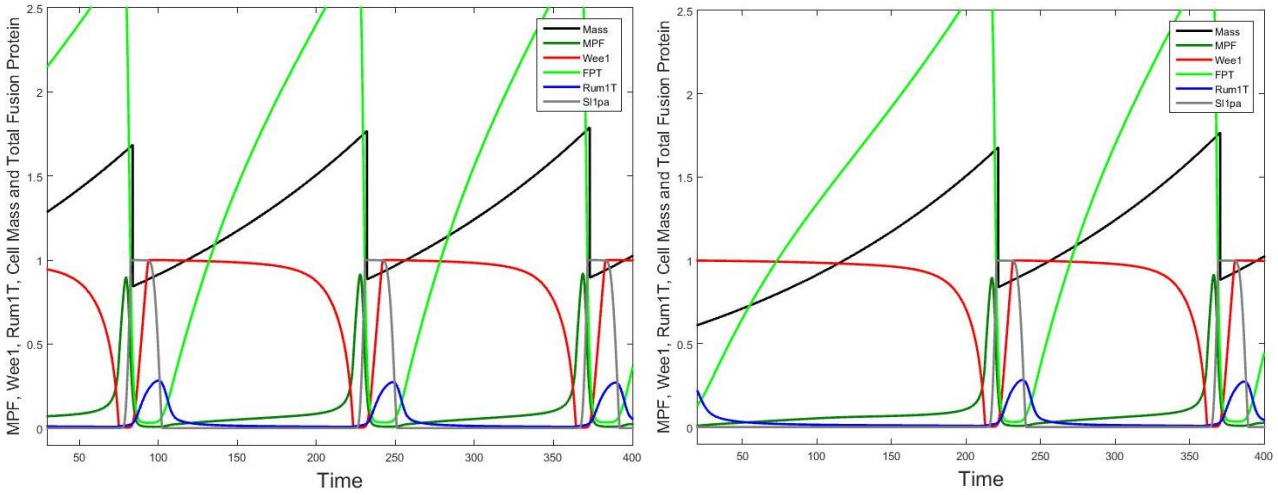

Fig. 3. Cdc25 inhibition on cell cycle dynamics. Dynamics of different cell cycle components after addition of Cdc25 inhibitors, PDC04 (Up) and PDC05 (Down). Concentrations of PDCs are listed on Table 1. Note the prolonged time between cycles (>200 min) due to an increase in G1/S time and the S/G2 block, this could be produced by a longer and sustained concentration of Wee1 especially with PDC05. There is a tendency for mitotic catastrophe of cells treated with both PDC04 and PDC05, although cells do not reach threshold for this process (mass ≈ 2).

To compare the concentration effect of PDCs we show in TABLE 1 the drug ranking for cell cycle inhibition after modification of MATLAB code for MPF and Cdc25 inhibition with their respective PDC inhibitors separately.

TABLE 1. RANKING OF PDC DRUGS IN ORDER OF THEIR EFFICACY ON CELL CYCLE ARREST

Drug	Concentration (mM)	Mass of Cell	Inhibition Target
PDC05	6×10^{-4}	1.79633	Cdc25
PDC02	2.45×10^{-3}	1.99138	MPF
PDC04	9×10^{-4}	1.83041	Cdc25
PDC03	4.97×10^{-3}	2.01400	MPF
PDC01	5.3×10^{-3}	1.97859	MPF

IV. DISCUSSION

MPF inhibition by PDC01, PDC02 and PDC03 showed a clear increase in cell mass, possibly due to a prolonged time in G1 stage. This increment in cell size has been reported to produce a conditional mitotic catastrophe, where cells do not complete correctly DNA replication and go directly to mitosis and thereby these cells death [11]. In fact, Gérard et al. [10] established a mitotic catastrophe threshold of mass ≈ 2. As we can see in Table 1, all MPF inhibitors reached easily this cell mass threshold indicating that concentrations in order of 10^{-3} mM were enough to induce an anti-proliferative effect and made possible the ranking. The tendency towards G1-phase (right in Fig. 1) because of the notorious increase in MPF-PDC and lack of active MPF could explain this phenomenon. Some research before have denoted this noteworthy effect of CDK inhibitors on cell cycle progression [9],[12].

On the other hand, Cdc25 inhibition with PDC04 and PDC05 had a highlighted effect on cell cycle delay. There was a distinguishable prolongation in time between cycles (>200 min), especially for PDC05 treatment, which could be associated with an increase in G1/S time and an S/G2 block. Furthermore, sustained concentrations of Wee1 (Fig. 3, down) could be related with the block on S/G2 due to its regulatory role on MPF activity, shifting the process to S phase (left in Fig. 1). According to this, we could infer tardiness in cell cycle progression which would produce disruption in intracellular processes and finally manifestation of cell death. Even though cells did not reach mitotic catastrophe

threshold (mass ≈ 2), there was a significant increase in cell mass which could induced cell death. In fact, over-expression of Cdc25 activity has been frequently observed in different types of cancer (thyroid, laryngeal, esophageal, gastric, hepatocellular, ovarian, endometrial, prostate, and colorectal as well as in non-Hodgkin lymphomas [13]. Over-expression is most often accompanied by over-activation of Cdc2 and correlates with aggressiveness, bad prognosis and high-grade tumors. In this sense, targeting inhibition activity of Cdc25 by PDCs appears a better therapeutic approach than MPF (Cdc2) inhibitors, taking into account the larger disruption of Wee1 control in comparison to effects on MPF inhibition in our model. This correlates with the fact that Cdc25 has become a promising target for the development of anticancer drugs recently [6], although more research and experimental confirmation is needed.

Finally, the results presented in TABLE 1 are the minimum concentrations of each PDC that leads to cell cycle arrest. A better comparison between PDCs can be done if we establish a threshold level for cell death (mass ≈ 2, for mitotic catastrophe [10]), perhaps using step function (*heaviside*) in MATLAB and assigning 0 for cell survival and 1 for cell death within the code.

V. CONCLUSION

Our results suggest that this *In Silico* strategy for drug comparison and discovery is optimal in terms of time and experimental predictions, but experimental validation is required. Some drawbacks of this strategy encompass the lack of kinetic data, biochemical signaling gaps and genetic/metabolic origins between patients. This approach can be added to the high amount of mathematical modeling that have been developed to analyze cell cycle dynamics, which allow researchers to initiate in new investigation lines for cancer treatment [2],[14]. As an experimental component of a systems biology pipeline, it seems that *In Silico* drug-discovery would play an impressive role on cancer treatment and other diseases [15]. Particularly in cancer, an *In Silico* method can provide with prominent previous advances that will help to accomplish research objectives and gain prior knowledge to understand the complex metabolic networks that involves this disease. Hence, this kind of computational strategy will lead to progression in future medicine, supporting the new paradigm of P4 medicine (preventive, personalized, participatory and predictive).

Appendix 1. *Hypothetical Experimental Data on Lead Compounds PDC01 to PDC05.*

1. All 5 compounds are moderately soluble in water or plasma. The maximum concentration that can be achieved is 0.1 mM.

2. From binding experiments the K_is for the five different compounds are:

Drug	Ki (nM)	Enzyme
PDC01	110.00	Cdc2
PDC02	240.50	
PDC03	120.76	
PDC04	200.12	Cdc25
PDC05	105.76	

Appendix 2. *MATLAB Code for Cdc25 Inhibition with PDC05.*

```
% This code is based on the model presented in: Gérard, Claude, et al. "Cell Cycle Control by a Minimal Cdk Network." PLoS Comput Biol 11.2 (2015): e1004056.

function cellcycle
clear;
clc;
close all;
n=1;
nbvar=11; %Number of variables and initial conditions:
%Initial conditions
MPF0=0;
MPFp0=0;
Slp1A0=0;
IEA0=0;
```

```
MPFrum10=0.01;
Rum10=0.001;
Rum1p0=0.01;
Wee10=0.01;
Cdc25p0=0.01;
M0=0.3;
Cdc25PDC0=0;
xini=[MPF0 MPFp0 Slp1A0 IEA0 MPFrum10 Rum10 Rum1p0 Wee10 Cdc25p0 M0 Cdc25PDC0];
fprintf('varia=30_MCN_allparam_X=0.1_10cells')
for n=1:3
% Time parameters:
trans=400;
tend=300;
ndiv=10;
tstep=0.1;
integration(xini,trans,tend,tstep,ndiv);
n=n+1
end
% Integration
function output=integration(x0,trans,tend,tstep,ndiv);

division=[];
options = odeset('Events',@events,'OutputSel',1);
tspan = [0:tstep:tend];
R=[0 x0];
treset=[];
for i=1:ndiv
fprintf('.')
[t x] = ode23s(@dxdt,tspan,x0,options);
x0=x(end,:);
x0(10)=x0(10)/2; % reset of the mass
tt=t+R(end,1);
R=[R;tt x];
if tt(end)>trans
treset=[treset; tt(end)];
end
end
fprintf('\n\n')
%%% Plots
t=R(:,1);
k=find(t>trans);
k=k(1);
t=R(:,1)-trans;
tend=t(end);
mass=R(:,11);
MPF=R(:,2); %%
MPFp=R(:,3); %%
Wee1=R(:,9); %%
Rum1T=R(:,6)+R(:,7)+R(:,8); %%
Slp1a=R(:,4); %%
figure (1)
plot(t,mass,'k','LineWidth',3);
hold on;
plot(t,MPF,'Color',[0 0.5 0],'LineWidth',3); %%
plot(t,Wee1,'r','LineWidth',3); %%
plot(t,MPF+MPFp,'g','LineWidth',3); %%
```

```
plot(t,Rum1T,'b','LineWidth',3); %%
plot(t,Slp1a,'Color',[0.5 0.5 0.5],'LineWidth',3); %%
xlabel('Time','fontsize',18);
ylabel('MPF, Wee1, Rum1T, Cell Mass and Total Fusion Protein','fontsize',14);
xlim([20 400]);
ylim([-0.1 2.5]);
legend('Mass','MPF','Wee1','FPT','Rum1T','Sl1pa'); %%
set(findobj(gca,'Type','line'),'LineWidth',2);
%%% Statistics
tdiv=treset(end)-treset(end-1);
mdiv=max(mass(k:end));
division=[division ; tdiv mdiv]
fprintf('t_div=%g \n',tdiv)
fprintf('m_div=%g \n',mdiv)
function y = dxdt(t,v)
%Parameters
kSMPF=0.05;
kD1CYC=0.0235;
kD2CYC=0.75;
kASS=100;
kDISS=0.0025;
kDRUM1=0.125;
kIRUM1=2;
k1SLP1=0.8;
J1SLP1=0.001;
V2SLP1=0.2;
J2SLP1=0.001;
kDMPFRUM1=0.35;
k1IE=0.2;
a=0.05;
J1IE=0.001;
V2IE=0.05;
J2IE=0.001;
k12RUM1=50;
kARUM1=35;
VWEE1=0.125;
J1WEE1=0.01;
J2WEE1=0.01;
k1CDC25=0.05;
k2CDC25=2.5;
J1CDC25=0.01;
J2CDC25=0.01;
VCDC25=0.2;
k1WEE1=0.05;
k2WEE1=2.5;
m=0.005;
Cdc25T=1;
Wee1T=1;
IET=1;
Slp1T=1;
VSRUM1=0.06;
kDRUM1P=250;
kDX=1;
CCP=0; %%
Ki=105.76;
PDC=0.0006;
```

```
%Variables
MPF=v(1);
MPFp=v(2);
Slp1A=v(3);
IEA=v(4);
MPFRum1=v(5);
Rum1=v(6);
Rum1p=v(7);
Wee1=v(8);
Cdc25p=v(9);
Mass=v(10);
Cdc25PDC=v(11);
%Algebric Equations
kWEE1=k1WEE1*Wee1T+(k2WEE1-k1WEE1)*Wee1;
kWEE1p=0.625; %%
kCDC25=k1CDC25*Cdc25T+(k2CDC25-k1CDC25)*Cdc25p;
kCDC25p=1; %%
Wee1p=Wee1T-Wee1;
Cdc25=Cdc25T-Cdc25p-Cdc25PDC;
IE=IET-IEA;
Slp1=Slp1T-Slp1A;
% ODEs
y = [
%MPF
kSMPF*Mass-(kWEE1*MPF)+(kCDC25*MPFp)-(kD1CYC+kD2CYC*Slp1A)*MPF-
kASS*Rum1*MPF+(kDISS+kDRUM1+kIRUM1)*MPFRum1;
%MPFp
kWEE1*MPF-kCDC25*MPFp-(kD1CYC+kD2CYC*Slp1A)*MPFp;
%Slp1A
k1SLP1*IEA*Slp1/(J1SLP1+Slp1)-V2SLP1*Slp1A/(J2SLP1+Slp1A);
%IEA
k1IE*(MPF)*IE/(J1IE+IE)-V2IE*IEA/(J2IE+IEA);
%MPFRum1
kASS*Rum1*MPF-(kDISS+kDRUM1+kIRUM1+kDMPFRUM1)*MPFRum1;
%Rum1
VSRUM1-kASS*Rum1*MPF+(kDISS+kDMPFRUM1)*MPFRum1-k12RUM1*MPFp*Rum1+kARUM1*Rum1p-
kDRUM1*Rum1;
%Rum1p
kIRUM1*MPFRum1+k12RUM1*MPFp*Rum1-kARUM1*Rum1p-kDRUM1P*(MPF)*Rum1p-kDRUM1*Rum1p;
%Wee1
VWEE1*Wee1p/(J1WEE1+Wee1p)-kWEE1p*(MPF+a*MPFp)*Wee1/(J2WEE1+Wee1); %%
%Cdc25p
kCDC25p*(MPF+a*MPFp)*Cdc25/(J1CDC25+Cdc25)-VCDC25*Cdc25p/(J2CDC25+Cdc25p); %%
%Mass
m*Mass;
%Cdc25PDC
Ki*PDC*Cdc25p;
];
% Event
function [value,isterminal,direction] = events(t,x)
% Locate the time when (testvar-threshold) passes through zero in a decreasing direction and stop integration.
testvar=x(1); % cdk
threshold=0.2; % threshold on cdk
value = testvar-threshold; % detect height = 0
isterminal = 1; % stop the integration
direction = -1; % negative direction
```

REFERENCES

[1] Tyson JJ, Novák B. Temporal Organization Of The Cell Cycle. *Current Biology*. 2008. 18 (17): R759-R768.

[2] Sible JC, Tyson JJ. Mathematical Modeling as a Tool for Investigating Cell Cycle Control Networks. *Methods*. 2007. 41 (2): 238-247.

[3] Echalier A, Cot E, Camasses A, Hodimont E, Hoh F, Jay P, Sheinerman F, Krasinska L, Fisher D. An Integrated Chemical Biology Approach Provides Insight into Cdk2 Functional Redundancy and Inhibitor Sensitivity. *Chem Biol*. 2012. 19 (8): 1028-1040.

[4] Sánchez C, Gelbert LM, Lallena MJ, de Dios A. Cyclin Dependent Kinase (CDK) Inhibitors as Anticancer Drugs. *Bioorg Med Chem Lett*. 2015. 25 (17): 3420-3435.

[5] Cho YC, Park JE, Park BC, Kim JH, Jeong DG, Park SG, Cho S. Cell Cycle-Dependent Cdc25C Phosphatase Determines Cell Survival by Regulating Apoptosis Signal-Regulating Kinase 1. *Cell Death Differ*. 2015. 22 (10): 1605-1617.

[6] Bana E, Sibille E, Valente S, Cerella C, Chaimbault P, Kirsch G, Dicato M, Diederich M, Bagrel D. A Novel Coumarin-Quinone Derivative SV37 Inhibits CDC25 Phosphatases, Impairs Proliferation, and Induces Cell Death. *Mol Carcinog*. 2015. 54 (3): 229-241.

[7] de la Cruz J, Kim DH, Hwang SG. Anti-Cancer Effects of Cnidium Officinale Makino Extract Mediated through Apoptosis and Cell Cycle Arrest in the HT-29 Human Colorectal Cancer Cell Line. *Asian Pac J Cancer Prev*. 2014. 15 (13): 5117-5121.

[8] [8].Mariaule G, Belmont P. Cyclin-Dependent Kinase Inhibitors as Marketed Anticancer Drugs: Where Are We Now? A Short Survey. *Molecules*. 2014. 19 (9): 14366-14382.

[9] Krystof V, Uldrijan S. Cyclin-Dependent Kinase Inhibitors as Anticancer Drugs. *Curr Drug Targets*. 2010. 11 (3): 291-302.

[10] Gérard C, Tyson JJ, Coudreuse D, Novák B. Cell Cycle Control by a Minimal Cdk Network. *PLoS Computational Biology*. 2015. 11 (2): e1004056. doi:10.1371/journal.pcbi.1004056.

[11] Rello S, Herrero D, Lagares L, López R, Mulet N, Huertas J, Garcia S, García X, Muñoz C, Tirado OM. The Importance of Being Dead: Cell Death Mechanisms Assessment in Anti-Sarcoma Therapy. *Front Oncol*. 2015. 5 (82). doi: 10.3389/fonc.2015.00082.

[12] Gérard C, Tyson JJ, Novák B. Minimal Models for Cell-Cycle Control based on Competitive Inhibition and Multisite Phosphorylations of Cdk Substrates. *Biophys J*. 2013. 104 (6): 1367-1379.

[13] Boutros R, Lobjois V, Ducommun B. CDC25 phosphatases in cancer cells: Key players? Good targets? *Nat Rev Cancer*. 2007. 7: 495–507.

[14] Tyson JJ, Baumann WT, Chen C, Verdugo A, Tavassoly I, Wang Y, Weiner LM, Clarke R. Dynamic Modelling Of Oestrogen Signalling And Cell Fate In Breast Cancer Cells. *Nature Reviews Cancer*. 2011. 11 (7): 523-532.

[15] Iyengar R, Zhao S, Chung SW, Mager DE, Gallo JM. Merging Systems Biology with Pharmacodynamics. *Sci Transl Med*. 2012. 4 (126): 126ps7. doi: 10.1126/scitranslmed.3003563.