IN SILICO DRUG DISCOVERY USING DYNAMICAL MODELING

Introduction

Cancers are a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. The cell cycle division plays a crucial role in the normal or abnormal growth, development, reproduction and cellular repair. [3,5] Insights into how the cycle behaves in a multitude of conditions can lead to fruitful achievements in drug development and cancer therapy.

Cyclin-dependent kinases (CDKs) mediate the cell cycle progression and regulate transcription, additionally in many cancers CDKs expression has been found to be overactive or its proteins not properly functional. Regulation happens by the association CDK to cyclins in a CDK:cyclin complex, hence making it clear that the abnormal expression of cyclins will have a direct impact on cell deregulation and may lead to tumor development.[1,2,3] This points to them as prime target candidates for cancer drug therapy as already proven by the success of Imatinib, [1] the 7-year update of phase III trials for Imatinib estimates an event-free survival (EFS) and overall survival (OS) of 81% and 86%, respectively.[9]

Unfortunately the introduction of a new drug is an expensive, time-consuming and strenuous process. It is estimated that a typical new drug discovery, from lead identification through to clinical trials, can take up to 14 years and cost 800 million US dollars. [9] Such high costs severely restrict new drug research and development.

By using Dynamical Modeling and constructing systems of equations capable of represent, even if in a simplistic manner, the cell pathways and regulatory mechanisms that can systematically capture the effect of a given drug on the complex molecular networks and on human physiology we hope to expedite the research process and eventually examine huge compound libraries in short time periods with lower costs. [6,7,9,11]

To assess this possibility we used the minimal Cdk network model, consisting of a single cyclin-Cdk fusion protein, that in the fission yeast cell can control DNA synthesis and mitosis.[3] We show that by adopting a simple dynamical model based on this minimal network we were able to screen 5 candidate drugs of cell cycle inhibition in a short period and select the best candidate among them for further development.

Computational Approach

Cells need to reach a critical size to divide, the dependency of the cell cycle on growth is thought to be established by size requirements for major cell cycle transitions. In eukaryotes, like the budding yeast such critical cell size thresholds are imposed at the G1/S phase transition and/or the G2/M phase transition. [5] They govern the time spent by cells in G1 phase and/or G2 phase and in the CDK model they are regulated by the reactions in Figure 1.[3] By perturbing these reactions

with different kinds of inhibition we are able to cause them to change, delaying or hastening these thresholds and causing cells to diverge in size and viability. [7,10,11]

To create a useful computational model we need to follow the cell cycle reactions through time and be able to see the oscillations, bistability behavior, size and mass variations, thresholds, reactions, etc. In the simplest form all the events of the cell cycle depend on chemical reactions and the progression of chemical reactions can be described by equations that relate time to changing quantities. [4,5]

Ordinary Differential Equations (ODEs) are known as chemical kinetics equations and are derived similarly to the Michaelis-Menten equation, they are able to generate both bistability and oscillation they also describe the functions of one independent variable and its derivatives moving through time. [4]

Furthermore we know the initial values of the variables and this system can be treated as a single compartment, from this we can assert that the cell cycle can be effectively modeled by a dynamical system of ODEs that involves continuous fluctuation of variables. From each of the 10 variables present on the CDK model one ODE was generated and then the 10 ODEs were coupled and integrated to form a dynamical system of equations, [3] thus we could generate a model that would account for the time evolution of the 10 variables and their derivatives. Using this system we were able track the concentrations and variables as they moved through time.

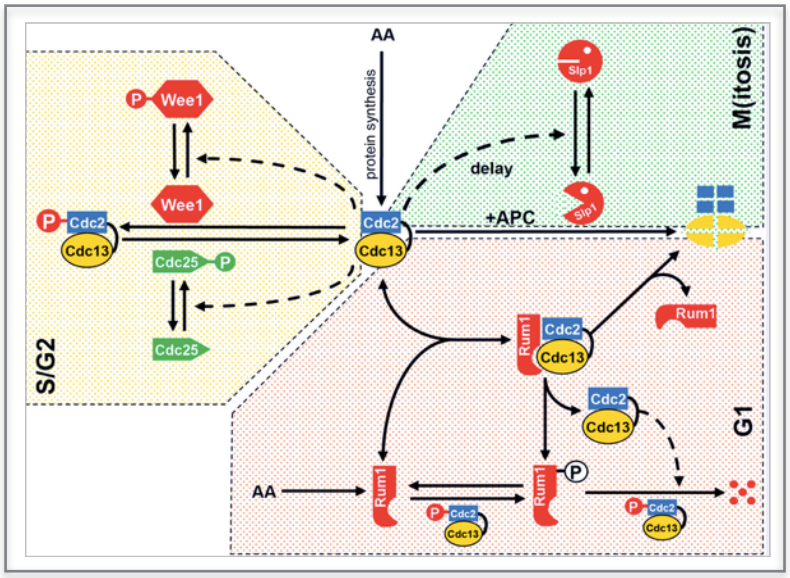

Figure 1. Reaction scheme for the minimal CDK network driving the cell cycle in fifteen yeast.
* From Gerard et al,2015 [3]

Results

The Minimal Cdk Network model was used to simulate non-competitive inhibition in the cell, it was expected that this inhibition would to increase phase G1 duration and decrease S/G2 thus preventing the cell from dividing or forcing the transition from phase S/G2 to M without proper DNA synthesis rendering these new cells unviable.This was done by applying different concentrations of the following candidate drugs:

I. PDC-01, PDC-2, PDC-03 which are selective inhibitors of cyclin-dependent kinases, and
II. PDC-04 and PDC05 which are selective inhibitors of the Cdc25 phosphatase.

The candidate drugs in **I** were modeled as non-competitive selective inhibitors of CDK (the active cyclin:CDK, Cdc13-L-Cdc2 is referred to as MPF). At the end of M phase, MPF is disassembled when the anaphase-promoting complex (APC) polyubiquitinates Cdc13, which tags the fusion protein of the minimal module for rapid proteasomal degradation (Fig. 1, M module).[3] Initial degradation of the fusion protein is mediated by the APC in conjunction with Slp1. This system introduces a negative feedback loop, as MPF promotes its own degradation by activating APC:Slp1, a feature that is common to all eukaryotes. Theoretical arguments and experimental evidence suggest that this negative feedback loop is time-delayed, but the underlying molecular mechanism of this delay is unclear. For the minimal CDK network the time delay was generated by inserting an intermediary enzyme (IE) between MPF and APC:Slp1.[3] We assumed that when blocking MPF activity the candidate drugs in **I** were also blocking MPF from delaying its own degradation via IE (Figure 2).

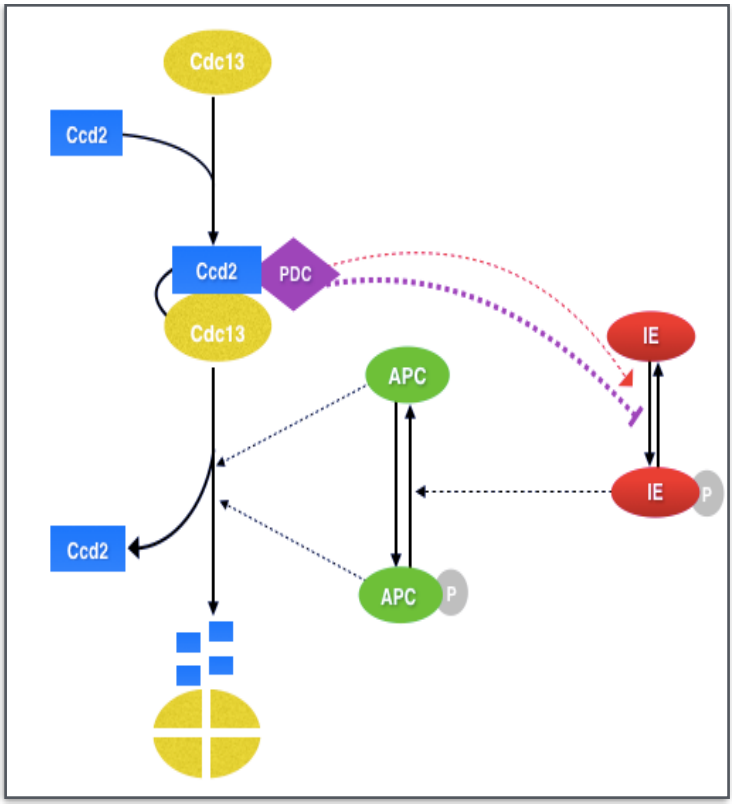

Figure 2. Assumed mechanism of action for candidate drugs I (PDC 01/02/03). PDC would bind to the active Cdc13-L-Cdc2 complex and inhibit MPF from delaying its own degradation. Red arrow represents usual route where MPF is involved in the negative feedback loop and delays APC trough IE. The purple reaction shows the same route but with reduced/inhibited phosphorylation of IE leading the reaction to shift closer to IE and reducing IEp leading to a reduction in the expected delay.

If we consider dMPF/dt as a representation of the enzymatic reaction maximum velocity, Vmax we can calculate the non-competitive inhibition as:

$$Vmax(Inhibition) = \frac{Vmax}{1 + \frac{[I]}{Ki}} \qquad \text{so,}$$

$$dMPF/dt(Inhibition) = \frac{dMPF/dt}{1 + \frac{[I]}{Ki}}$$

In the model this was achieved by dividing both the MPF and IE ODEs by dividing by A:

$$A = 1 + \frac{[I1]}{Ki1}$$

Were `I1` is the selective CDK inhibitor concentration and `Ki1` the inhibition constant.The same was done for the IE and the modified ODEs are as follows:

```
MPF: kSMPF*Mass-kWEE1*MPF+kCDC25*MPFp-(kD1CYC+kD2CYC*Slp1A)*MPF-
kASS*Rum1*MPF+(kDISS+kDRUM1+kIRUM1)*MPFRum1/A;

IE: k1IE*(MPF+a*MPFp)*IE/(J1IE+IE)-V2IE*IEA/(J2IE+IEA)/A;
```

The candidate drugs in **II** were modeled as selective inhibitors of the Cdc25 phosphatase, since the inhibition of Cdc25p would affect the reaction and shift it closer to MPFp resulting in less active MPF (Figure 3) both the MPF and CDC25p ODEs were divided by B.

$$B = 1 + \frac{[I2]}{Ki2}$$

Were `I2` is the Cdc25 phosphatase inhibitor concentration and `Ki2` the inhibition constant.

We also tested the effect of the combined inhibition of Cdc25 and MPF - The ODEs were simultaneously divided by A and B (MPF_ODE/ B/A; IE_ODE/A and CDC25p_ODE/B). The full modified code is provided in the attached ***.m*** files.

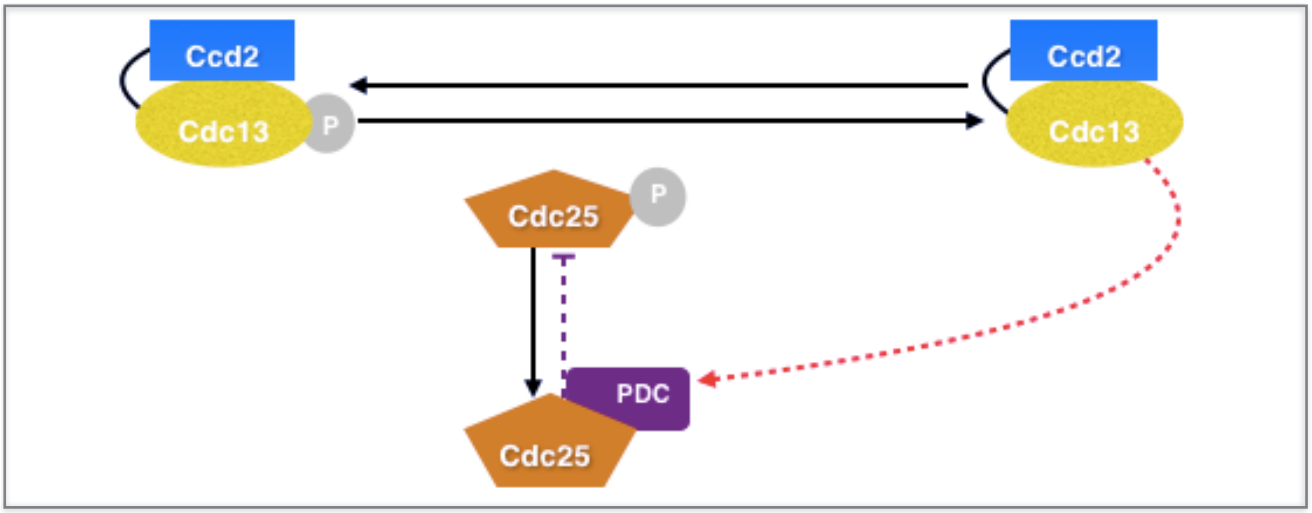

Figure 3. Assumed mechanism of action for candidate drugs in II (PDC 04/05) as Cdc25 specific inhibitors. PDC would bind to Cdc25 and reduce/inhibit the phosphorylation of Cdc25 causing an decrease in Cdc25p, consequentially this inhibition would affect the positive feedback loop between Cdc25 and MPF. Red arrow shows usual MPF phosphorylation of Cdc25 and purple shows that PDC blocks Cdc25 activation.

The 5 potential drugs were computationally tested in multiple concentrations, first for single inhibition of MPF (Figure 4-B, D, C) or CDC25 (Figure 4-E, F, G) and then for combined inhibition of MPF and CDC25 (Figure 5). In fission yeast cells the phase of DNA synthesis is quite brief (20–30 min), after which the cell is in G2 phase. When phase G2 total duration (ΔG2/S) fell bellow 20 minutes the inhibition was deemed successful, this would drive them into mitosis before they have completed DNA synthesis, a lethal situation called “mitotic catastrophe”, [3] these cells cannot properly re-license the DNA replication origins after division, inhibition was also successful when the cell showed mitotic arrest.

The arrested cell enters the ‘resting phase’ (G0 phase), a period where they exist in a quiescent state. During the G0 phase, the cell cycle machinery is dismantled and cyclins and cyclin-dependent kinases disappear. These cells will remain in the G0 phase until there is a reason for them to divide.

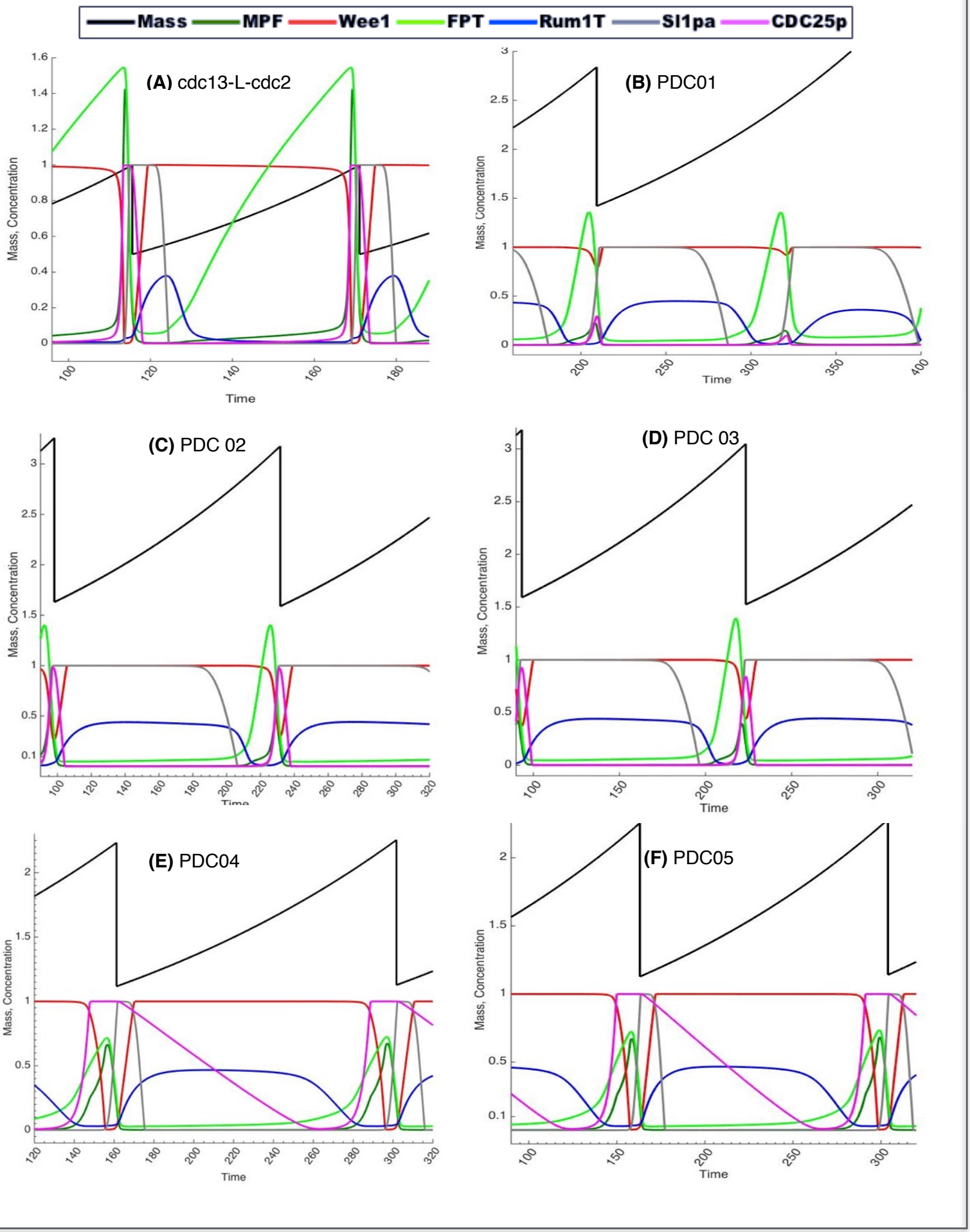

Figure 4. **(A)** Cell cycle of control condition cell (cdc13-L-cdc2). **(B)** PDC01 which arrested the cycle at 775nM. Concentrations of PDCs in **(C)**, **(D)**, **(E)** and **(F)** that caused the cell to divide with $\Delta S/G2 < 20$ minutes. **(C)** PDC02 at 1550nM. **(D)** PDC03 at 794nM. **(E)** PDC04 at 2850nM. **(F)** PDC05 at 1525nM. *Legend depicts cell cycle enzymes through time characterizing the cycle phases.

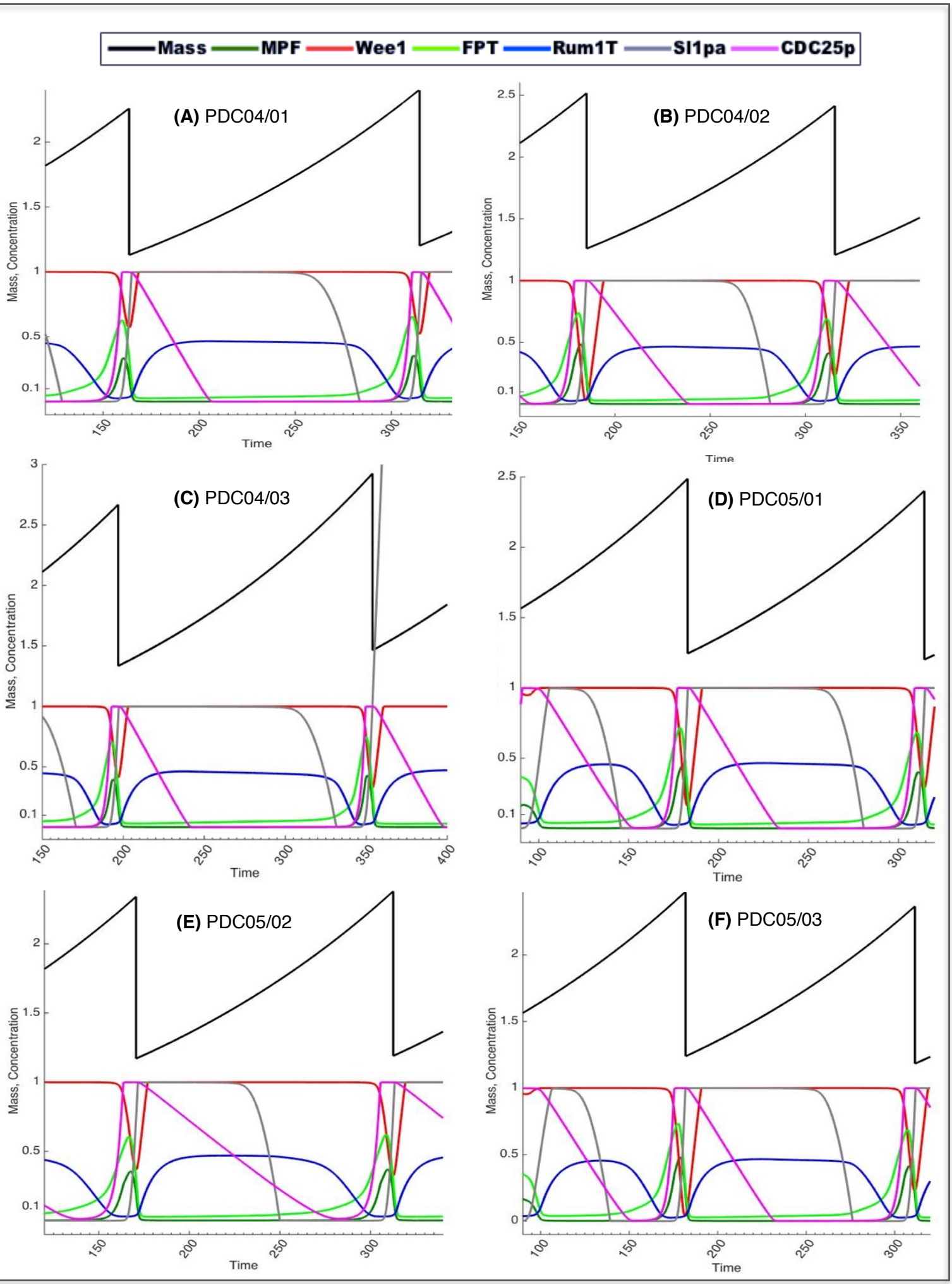

Figure 5. Cell cycle of combined inhibition of MPF and Cdc25. PDCs concentrations that caused the cell to divide with ΔS/G2 < 20 minutes. **(A)** PDC04/01 at 1250nM. **(B)** PDC04/02 at 1600nM. **(C)** PDC04/03 at 1300nM. **(D)** PDC05/01 at 799nM. **(E)** PDC05/02 at 1650nM. **(F)** PDC05/03 at 795nM.
*Legend depicts cell cycle enzymes through time characterizing the cycle phases.

Candidate Drug	Concentration *
(A) SINGLE INHIBITION	
PDC 01	775
PDC 03	794
PDC 05	1525
PDC 02	1550
PDC 04	2850
(B) COMBINED INHIBITION	
PDC 05 / 03	795
PDC 05 / 01	800
PDC 04 / 01	1250
PDC 04 / 03	1300
PDC 04 / 02	1600
PDC 05 / 02	1650
(C) SINGLE AND COMBINED INHIBITION	
PDC01	775
PDC03	794
PDC05 / 03	795
PDC05 / 01	799
PDC04 / 01	1250
PDC04 / 03	1300
PDC05	1525
PDC02	1550
PDC04 / 02	1600
PDC05 / 02	1650
PDC04	2850

For all drugs, as the inhibitor concentration increased, ΔG2/S decreased and phase G1 duration (ΔG1) increased this resulted in cells with larger size/mass but overall the cell cycle duration remained the same except for when the cycle was arrested (Figure 4-B). The candidate drug PDC01 was the strongest inhibitor among both drug groups causing the cell to promptly arrest while the other drugs caused cells to decrease ΔG2/S under 20 minutes but still divided. Most drugs were able to arrest the mitotic cycle when present in higher concentrations (Figure 6).

From these observations we were able to create a ranked list of candidate drugs according to their efficacy to inhibit the cell cycle (Table 1) for future further analysis.

Table 1 - Candidate drugs ranked by most to least effective.
(A) Concentration of effective inhibition for inhibition of only MPF or Cdc25.
(B) Concentration of effective inhibition when MPF and Cdc25 inhibition is combined.
(C) Ranking of most to least effective candidate drugs for both single and combined inhibition.

* Concentration is given in nM.

Discussion

In this study we used a modified Minimal CDK model to computationally predict drug induced inhibition in the cell cycle. Using the dynamical model created we were able to find how the different drugs inhibited different reactions and the overall phases of the cell cycle.[5] These modifications in the cycle as drugs and concentrations change is indicative that this dynamical model is a good representation of the cycle and that the cells are indeed being influenced by our simulations.

Computational simulations can also show unexpected behaviors, e.g. a drug might be expected to greatly inhibit a certain reaction from a strict chemical point but if that reaction is in a robust and redundant pathway with extra positive or negative feedback loops the expected inhibition can be easily overcome. Dynamical models provide a way to integrate the chemical and biological

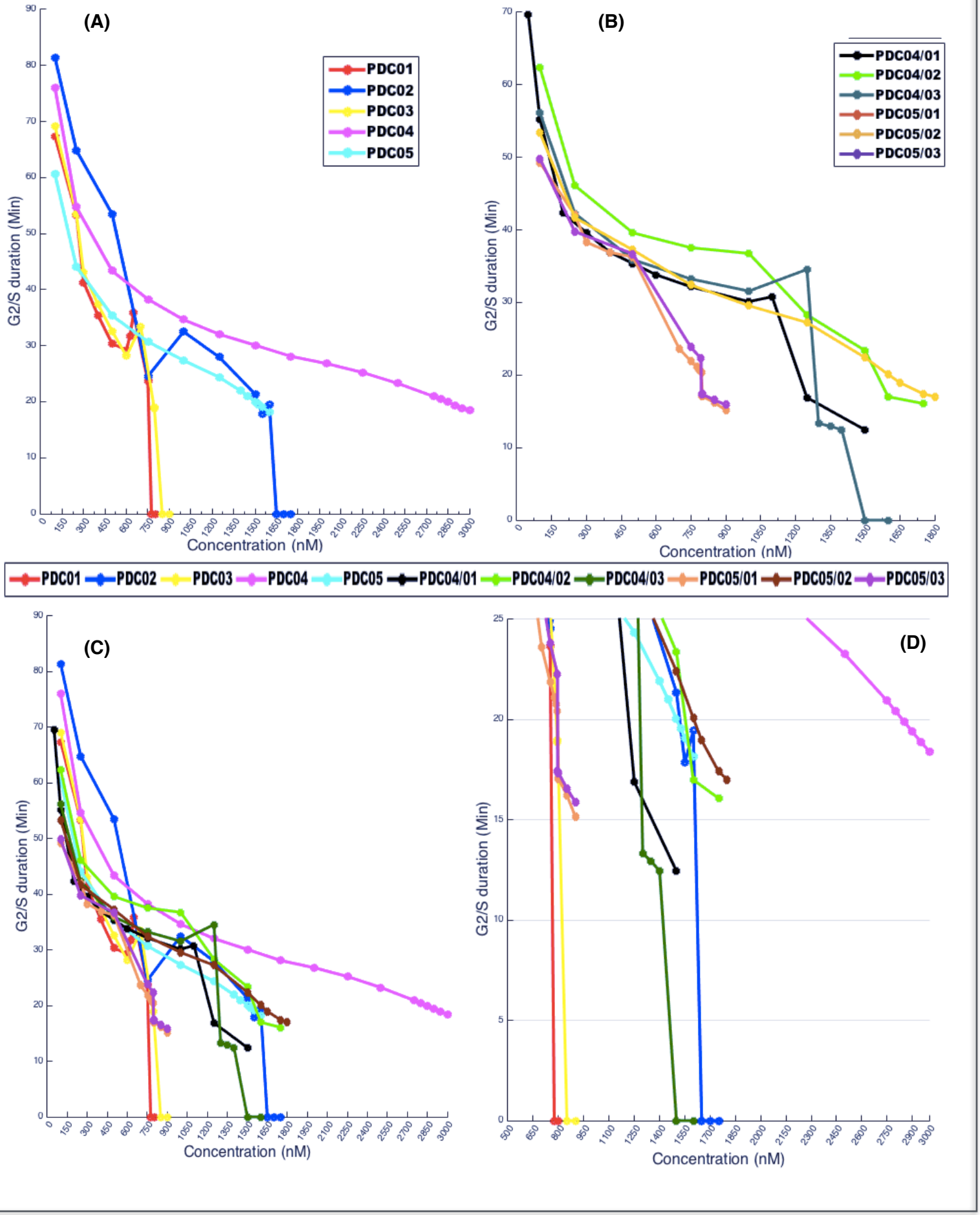

Figure 6. Variation in ΔG2/S phase duration according to inhibitor and concentration. **(A)** Single inhibition. **(B)** Combined inhibition. **(C)** Single and Combined inhibition. **(D)** Closer view of plot **(C)** highlighting ΔG2/S < 20 minutes.

*In cells that stopped diving (arrested), ΔG2/S is shown at 0 minutes for better graphical representation.

approach, the theoretical and experimental knowledge already known in the literature to reveal important structural and biochemical information, predict targets and interactions that can then be used to create better designed experiments.[6,7]

An important aspect of CDK inhibitors is the existence of pre-existing and/or acquired resistance as it happens with the Imatinib-resistant chronic myeloid leukemia.[8] Hence the next important question would be whether combinations of targeted drugs may (better) overcome cell resistance. The differential target profiles of CDKi and inhibitors of other enzymes may also explain why several of them, when combined, produce synergistic effects.[8] This was shown in our model, when the 2 different groups of PDCs were tested simultaneously. As the combination of drugs from group I (MPF inhibition) and of group II (CDC25 inhibition) did indeed produce synergistic effects, in Table 1-C the tested cases of combined inhibition (PDC05/03; PDC05/01; PDC04/ 03 and PDC04/01) showed better results than the single inhibition by PDC05, PDC02 and PDC04.

Modern drug discovery is characterized by the production of vast quantities of compounds and the need to examine these huge libraries in short periods of time. [7] All the dada generated in the dynamical modeling approach enabled us to produce a ranked list (Table 1) of the candidate drugs in a short time interval, this is specially interesting and useful since it permits researchers to jump start the experimentations and effectively reduce costs. Biological systems are highly complex but the use of even a simple dynamical model as shown in this study can be used to facilitate the design and discovery of new drugs, this systematic evaluation of compounds (and hopefully of huge libraries of compounds) to identify potential lead candidates that can then be synthesized and tested is a functional and effective tool to create better drugs with lower/reduced costs.

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