Quantitative modeling with Petri nets: perspectives for the target-based treatment of β-globin disorders

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Abstract: A novel approach proposed in the present work for alleviating the severity of β-globin disorders is centered upon induction of γ-globin gene expression in fetal hemoglobin and reduction of free and toxic α-chains in adult hemoglobin. We use hybrid functional Petri nets in Snoopy framework to create quantitative model of fetal-to-adult hemoglobin switching network and validate its coherence with qPCR data available for existing target-based drug and gene therapies of β-globin disorders. Then we apply simulation-based model checking to underlying biological network to predict the most efficient molecular targets for β-globin disorders. To measure the effectiveness of β-globin disorder treatments we devise a unique criterion, $F(\alpha, \beta, \gamma) = \alpha - (\beta + \gamma) + c$, and justify the reason why this formula can be used as unique criterion. Simulation results show that our approach has potential more beneficial effect on β-globin disorders than existing target-based therapies as it minimizes $\mid F \mid$.

Key–Words: Hybrid functional Petri net, quantitative modeling, target-based gene therapy, fetal-to-adult hemoglobin switching network, β-thalassemia

1 Introduction

Sickle-cell disease (SCD) and β-thalassemia are caused by defective or insufficient production of β-globin chains in adult hemoglobin (HbA). The current SCD and β-thalassemia treatments lack the desired efficacy and therefore remain insufficient to cure these diseases [10]. Instead, target-based therapies offer the promise of breakthrough disease treatments. For instance, it was observed that even modest induction of fetal haemoglobin (HbF) in infancy by an increase of γ-globin chains may be sufficient to compensate the deficit in HbA and consequently alleviate the severity of SCD and β-thalassemia [10]. It was also reported that decreasing the concentration of free toxic α-globin chains can be considered as potential pathway to reach improved treatment option for such diseases as reduction of free toxic α-globin chains in patients with β-thalassemia was in their advantage in terms of side effect of their lack of β-globin production [13]. This is the pressing motivation for the current work to explore basic molecular interactions in fetal-to-adult hemoglobin switching network in order to predict the optimal molecular targets leading to the most effective target-based therapy of β-globin disorders. Guided by this motivation, we use Snoopy software to create hybrid functional Petri net (HFPN) model of fetal-to-adult hemoglobin switching network and perform simulation-based model checking to predict molecular targets having desired characteristics.

2 Related work

Small interfering RNA (siRNA) and short hairpin RNA (shRNA) are two alternative mechanisms in line with RNAi-based gene therapy. Although both drug and RNAi approaches behave similar recognizing molecular targets, the former approach uses a drug to silence the targets, while the latter one knocks down the targets with siRNA or shRNA mechanisms. Side effects remain a major concern for the target-based drug therapies. However, no adverse side effects have been reported for RNAi approaches. This makes RNAi approaches advantageous over drug therapy.

Improved understanding of molecular interactions in fetal-to-adult hemoglobin switching mechanism holds the key to the identification of novel therapeutic targets not only for SCD and β-thalassemia but also for other β-globin disorders. The importance of B-cell lymphoma/leukemia 11A (BCL11A), Kruppel-like transcription factor 1 (KLF-1), myeloblastosis (Myb), histone deacetylase 1 and 2 (HDAC1/2) and SOX6 as the drug targets for induction of γ-globin gene in humans is discussed in [9].

A number of target-based drugs currently available or in clinical trials have been examined for in-

duction of γ-globin gene. In [9], we compared relative efficacy of these drugs on induction of γ-globin mRNA levels. Simulation-based computer experiments showed that concentrations of γ-globin mRNA in the untreated cells and in cells treated with the combination of Simvastatin and tBHQ [11], the two drugs in clinical trials, MS-275 [5], a drug in clinical trials, ST-20 [6], a drug already available, ACY-957 (two cases depending on dosage) [18], a drug in clinical trials, and our proposed strategy of inhibiting the multiprotein complex of erythroid transcription factors (ETF) were 0.08, 0.027, 0.033, 0.025, 0.035, 0.04 and 0.0435, respectively, 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 1) by 4.4-fold, ACY-957 (case 2) by 5.0-fold and finally our strategy by 5.4-fold over the untreated control. Thus, ETF turns out to be more efficient drug target as its silencing leads to greater induction of γ-globin gene compared to the ones proposed in [5, 6, 11, 18].

siRNA- and shRNA-mediated gene therapeutic strategies inducing γ-globin gene expression, that are reported so far in the literature include: (1) reducing methyl-binding domain (MBD2) mRNA gene expression by siRNA-mediated knockdown of MBD2 [11]; (2) shRNA-mediated knockdown of Myb followed by silencing gene expression of KLF-1 and BCL11A; (3) shRNA-mediated inhibiting BCL11A gene expression followed by silencing gene expression of KLF-1 and BCL11A, (4) siRNA-mediated knockdown of chromodomain helicase DNA (CHD4) binding protein followed by silencing gene expression of KLF-1 and BCL11A [10]. In [2], we reported that the strategies (1)-(4), respectively, result in 1.9-, 3.4-, 4.0-, 5.0-fold induction of γ-globin gene expression. In the same research we proposed a new RNAi-mediated strategy that targets BCL11A, friend of GATA protein 1 (FOG1) and HDAC1/2 mRNAs, and showed that this strategy results in 6-fold induction of γ-globin gene expression.

The approaches mentioned above might give reasonable assurance that the reactivation of HbF has a significant impact in treating β-globin gene disorders in many cases, but they are not sufficient alone to create a convincing treatment option. Another important factor that we should take into account is related to excess of free α-globin chains in patients with β-globin gene disorders. It was reported that natural reduction of free -globin chains in patients with β-thalassemia was in their advantage in terms of side effect of their lack of β-globin production [13]. Therefore, decreasing the concentration of toxic free α-globin chains should be considered as potential pathway to reach improved treatment option for such diseases. Alpha-hemoglobin-stabilizing protein (AHSP) is a chaperone molecule which binds to free α-globin chain of HbA and decreases level of free toxic monomeric -subunits of HbA and avoids ineffective erythropoiesis consequently [14]. Therefore, AHSP can be considered as a target to be beneficial for patients who suffer from β-globin gene related diseases [7].

2.1 Contribution

Based on the balance between α, β and γ chains we devise the unique criterion $F(\alpha, \beta, \gamma) = \alpha - (\beta + \gamma) + c$ to measure the effectiveness of the approaches for β-globin disorders and justify the reason why this formula provides a unique criterion. Then we measure efficacy of three strategies for a minor case of β-globin gene disorder β^+ and its severe case β^0. These strategies (1) knock down KLF1 gene expression by Simvastatin and tBHQ [11], (2) down regulate BCL11A and SOX6 gene expression using ACY-957, and (3) knock down MBD2 gene expression using MBD2 siRNA [16]. After that, we offer a compound strategy which not only induces γ-globin gene expression, but also avoids excessive free toxic α-globin chains by increasing AHSP gene expression with RNAa method. Simulation results show that our compound strategy improves results reported in [8, 11, 18] and, therefore, has improved beneficial therapeutic effect on β-globin disorders. To the best of authors' knowledge, this line of research is the first attempt to offer a compound strategy that is based on both induction of γ-globin gene expression and reduction of excessive α-globin chains.

We create HFPN model of fetal-to-adult hemoglobin switching network on Snoopy platform [5], validate the model with available qPCR data, and perform simulations to identify the most effective molecular targets for treatment of β-globin gene disorders.

3 Quantitative modeling with Petri nets

It is quite regular to express, interpret and predict the characteristics of a biological system in terms of quantitative change of biological components. Creating quantitative models is therefore crucial to make meaningful deductions regarding the behavior of biological systems. Over the last two decades, Petri net technologies have been extensively used for creating quantitative models of metabolic networks, signal transduction pathways and gene regulatory networks. When modeling biological processes, Petri net components such as places, transitions and arcs are respectively

used to represent biological entities, biological phenomena and flow of biological information. Concentration, reaction rate and reaction stoichiometry are usually assigned as parameters to places, trhaaansitions and arcs, respectively. Biological systems often comprise continuous, Boolean and discrete processes. Corresponding Petri nets are expected to be continuous [7], hybrid [7] and functional [12].

3.1 Creating HFPN model

We create HFPN model based on fetal-to-adult hemoglobin switching network [3] and qPCR data available to date [8, 11]. The snapshot of HFPN model is represented in Fig. 1.

The model is composed of 36 continuous and 8 discrete entities. The discrete entities represent presence/absence of β-globin gene mutation, drug, siRNA, RNAa treatments and delay in the expression of β-globin gene relatively to expression of α-globin and γ-globin genes. A discrete place takes values of 0 or 1. The continuous entities represent γ-globin gene, mRNAs, proteins, and their complexes. The model comprises 83 continuous processes; 49 processes representing activation, transcription, translation and binding and 34 processes standing for natural degradation of mRNAs and proteins. The only discrete process indicates delay in the expression of β-globin gene. The model uses 127 regular arcs (edges) and 13 inhibitor arcs to indicate interactions between components and processes.

We assume that a protein is produced according to the central dogma of biology. This is why, the initial concentrations are set to 0, and transcription of DNA into mRNA is represented by source transition, which continuously feeds mRNA.

It is hard to determine the rates solely based on wet lab observations. We adopt transcription and translation rates described in similar papers [9], but those not following the routine rates are obtained by applying reverse engineering method to reach the closest fit for hemoglobin switching developmental process. In this study, we refer to wet lab observations for Simvastatin together with tBHQ, and ACY-957 drug treatments, and MBD2 siRNA-based treatment to validate our model. We carefully calibrate results wherever and whenever possible to have clear trend and similarity between in silico results and qPCR data [8, 11].

4 Simulation-Based Validation of the Model

We plot the concentrations (on the y axis) versus time (on the x axis) measured in Petri time (pt), with the time interval of 5 pt corresponding to 3 months of gestational age. In these plots, it is assumed that fetal life starts at 15 pt (0 months) and child is born at 30 pt (9 months). We measure a concentration at 100 pt, when its level clearly remains in a stable steady-state.

To find the closest fit to inclinations of α-, β- and γ- globin mRNA levels in fetal-to-adult hemoglobin switching developmental stage, we extrapolated the trends between wild-type β-genes family [3]. We considered two cases of β-globin disorder to choose the correct treatment. These are severe case, β^0, in which the production of β-chains is almost stopped and a minor one, β^+, in which β-globin chains are suspended, but not fully stopped. Based on simulation results, we found that ratio $\frac{\beta}{\alpha}$ is 99% for a healthy person, and it is 80% in the case of β^+. These results agree with wet lab observations [17]. Simulation results related to the ratios between AHSP, β-, γ- and α-globin gene expressions are also validated in accordance with data provided in [17]. When there is lack of β-chains due to β-globin gene mutation drug treatments in primary human erythroid cells with a combination of Simvastatin and tBHQ decrease KLF1 gene expression by 44%, and induce γ-globin gene expression [11]. As it can be seen from Fig. 2, simulation results for KLF1 mRNA concentration is a good fit to corresponding wet lab observations.

To study human γ-globin gene regulation, experiments were carried out in CID-dependent mouse bone marrow cells carrying β-YAC. It is known that human γ-globin gene is repressed in these adult phenotype erythroid cells [4]. It was also observed that siMBD2 treatment in CID cells reduces expression of MBD2 by approximately 80%, derepressing γ-globin expression [8]. Simulation results for MBD2 gene expression are shown in Fig. 3. By carefully calibrating the rate of binding between siMBD2 and MBD2 mRNA we achieved 5-fold decrease from 5 down to 1 for MBD2 mRNA concentration, which is numerically validated by wet lab observations [16].

As it is reported treatment of CD71lowGlyAneg cells with ACY-957 decreases BCL11A and SOX6 gene expressions by 2- and 10-fold, respectively. Simulation results for BCL11A and SOX6 mRNA levels are illustrated in Fig. 4 and Fig. 5. Simulation results for β^0 show change of BCL11A mRNA concentration from 0.764096 down to 0.38212 by 2-fold and SOX6 mRNA concentration from 5 down to 0.5 by 10-fold, respectively.

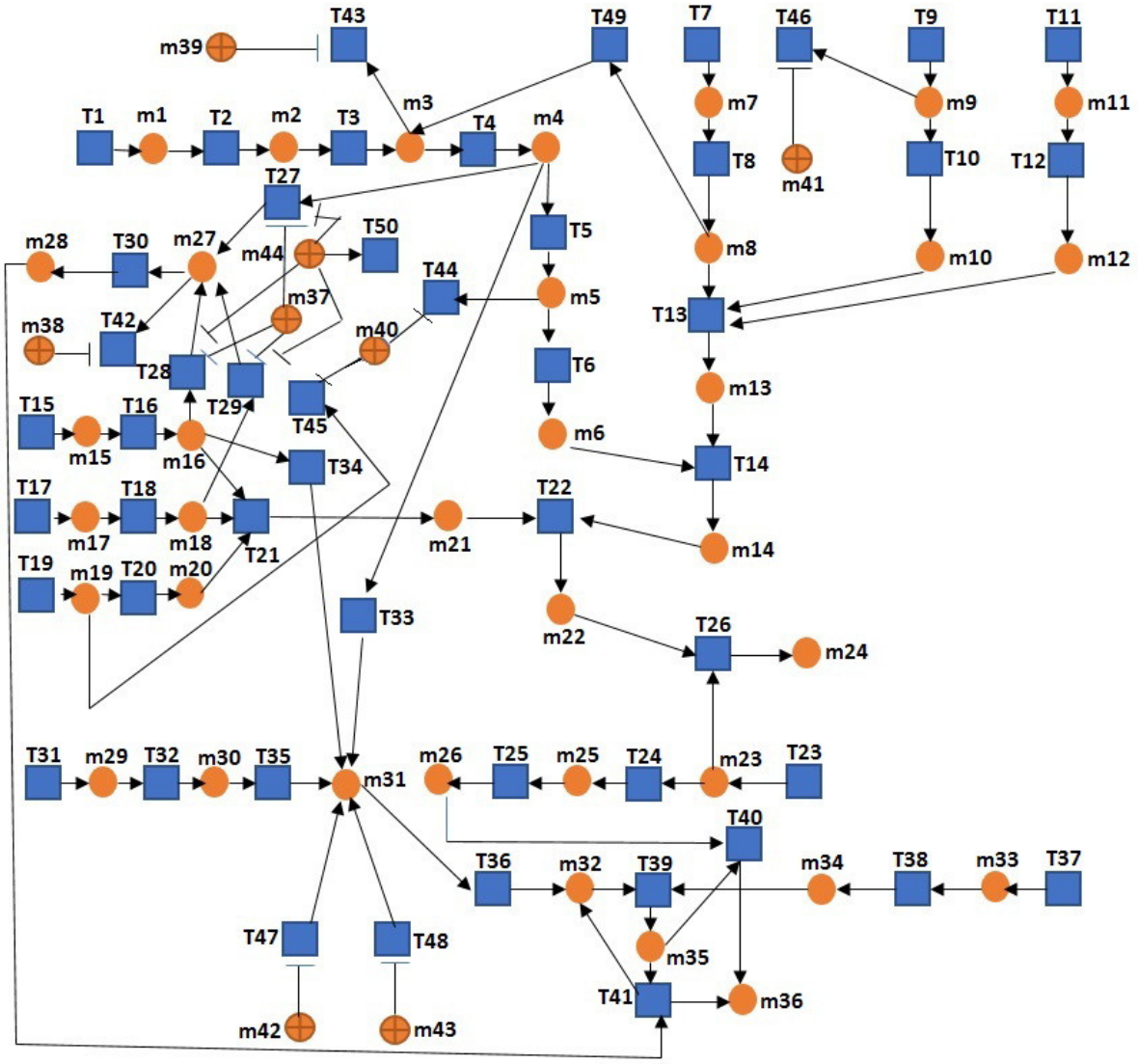

Figure 1: HFPN model of fetal-to-adult hemoglobin switching network.

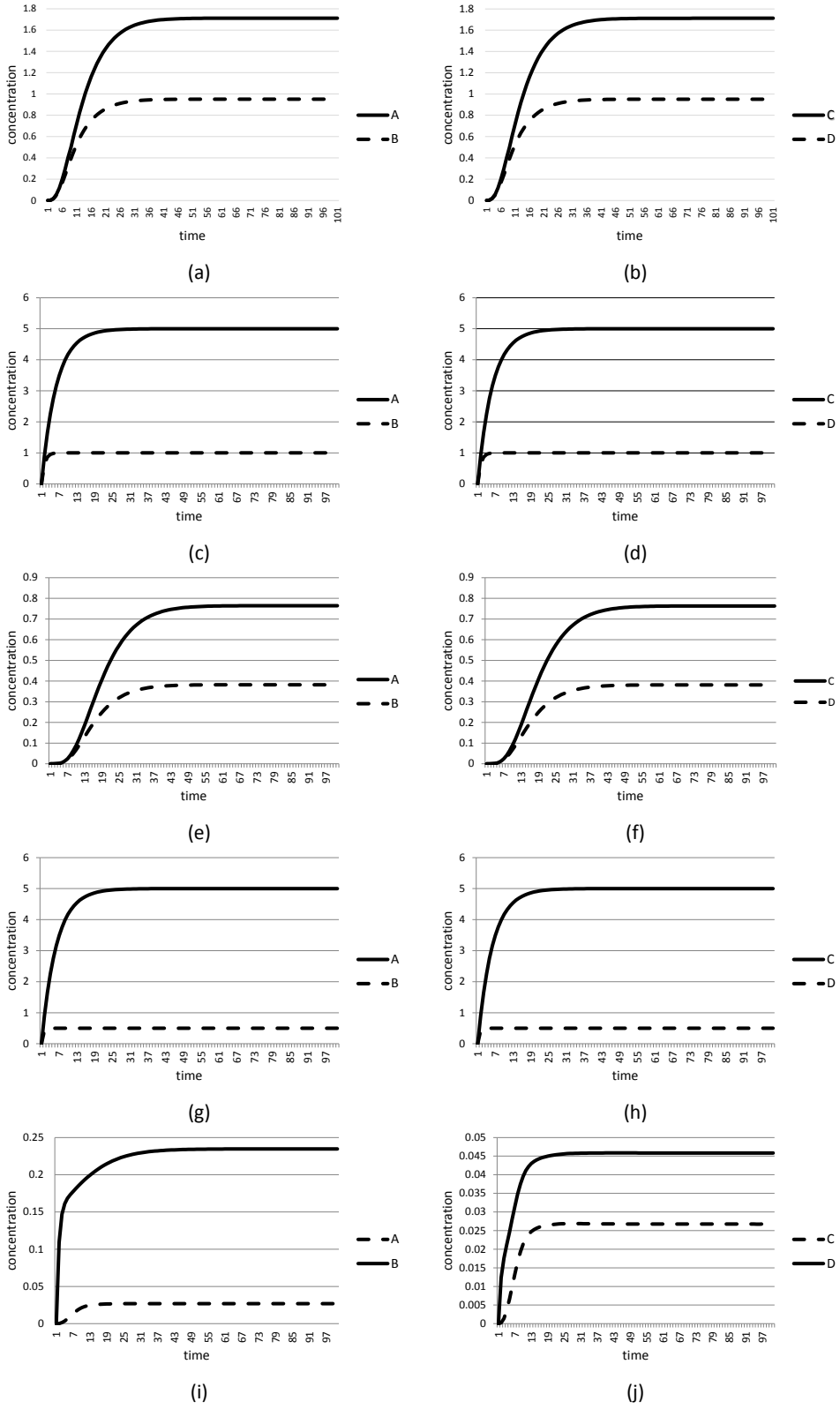

Figure 2: Simulation results for expression of (a) KLF1 mRNA (β^0), (b) KLF1 mRNA (β^+), (c) MBD2 mRNA (β^0), (d) MBD2 mRNA (β^+), (e) BCL11A mRNA (β^0), (f) BCL11A mRNA (β^+), (g) SOX6 mRNA (β^0), (h) SOX6 mRNA (β^+), (i) AHSP mRNA (β^0), (j) AHSP mRNA (β^+).

In the case of β^+, we observed change of BCL11A mRNA levels by 2-fold from 0.762913 to 0.381529 and SOX6 mRNA levels by 10-fold from 5 down to 0.5. These results are in strong agreement with qPCR data.

In the proposed strategies, we have increased AHSP gene expression by potential RNAa technique to reduce free toxic α-subunits in human adult hemoglobin. Simulation results for our strategies show increase of AHSP levels by 8.7-fold from 0.0269213 to 0.234716 for β^0, and by 1.7-fold from 0.0262398 to 0.045885 in the case of β^+, respectively (see Fig. 7). Because of lack of the wet lab observations we were not capable to compare and validate simulation results for MBD2, BCL11A, and SOX6 mRNAs.

5 Identifying improved treatment for β-globin disorders

In normal population, there is a balance between globin chain synthesis and production of α and non-α (β plus γ) chains [13]. In patients affected by β-thalassemia and SCD, the balance is achieved by increase of γ-globin production which in turn compensates deficit in β-globin mRNA. However, inducing $gamma$-globin gene expression too much can lead to side effects [15], so that it is reasonable to keep total concentration of β- and $gamma$-globin mRNAs at a constant level. There is also relationship between ratio of β- and α-globin mRNAs and β-thalassemia disease severity. It was reported that this ratio is 0.99 in normal controls, but decreases with increase of disease severity [13]. In β-thalassemia patients the balance between α- and non- α-globin expressions is achieved by decreased α-globin gene expression. On the other hand, reducing α-globin expression excessively may result in phenotype in patients who deal with excess of γ chains before birth, and β chains after birth [3]. Bringing together all these observations we devise a unique criterion, $F(\alpha, \beta, \gamma) = \alpha - (\beta + \gamma) + c$, to measure the effectiveness of a treatment, where c is expected to be a constant related to δ-chain concentration level. The optimal treatment should establish potential ideal balance between α-, β- and γ-globin mRNAs. Thereby the most effective treatment can be found by minimizing $|F|$.

There were several attempts to find effective treatment for β-globin disorders by targeting components of fetal-to-adult hemoglobin switching network. To the best of our knowledge the present research is the first work that proposes a compound strategy based on inducing γ-globin gene expression as well as reducing free α-globin chains. We compare efficacy of compound treatments by measuring the value of $|F|$ at 70 pt. After validating HFPN model for a healthy person with available qPCR data [13] and taking into account proportions of the chains in HbA, we find that $c = 0.00703463$. When $|F| > 0$ there exist excessive free toxic α-subunits in HbA, and when $|F| < 0$ there might be excessive γ-chains in HbF or β-chains in HbA. A potential optimal strategy is the one that prevents both unwanted cases. This is achieved when $|F| \to 0$. This is main intention behind of considering $|F|$ rather than $|F|$. The optimal strategy is found by minimizing $|F|$.

Simulation results for this case show 1.7-, 5.6-, and 1.7-fold induction of γ-globin gene expression from 0.00289782 of normal control to 0.00497709, 0.0161972, and 0.00478446 for treatments with a combination of Simvastatin and tBHQ, ACY-957, and siRNA, respectively. We observed that drug treatment with ACY-957 leads to more γ-globin gene induction compared to the other two treatments. However, $|F|$ reaches its minimum value at 0.00243087 for MBD2 siRNA technique. Thus, we conclude that MBD2 siRNA technique along with AHSP RNAa technique the most prominent strategy in the case of β^+. Simulations results performed for this compound strategy results in 1.7-fold increase in both γ-globin mRNA and AHSP mRNA levels and $|F| = 0.00015858$.

6 Conclusion

In the present work, we exploit quantitative modeling with HFPN to identify the most efficient therapeutic strategy for minor and severe forms of β-globin diseases, proposing this approach to the benefit of both target-based therapy and quantitative modeling with HFPN. We focus on determining the optimal targets that lead to the maximum γ-globin gene induction and minimum production of free toxic α-globin chain. In the meantime, we try to shed light on how quantitative modeling with HFPN can be used to recognize targets for target-based treatment of the β-globin disorders without emphasizing how this can be achieved from pharmacological point of view. Although it is not within the scope of this paper, as prospects, we are sure that a more detailed discussion of biological consequences should be considered in collaboration with a pharmacogenetics group. It should also be noticed that the present approach can be successfully used for identifying optimal molecular targets not only for β-globin disorders but also for other diseases.

By developing deterministic quantitative model, we tried to create the closest approximation of underlying biological system based on rigorous review of biological literature available to date. As we hope our

model is trustful enough as we tried to be as punctual as possible when constructing molecular interactions between the biological components of fetal-to-adult hemoglobin switching network. As a further work we are planning to integrate stochastic and fuzzy parameters to the model to see the effect of the noise, randomness and uncertainty to underlying biological system.

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