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Procedia Computer Science 00 (2016) 1–12

Procedia Computer Science

Target based drug discovery for β-globin disorders: Drug target prediction using quantitative modelling with hybrid functional Petri nets

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Abstract

Recent molecular studies provide important clues into treatment of β-thalassemia, sickle-cell anaemia and other β-globin disorders revealing that increased production of fetal hemoglobin, that is normally suppressed in adulthood, can ameliorate the severity of these diseases. In this paper, we present a novel approach for drug target prediction for β-globin disorders. Our approach is centered upon quantitative modelling of interactions in human fetal-to-adult hemoglobin switch network using hybrid functional Petri nets. In accordance with the reverse pharmacology approach we pose a hypothesis regarding modulation of specific protein targets that induce γ-globin and consequently fetal hemoglobin. Comparison of simulation results for the proposed strategy with the ones obtained for already existing drugs shows that our strategy is the optimal as it leads to highest level of γ-globin induction and thereby has potential beneficial therapeutic effects on β-globin disorders. Simulation results enable verification of model coherence demonstrating that it is consistent with qPCR data available for known strategies and/or drugs.

Keywords: Reverse pharmacology; drug discovery; fetal-to-adult hemoglobin switch network; β-thalassemia; sickle-cell anaemia; quantitative modelling; hybrid functional Petri net

1. Introduction

β-thalassemia, sickle-cell anaemia and other β-globin disorders caused by mutations in adult hemoglobin (HbA) are among most common genetic disorders in the world. According to the recent estimates, hundreds of thousands of children with these diseases are born every year, and there are tens of millions of patients with these disorders in the world. It was also reported that these diseases are among major sources of mortality worldwide [1]. The prevalence of these diseases is expected to rise dramatically over the next century as the world's population grows. Therefore, developing improved treatment for these disorders is of utmost interest.

The current curative therapies and treatments for these diseases involve bone marrow transplantation [2], gene therapy [3], and symptomatic care followed by transfusion of red blood cells as it is clinically necessary [4]. Numerous

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challenges are encountered in the implementation of curative therapies and treatments. Firstly, these therapies are expensive and therefore have significant limitations for widespread use [5]. Secondly, despite significant scientific and clinical advances in these approaches they still remain largely experimental [6, 7]. Finally, when using regular blood transfusions, iron overload can lead to major clinical complications [4].

It has been reported that increase in fetal hemoglobin (HbF) levels can significantly ameliorate the clinical severity and decrease mortality in sickle-cell anemia [8]. Similar clinical observations have been made in patients with β-thalassemia [9, 10]. These clinical observations have been confirmed by epidemiological studies of thalassemia populations [11–13]. Recent molecular findings provide new insights into hemoglobin biology and new ensemble of therapeutic targets for treatment of β-globin disorders. The discovery that BCL11A and its interacting partners, as the major regulators of human fetal-to-adult hemoglobin switch, directly or indirectly influence HbF silencing has given rise to concern that these regulators could be novel molecular targets for drugs that induce HbF [14, 15].

In this paper, we present a comparative analysis of six target-based strategies that induce HbF through inducing γ-globin gene: (1) suppressing expression of KLF1 by Simvastatin [16] and tBHQ [16], the two drugs in clinical trials; (2) suppressing expression of KLF1 by MS-275 [17–19], a drug in clinical trials; (3) suppressing expression of KLF1 and HDAC1/2 by ST-20 [19], a drug already available; (4-5) suppressing expression of BCL11A and SOX6 by ACY-957, [20, 21] a drug in clinical trials; and (6) inhibiting ETF, a complex of Erythroid Transcription Factors GATA1, FOG1 and SOX6, and thereby decreasing concentrations of BCL11A and SOX6. The treatment in strategies (4-5) depends on dosage of ACY-957 and time. The case (6) represents our target-based drug target discovery strategy. In this strategy, we develop a hypothesis regarding biological component that influence HbF silencing, select ETF as the biological target, inhibit its function by hypothetical ETF inhibitor (ETFI for short) to regulate HbF activity. No specific drug has been developed yet for the latter strategy.

We exploit hybrid functional Petri net (HFPN) as computational platform to create quantitative model of human fetal-to-adult hemoglobin switch network, and perform a series of simulations in accordance with the above strategies. Simulation results for strategies (1)-(5) show that our model is consistent with available qPCR data, demonstrating expected distribution of mRNA and protein concentrations. The series of simulation results we carried out reflect the major aim of the research which is finding the optimal strategy leading to maximum γ-globin mRNA fold increase. Comparative analysis of the simulation results on γ-globin mRNA upregulation show that the strategy (6) is the optimal case as it leads to the highest level of γ-globin mRNA concentration.

The paper is organized as follows. We start with introducing the molecular mechanism driving human fetal-to-adult hemoglobin switch network to make it easy for the readers to understand biological context behind the present research. After that, we briefly review Petri nets. Then we present our HFPN model of human fetal-to-adult hemoglobin switch network. Following this, we discuss the computational validation of the model based on known wet lab results and present our target-based drug discovery strategy. Finally, we summarize our findings.

2. Fetal-to-adult hemoglobin switch

In this section we discuss biological context behind our research and refer readers to papers in the field for detailed information [14, 22–27]. Human fetal-to-adult hemoglobin switch and associated molecular regulatory network are

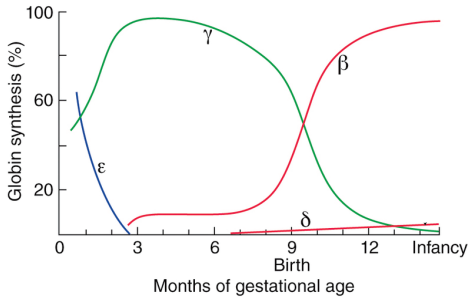


Figure 1. (Reprinted from [15]) There are two developmental switches in expression from the β-globin gene cluster, from embryonic-to-fetal during the first three months of conception, and from fetal-to-adult during the next six months ending at the time of birth.

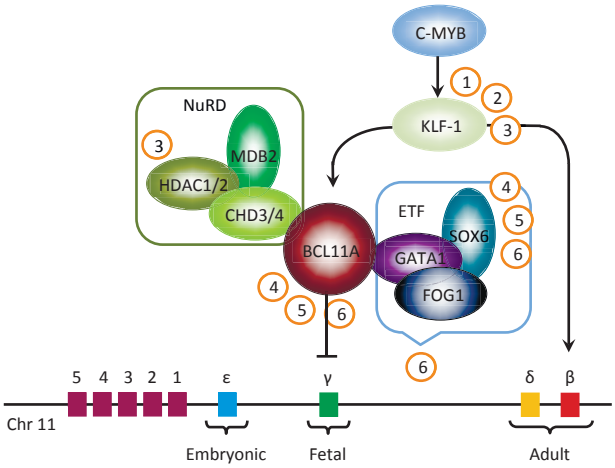


Figure 2. The switch from fetal to adult hemoglobin is regulated by numerous biological components. Positive and negative interactions between these components are denoted by regular and blunted arrows, respectively. Regulators of this process are potential therapeutic targets for patients with β-globin disorders. Circle surrounding a biological component indicates number of the strategy that targets specified component.

illustrated in Fig. 1 and Fig. 2, respectively. In the beginning of the first three months, there is robust expression of an embryonic form of a β-globin known as ε-globin [24]. Concurrently while, when ε-globin starts being down regulated, the β-like globin molecule known as γ-globin is produced [25]. This is known as embryonic-to-fetal hemoglobin switch, which is the first developmental switch in expression of human globin genes. γ-globin remains the predominant hemoglobin for much of gestation until after birth. Close to the end of third month ε-globin is almost completely silenced. This event is followed by up regulation of β-globin gene. The second developmental switch in expression of human hemoglobin known as fetal-to-adult hemoglobin switch takes place around time of birth. This event triggers increase of HbA production and decrease of HbF production.

BCL11A is the major protein that represses the expression of γ-globin genes [23]. It was observed that down regulation of BCL11A robustly induces γ-globin gene expression [14]. Its protein partners, including HDAC1/2 and GATA1 contribute to repression of γ-globin gene by binding to BCL11A [26]. KLF1 contributes to this process by positively regulating the expression of BCL11A [14]. KLF1 also promotes transcription of β-globin gene. Additionally, it has been reported that the transcription factor SOX6 cooperates with BCL11A to silence the γ-globin genes in humans [27].

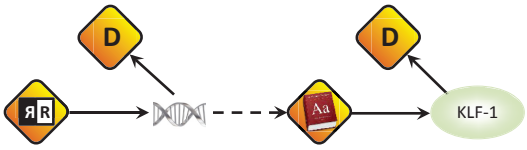


Figure 3. The central dogma of molecular biology illustrated for KLF-1: mRNA transcribed from DNA is then translated into protein. Both mRNA and protein levels are kept low by natural degradation.

3. Petri Nets

In context of biological systems Petri net is a bipartite graph composed of two types of nodes, places and transitions; the former being suitable for representing biological entities, and the latter biological phenomena. In such a net, the arcs connect places with transitions or vise versa, and keep information on reaction stoichiometry. The places encode the molecular concentration. Continuity is perhaps the only characteristic that distinguishes Petri nets

for biological systems from classical or original ones. In continuous Petri nets each transition has detailed information about the kinetics of the related biochemical reaction. Biological systems are characterized by often interaction between different structured processes. It is quite regular that a Petri net model of biological system comprises continuous, boolean and discrete processes. For instance, biochemical reactions are continuous processes, while the presence/absence of a biological phenomenon is a boolean process. A counter-like mechanism on the other hand is a typical discrete process. HFPN is inherited from hybrid Petri net in which a function is associated with each continuous process. HFPN has been successfully implemented to modelling and simulating of various biological processes [28–31].

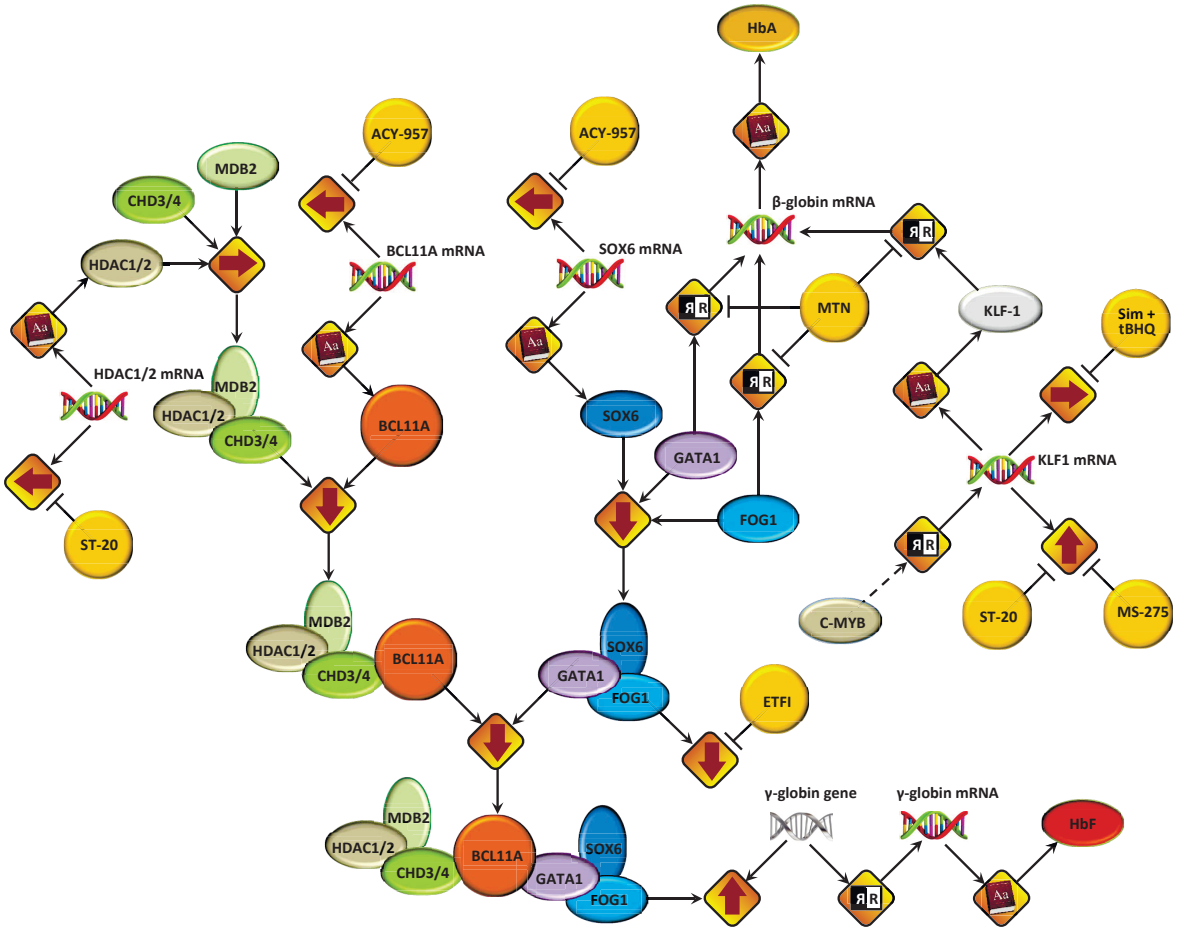


Figure 4. HFPN model of fetal to adult hemoglobin switch network.

4. Creating the model

We create HFPN model of human fetal-to-adult hemoglobin switch network in accordance with biological context extracted from the literature[14, 23, 26, 27]. In this model, it is assumed that major proteins are made up by the central dogma of molecular biology, so that mRNA transcribed from DNA is then translated into protein. It is also supposed that the mRNA and protein levels are kept low by natural degradation. Fig. 3 exemplifies the central dogma and degradations for KLF-1. Our model incorporates similar net fragments for all major proteins, though for the the sake of clarity corresponding net fragments are not included in the graphical description of the model. The Fig. 4 shows a skeleton of human fetal-to-adult hemoglobin switch network. In this figure we focus on protein activations,

gene-protein and protein-protein interactions, and suppression of mRNA expressions by binding drugs. The Fig. 5 is a Cell Illustrator screen snapshot illustrating HFPN model of human fetal-to-adult hemoglobin switch network. Our HFPN model comprises 9 generic entities, 27 continuous entities, 60 processes, 9 boolean variables and 27

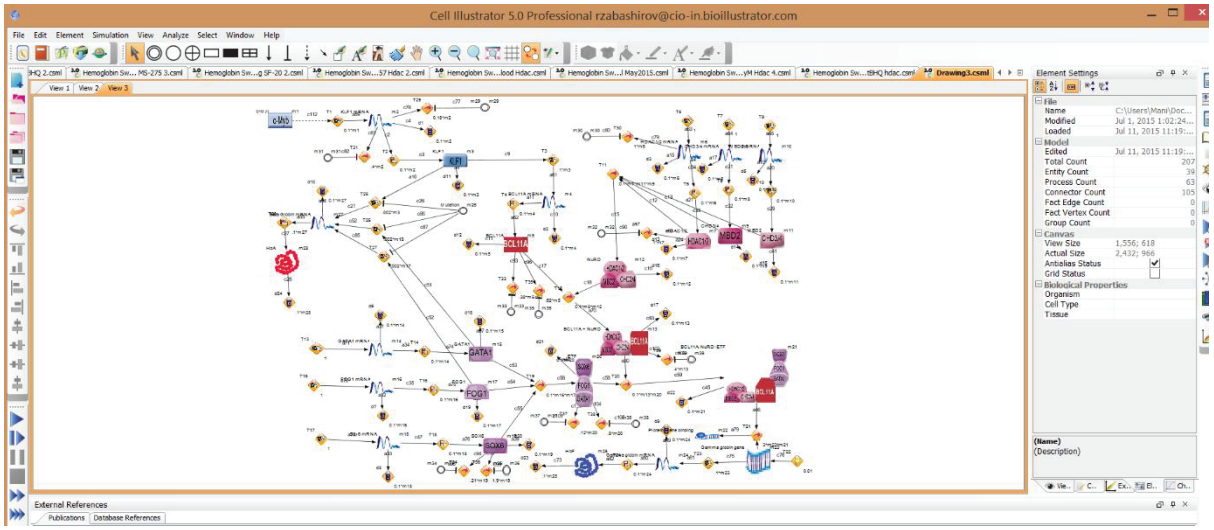


Figure 5. Cell Illustrator screen snapshot illustrating HFPN model of fetal-to-adult hemoglobin switch network.

continuous variables. The generic entities stand for the drugs Simvastatin, tBHQ, ACY-957, ST-20, MS-275, ETF inhibitor (our predicted drug) and β-globin gene mutation (MTN). Continuous entities represent genes, mRNAs, proteins, and their complexes. The processes act for biological phenomena such as transcription, translation, binding, mRNA and protein degradations. Boolean variables are used to check presence/absence of drugs and β-globin gene mutation, whereas continuous variables to measure concentrations of biological components. Relationship between biological components and HFPN entities, biological phenomena and HFPN processes as well as information on natural degradations and connectors are detailed in Tables I–IV.

In molecular biology, it is quite regular that two identical experiments lead to not identical observations. Unfortunately, wet lab results for certain biological phenomena are scarce and sometimes contradictory. This is why it is rather cumbersome task to determine kinetic parameters such as reaction rates based on wet lab results only. In this work, we set the rates of biological phenomena in accordance with [28–31], and then carefully calibrate them to validate the model of fetal-to-adult hemoglobin switch network with available qPCR data for wild type β-globin gene (Fig. 1-2). The process rates adopted in the present research are presented in Table II.

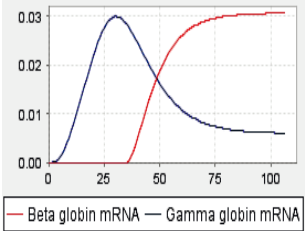


Figure 6. Simulation results for expression of wild type β-globin and γ-globin molecules.

5. Computational validation of the model

In the present research, we use Cell Illustrator software to create HFPN model of human fetal-to-adult hemoglobin switch network and perform simulations to validate the model. Validation is achieved through altering calibration

Table 1. Entities in the HFPN model of human fetal-to-adult hemoglobin switch.

Entity name	Entity type	Variable	Value	Type
C-MYB	Continuous	m1	1	Double
KLF-1mRNA	Continuous	m2	0	Double
KLF-1	Continuous	m3	0	Double
BCL11AmRNA	Continuous	m4	0	Double
BCL11A	Continuous	m5	0	Double
HDAC1/2mRNA	Continuous	m6	0	Double
HDAC1/2	Continuous	m7	0	Double
MDB2mRNA	Continuous	m8	0	Double
MDB2	Continuous	m9	0	Double
CHD3/4mRNA	Continuous	m10	0	Double
CHD3/4	Continuous	m11	0	Double
NuRD	Continuous	m12	0	Double
BCL11A_NuRD	Continuous	m13	0	Double
GATA1mRNA	Continuous	m14	0	Double
GATA1	Continuous	m15	0	Double
FOG1mRNA	Continuous	m16	0	Double
FOG1	Continuous	m17	0	Double
SOX6mRNA	Continuous	m18	0	Double
SOX6	Continuous	m19	0	Double
ETF	Continuous	m20	0	Double
BCL11A_NuRD_ETF	Continuous	m21	0	Double
γ-globin_BCL11A_NuRD_ETF	Continuous	m22	0	Double
γ-globin gene	Continuous	m23	0	Double
γ-globin mRNA	Continuous	m24	0	Double
HbF	Continuous	m25	0	Double
Mutation	Generic	m26	0	Boolean
β-globin mRNA	Continuous	m27	0	Double
HbA	Continuous	m28	0	Double
Simvastatin+tBHQ as KLF-1 mRNA suppressor	Generic	m29	1	Boolean
MS-275 as KLF-1 mRNA suppressor	Generic	m30	1	Boolean
ST-20 as KLF-1 mRNA suppressor	Generic	m31	1	Boolean
ST-20 as HDAC1/2 mRNA suppressor	Generic	m32	1	Boolean
ACY-957 as BCL11A mRNA suppressor (case 1)	Generic	m33	1	Boolean
ACY-957 as SOX6 mRNA suppressor (case 1)	Generic	m34	1	Boolean
ACY-957 as BCL11A mRNA suppressor (case 2)	Generic	m35	1	Boolean
ACY-957 as SOX6 mRNA suppressor (case 2)	Generic	m36	1	Boolean
ETFI (ETF inhibitor)	Generic	m37	1	Boolean

Table 2. Processes in the HFPN model of human fetal-to-adult hemoglobin switch.

Phenomenon	Pr.	Type	Rate	Delay
Transcription of KLF-1 mRNA	T1	Continuous	m1*0.1	0
Translation of KLF-1	T2	Continuous	m2*0.1	0
Transcription of BCL11A mRNA	T3	Continuous	m3*1	0
Translation of BLC11A	T4	Continuous	m4*0.1	0
Transcription of HDAC1/2 mRNA	T5	Continuous	1	0
Translation of HDAC1/2	T6	Continuous	m6*0.1	0
Transcription of MDB2 mRNA	T7	Continuous	1	0
Translation of MDB2	T8	Continuous	m7*0.1	0
Transcription of CHD3/4 mRNA	T9	Continuous	1	0
Translation of CHD3/4	T10	Continuous	m10*0.1	0
Binding of HDAC1/2, MDB2 and CHD3/4	T11	Continuous	m7*m9*m11*0.1	0
Binding of NuRD with BCL11A	T12	Continuous	m5*m12*0.1	0
Transcription of GATA1 mRNA	T13	Continuous	1	0
Translation of GATA1	T14	Continuous	m14*0.1	0
Transcription of FOG1 mRNA	T15	Continuous	1	0
Translation of FOG1	T16	Continuous	m16*0.1	0
Transcription of SOX6 mRNA	T17	Continuous	1	0
Translation of SOX6	T18	Continuous	m18*0.1	0
Binding of GATA1, FOG1 and SOX6	T19	Continuous	m15*m17*m19*0.1	0
Binding of ETF with BCL11A_NuRD	T20	Continous	m13*m20*0.1	0
Binding of BCL11A_NuRD_ETF with γ-globin gene	T21	Continuous	m21*m23*0.1	0
Activation of γ-globin gene	T22	Continuous	0.01	0
Transcription of γ-globin mRNA	T23	Continuous	m23*0.1	0
Translation of HbF	T24	Continuous	m24*0.1	0
Activation of β-globin mRNA by KLF-1	T25	Continuous	m3*0.002	35
Activation of β-globin mRNA by GATA1	T26	Continuous	m15*0.002	35
Activation of β-globin mRNA by FOG1	T27	Continuous	m17*0.002	35
Translation of HbA	T28	Continuous	m27*0.1	0
Binding of Simvastatin+tBHQ to KLF-1 mRNA	T29	Continuous	m2*0.18	0
Binding of MS-275 to KLF-1 mRNA	T30	Continuous	m2*0.4	0
Binding of ST-20 to KLF-1 mRNA	T31	Continuous	m2*0.37	0
Binding of ST-20 to HDAC1/2 mRNA	T32	Continuous	m6*1	0
Binding of ACY-957 to BCL11A mRNA (case 1)	T33	Continuous	m4*0.38	0
Binding of ACY-957 to SOX6 mRNA (case 1)	T34	Continuous	m18*0.21	0
Binding of ACY-957 to BCL11A mRNA (case 2)	T35	Continuous	m4*0.62	0
Binding of ACY-957 to SOX6 mRNA (case 2)	T36	Continuous	m18*1.9	0
Binding of ETF and its inhibitor	T37	Continuous	m20*0.12	0

Table 3. Degradations in the HFPN model of human fetal-to-adult hemoglobin switch.

Phenomenon	Process	Type	Rate
mRNA degradation	d1–d10	Continuous	mi*0.05
Protein degradation	d11–d24	Continuous	mi*0.01

Table 4. Connectors in the HFPN model of human fetal-to-adult hemoglobin switch.

Connector	Firing style	Firing script	Connector type
c1–c59	Threshold	0	Input process
c60–c87	Threshold	0	Output process
c88–c99	Threshold	0	Input inhibitor
c100	Threshold	0	Input association

parameters (e.g., process rates and initial concentrations), and comparing validation parameters (e.g., gene, mRNA and protein concentrations) to the wet lab observations. The concentrations (y-axis) are plotted against time units (x-axis) called Petri time or pt, for short. In these plots, each 10 pt in the time axis corresponds to 3 months of gestational age, so we suppose that fetal life starts at 20 pt (0 months), and that a child is born at 50 pt (9 months). We gather results for γ-globin gene induction at 70 pt, that is, 6 months after the birth.

In order to obtain the closest approximation of concentration levels of wild type β-globin mRNA and γ-globin mRNA (Fig. 1) we started validating our model by extrapolating simulation results for wild type β-globin mRNA and γ-globin mRNA on the basis of their relationship with biological components involved in human fetal-to-adult hemoglobin switch network in Fig. 2. Simulation results for expression of wild type β-globin mRNA and γ-globin mRNA are illustrated in Fig. 6.

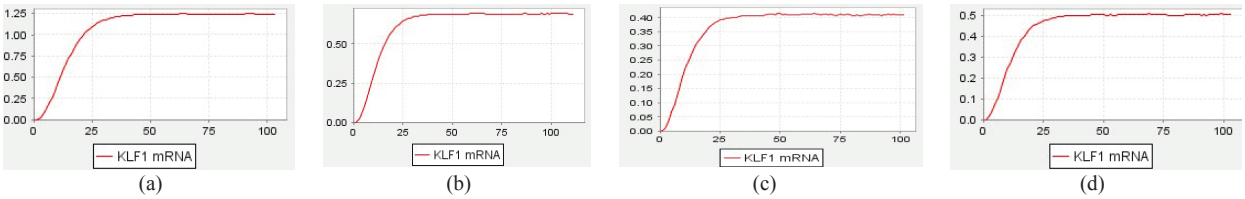


Figure 7. Simulation results for expression of KLF1 mRNA in (a) an untreated cell; a cell treated with (b) combination of Simvastatin and tBHQ; (c) MS-275; and (d) ST-20. Treatments with combination of Simvastatin and tBHQ decrease KLF1 mRNA concentration by approximately 44%, with MS-275 by 3-fold and with ST-20 by 2.5-fold over the untreated control.

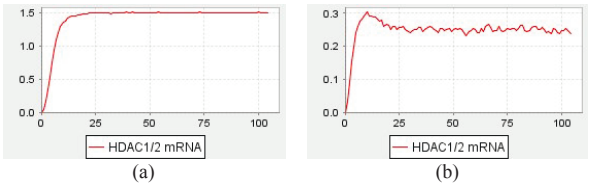


Figure 8. Simulation results for expression of HDAC1/2 mRNA in (a) an untreated cell; and a cell treated with (b) ST-20. ST-20 treatments of erythroid progenitors cultured from sickle cell anemia and beta thalassemia patients decrease HDAC1/2 mRNA levels by 6-fold over the untreated control.

When β-globin gene is mutated the drug treatments with Simvastatin, tBHQ, the combination of these two drugs, MS-275 and ST-20 suppress the levels of KLF1 mRNA and thereby BCL11A mRNA and protein, as a consequence increasing γ-globin mRNA and HbF levels. In vitro experiments in primary human erythroid cells showed that Simvastatin alone decreases KLF1 mRNA levels by approximately 20% of that seen in untreated cells, tBHQ alone by approximately 25%, and the combination of the two drugs by approximately 44% [16]. Drug treatments of erythroid progenitors cultured from sickle cell and β-thalassemia patients showed that MS-275 and ST-20 suppress KLF-1 mRNA by 3- and 2.5-fold, respectively [19]. Simulation results for concentration levels of KLF1 mRNA in untreated cells and in cells treated with the combination of Simvastatin and tBHQ, MS-275 and ST-20 are illustrated in Fig. 7(a-d), respectively. In all four cases KLF1 reaches the steady state at time point 25 pt, so that its concentration remain

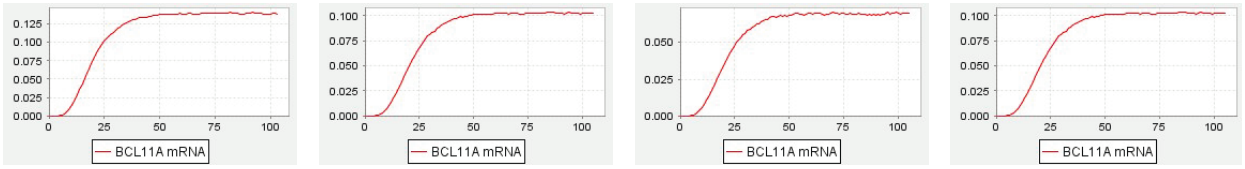


Figure 9. Simulation results for expression of BCL11A mRNA in (a) an untreated cell; a cell treated with (b) ACY-957 (case I); (c) ACY-957 (case II); and (d) our strategy. In case I, ACY-957 treatments with differentiation of cells for 5 days with 1 μM ACY-957 leads to a decrease of BCL11A mRNA by 1.4-fold, in case II by 2-fold and treatments with our strategy by 1.4-fold in over the untreated control.

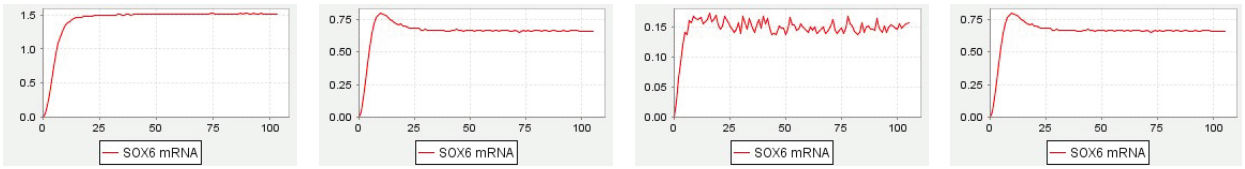


Figure 10. Simulation results for expression of SOX6 mRNA in (a) an untreated cell; a cell treated with (b) ACY-957 (case I); (c) ACY-957 (case II); (d) and our strategy. In case I, ACY-957 treatments with differentiation of cells for 5 days with 1 μM ACY-957 leads to a decrease in SOX6 mRNA by 2.3-fold, in case II by 10-fold and with our strategy by 2.3-fold over the untreated control.

continuously stable starting 25 pt. The concentration levels of KLF1 mRNA in untreated cells and in cells treated with the combination of Simvastatin and tBHQ, MS-275 and ST-20 at time point 70 pt were respectively 1.25, 0.70, 0.41 and 0.50, providing a good fit to above mentioned wet lab results.

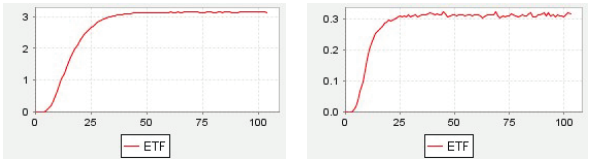
It was reported that drug treatments with ST-20 in erythroid progenitors cultured from sickle cell anemia and beta thalassemia patients decrease HDAC1/2 mRNA levels by 6-fold of untreated control [19]. As it can be seen from Fig 8, concentration levels of HDAC1/2 measured at time point 70 pt in an untreated cell and in a cell treated with ST-20 were 1.5 and 0.25, respectively, demonstrating a good agreement with the wet lab observations.

GeneChip and quantitative real-time PCR time course experiments with $CD71^{low}GlyA^{neg}$ cells differentiated for 5 days with 1 μM ACY-957 show ACY-957 treatments decrease BCL11A mRNA by 1.4-fold [21] and SOX6 mRNA by 2.3-fold [20]. It was also observed variations in differentiation time and ACY-957 dosage affect the rate of suppression so that in another series of experiments BCL11A mRNA and SOX6 mRNA were suppressed by 2- and 10-fold, respectively [20, 21]. Simulation results for BCL11A and SOX6 are illustrated in Fig. 9 and Fig. 10, respectively. Numerical simulation results for concentration levels of BCL11A mRNA in untreated cells found to be 0.14. In cells treated with ACY-957 in case I it is decreased by 2-fold down to 0.1, and in case II by 1.4-fold down to 0.07. Likewise, numerical values for concentration levels of SOX6 mRNA in untreated cells and in cells treated with ACY-957 in accordance with cases I-II were 1.5, 0.65 and 0.15, demonstrating decrease of concentration level by 2.3- and 10-fold in cases I and II, respectively. Both experimental and simulation results for treatments with ACY-957 on BCL11A and SOX6 mRNA are in strong quantitative agreement.

Finally, simulation results showed that treatments with our strategy decreases BCL11A mRNA concentration levels from 0.14 to 0.1 by 1.4-fold, SOX6 mRNA from 1.5 to 0.65 by 2.3-fold and ETF concentration levels from 3.1 to 0.31 (see Fig. 11), demonstrating decrease by 10-fold, over the untreated control.

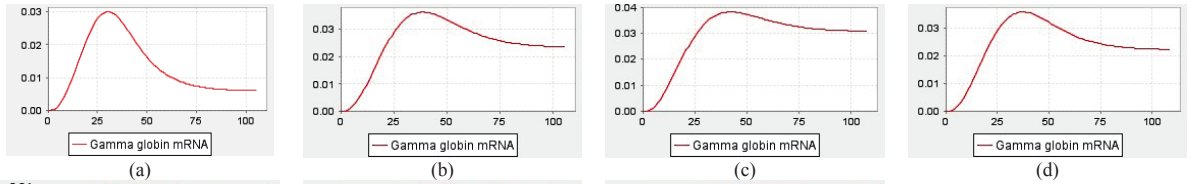
6. Drug target prediction for β-globin disorders

Once the model validated next we determined the effects of drug treatments on γ-globin mRNA. Concentration levels of γ-globin mRNA for untreated cells as well as cells treated in accordence with strategies (1) to (6) are shown in Fig. 12. As it can be seen from the figure the concentration measured at time point 70 pt in the untreated cells and in cells treated with the combination of Simvastatin and tBHQ, MS-275, ST-20, ACY-957 according to cases I-II,



(a) (b)

Figure 11. Simulation results for expression of ETF (a) in an untreated cell; and (b) in a treated one. ETF inhibition with our strategy decreases EFT concentration levels from 3.1 to 0.31, demonstrating decrease ETF concentration levels by 10-fold over the untreated control.



(a) (b) (c) (d) (e) (f) (g)

Figure 12. Simulation results for expression of γ-globin mRNA in (a) untreated cell; and in cells treated with (b) the combination of Simvastatin and tBHQ; (c) MS-275; (d) ST-20; (e) ACY-957 (case I); (f) ACY-957 (case II); and (g) ETF inhibitor. Simulation results show that the combination of Simvastatin and tBHQ increases γ-globin mRNA levels by 3.4-fold, MS-275 by 4.1-fold, ST-20 by 3.1-fold, ACY-957 (case I) by 4.4-fold, ACY-957 (case II) by 5.0-fold and finally our strategy by 5.4-fold over the untreated control.

and our proposed strategy were respectively 0.08, 0.027, 0.033, 0.025, 0.035, 0.04 and 0.0435, indicating that the combination of Simvastatin and tBHQ increases γ-globin mRNA levels by 3.4-fold, MS-275 by 4.1-fold, ST-20 by 3.1-fold, ACY-957 (case I) by 4.4-fold, ACY-957 (case II) by 5.0-fold and finally our strategy by 5.4-fold over the untreated control. These observations lead to the conclusion that the strategy proposed in the present research is the optimal among six strategies detailed in current work as it leads to the maximum increase of γ-globin mRNA levels.

7. Discussions

In the present study, we propose a multiprotein complex ETF as a target for drug discovery for β-globin disorders. Currently, multiprotein complexes are widely recognized as key targets for drug discovery though they tend to be more challenging targets than single protein targetting. Evidently, it is a real challenge to discover a small drug molecule that binds at the large and flat interfaces and disrupts the formation of multiprotein complexes. These kind of applications are largely avoided by many pharmaceutical companies. Another contradiction of drug discovery in practice is that while most regulatory proteins are components of multiprotein systems, pharmaceutical industry focus so much on the active sites of monomeric proteins. The question posed in [32] is whether this is really sustainable. Some attempts have been made by biotechnology companies [33] and academia [34] to set up work in this area. But perhaps more efforts are required for significant departure from conventional monomeric targets to multiprotein targets.

8. Conclusion

This paper exploits the relationship between reverse pharmacology and quantitative modelling with HFPN to the benefit of both fields. We demonstrate that a quantitative modelling with Petri net technologies can be efficiently implemented in target-based drug discovery. More specifically, we pose a hypothesis regarding protein and multiprotein

targets to discover a drug for β-globin disorders. In order to assess the precision of our prediction we create quantitative model of human fetal-to-adult hemoglobin switch network, use data from literature to validate the model and then perform simulations to compare our proposed strategy with already existing ones. When compared to the other strategies, our strategy results in maximum γ-globin gene induction which approves the consistency of the hypothesis.

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