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COMPARISON OF QUANTITATIVE MODELLING TYPES FOR BETA-THALASSEMIA AND SICKLE CELL DISEASE

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Abstract. β-globin gene disorders such as β-thalassemia and Sickle Cell Disease (SCD) are caused by various types of mutations in β-globin gene. There are several known strategies to treat them, but the lack of strategy comparison is vivid. In addition, there is not a common voice among scientists to select one of the common strategies as the most effective treatment for β-globin gene disorders. Running several tests on human beings who are suffering from any β-globin gene disorders can be time consuming, costly, and even not ethical in some cases. Therefore, computational modeling can play an important role to shed light on how biological systems work and how it is possible to identify new targets as predictions for possible novel treatments. However, the lack of model comparison among quantitative approaches is obvious as well. In this study, deterministic Petri nets are compared with Fuzzy Stochastic Petri nets for both drug-based and gene-therapies of β-globin gene disorders to check how it is possible to deal with randomness of biological systems and vagueness of their kinetic parameters. The comparison of deterministic Petri nets with fuzzy stochastic ones showed that deterministic models can be used as pre-tests with a single simulation run to make some naïve predictions, but Stochastic Petri nets with fuzzy parameters were promising to describe the biological system more accurately. This comparison made it possible to select Fuzzy Stochastic Petri nets to identify potential drug-based and gene therapies to treat β-globin gene disorders as well.

Keywords: Fuzzy Stochastic Petri Nets, β-globin gene disorders, quantitative modeling, simulation.
AMS Subject Classification: 68U99.
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1 Introduction

A point mutation or combination of mutations in β-globin gene may cause β-globin gene disorders such as β-thalassemia and Sickle Cell Disease (SCD) (Fortin et al., 2016). Such mutations in β-globin gene lead to lack of β-subunits in hemoglobin, and result in variable phenotypes in human adults. The type of thalassemia, where mutations lead to reduction in synthesis of β-globin chains is called β^+ thalassemia. Otherwise, it is referred to as β^0 thalassemia, where the mutations cause absence of the synthesis of β-globin chains. β-thalassemia can be categorized into β-thalassemia minor and major in terms of the severity of the disease (Cao et al., 2011). The overall annual incidence rate of symptomatic individuals (Either thalassemia minor or thalassemia major) is estimated at 1 in 10000 in the European Union and 1 in 100000 across the world (Galanello et al., 2010). Hemoglobin molecules contain two subunits α-chains and two subunits of γ-chains in fetal hemoglobin (HbF) and two subunits of β-chains. Hemoglobin molecule structures are illustrated in Figure 1.

There exist several treatments to cure β-globin gene disorders such as bone marrow trans-

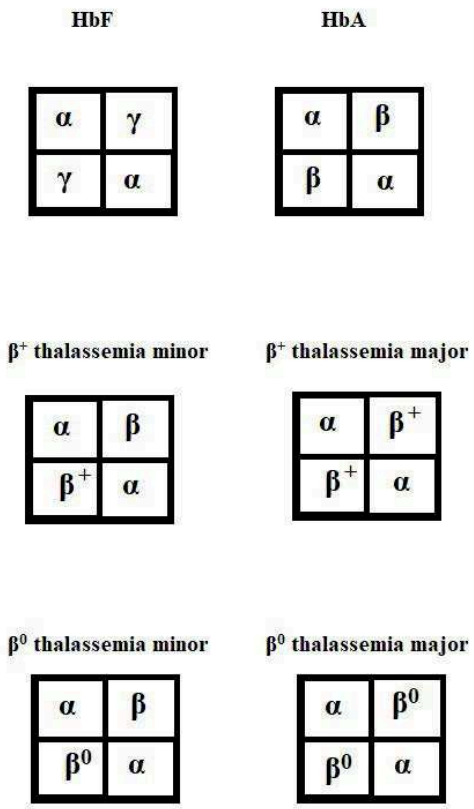

Figure 1: Hemoglobin molecule structure in a healthy person versus patients with different types of β-thalassemia

plantation, gene therapy (Persons et al., 2009), red blood cells transfusion, and chelation therapy particularly for patients who have β-thalassemia. However, there is not yet a global treatment to cure all patients, who are suffering from β-globin gene disorders. Therefore, identifying new strategies to ideally treat such diseases is essential. In this study, the concentration is on drug-based and gene therapies as effective therapies for β-globin gene disorders. Gene therapy has been introduced as a potential technique to ameliorate genetic related diseases since few decades ago. Gene therapies have improved rapidly during these years and there exist different techniques of gene therapy to transfer or repair of genes or knocking down gene expressions. Gene therapy also has been developed as a potential strategy to treat β-thalassemias as a group of hereditary hematological genetic diseases. Theoretical and experimental systems in gene therapy approaches in β-globin gene disorders have been created such as in silico (Bashirov et al., 2017) and in vitro (Italia et al., 2013) to predict the in vivo response. The advantage of gene therapy over drug therapy in treating β-globin gene disorders through γ-globin induction is related to the way it alters the specific gene expressions directly without worrying about on-target and off-target side effects (Bashirov et al., 2017). The crucial step to reach this aim is to have better understanding of hemoglobin switching network, which will be described in materials and methods in detail. Fuzzy Stochastic Hybrid Functional Petri nets (FSHFPNs) will be used in materials and methods section to describe the proposed models. In the same section, the numerical initial markings and values for process rates will be validated by using current available experimental data. The simulation results and their analysis will be demonstrated in the results section, and There will be a comparison between Fuzzy Stochastic Petri nets versus Deterministic Petri nets to show which one can be preferred in different cases. In case of gene therapy, the proposed quantitative modeling is conducted on Snoopy platform (Heiner et al., 2012) to compare known gene therapy approaches in treating β-globin gene disorders through

γ-globin induction using available qPCR data and to identify a novel gene therapy to improve the efficacy of the treatment. Finally, novel targets for drug therapy and gene therapy were predicted by using a FSHFPN model.

2 Materials and Methods

2.1 Biological Context

2.1.1 Fetal to Adult Hemoglobin Switching Network

Hemoglobin switching network is the term used for two switches in developmental stages of β-globin gene family regulation. The first switch happens from embryonic to fetal developmental stage, which occurs within the first six weeks of prenatal age. At this point, γ-globin gene expression is at its highest level while ε-globin gene expression is down-regulated. The second switch happens within the first six weeks of postnatal age, and it is called fetal to adult hemoglobin switching. At this point, β-globin gene expression starts replacing γ-globin gene expression by being up-regulated. β-globin gene expression reaches its highest level and γ-globin gene expression reaches its lowest level about 6 months after birth. Therefore, the dominant hemoglobin molecules at each stage of ontogeny are embryonic hemoglobin (Hbε) in the first three months after conception, fetal hemoglobin (HbF) in fetus life from the third month after conception to the time of birth, and adult hemoglobin (HbA) after birth to the end of life in a healthy person. β-globin gene family gene regulations are illustrated in Figure 2 in all these developmental stages known as hemoglobin switching network.

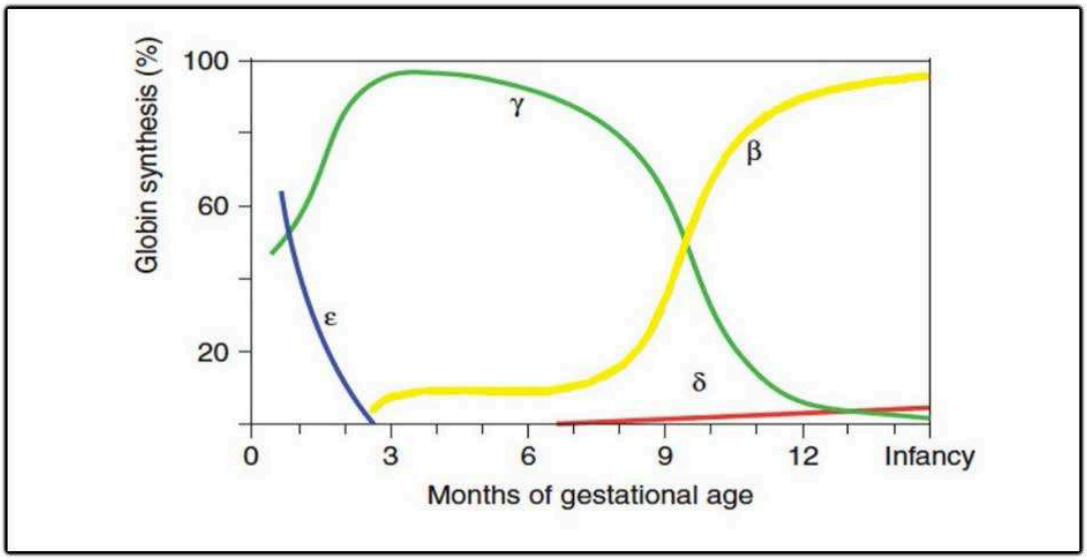

Figure 2: Hemoglobin switching developmental stages (Adopted from Sankaran (2008))

It is essential to know which entities (e.g. Genes) are involved in causing up/down regulation of specific gene expression during fetal-to-adult hemoglobin switching stage. At this developmental stage, it is known that the gene expression of B-cell lymphoma/leukemia 11A (BCL11A) is up regulated indirectly by Myeloblastosis (Myb) through Kruppel-Like transcription Factor 1 (KLF-1) (Persons et al., 2009), and BCL11A binds with Nucleosome Remodeling Deacetylase (NuRD) complex and silences the gene expression of γ-globin gene (Bauer et al., 2012). It was revealed that there are three other transcription factors which are involved in silencing γ-globin gene expression named GATA1, FOG1 (Miccio et al., 2010) and Sox6 (Xu et al., 2010). On the other hand, the transcription factors which are responsible for up regulating β-globin gene expression are KLF1, GATA1, and FOG1. Therefore, reactivation of γ-globin gene expression has

become a potential approach to ameliorate the lack of β-globin production, the clinical severity, and decrease the mortality rates of patients suffering from β-thalassemia (Roosjen et al., 2014). However, identifying target-based treatments to increase the level of γ-globin gene expression is still challenging (Doi et al., 2006). Thus, mathematical and quantitative computational modeling can be useful to describe and analyze such complex biological systems.

2.1.2 siRNA-and shRNA-Mediated Gene Therapy

RNA interference (RNAi) is a biological process which was discovered as a technique of gene therapy in the past two decades. RNAi is a post-transcriptional gene silencing process that inhibits gene expression using the gene's own DNA sequence. The most frequent used RNAi-mediated gene therapy approaches are small interfering RNA (siRNA) and short hairpin RNA (shRNA). In RNAi process, long double stranded RNA (dsRNA) cleaves into short dsRNA with typically 20-25 nucleotide length by Dicer enzyme. Then each of selected siRNA enters the cell and is unwound into single-stranded RNAs (ssRNAs) by binding to RNA-induced silencing complex (RISC). Following this, ssRNAs binds to the target mRNA with complementary sequence, resulting in significant degradation of the target mRNA. The degradation of target mRNA leads to suppression of its translation and stops production of the target protein. The mechanism of siRNA and shRNA are very similar except for this fact that shRNA is exported to the cytoplasm where it is converted into siRNA after the expression in the nucleus by removal of unnecessary fragments.

During the last few years, molecular biologists used RNAi approaches to inhibit gene expression of Myb, KLF-1, BCL11A, MBD2, and CHD4 to induce γ-globin gene expression to ameliorate the lack of β-globin level in patients suffering from β-globin gene disorders (Roosjen et al., 2014). The experiments to study γ-globin induction in chemical inducer dimerization (CID) dependent mouse bone marrow cell carrying β-globin yeast artificial chromosome (YAC) showed that siMBD2 treatment decreases the level of MBD2 mRNA by approximately 80% and leads to induction of γ-globin Gnanapragasam et al. (2011). The transduction of murine erythroleukemic (MEL) cells with virally encoded shRNA showed that shMyb501 as one of the two proposed shMyb constructs (shMyb500 and shMyb501) decreases the level of KFL1 mRNA and BCL11A mRNA by approximately 75% and 76%, respectively (Roosjen et al., 2014). It was also reported that gene therapy by shBCL11A in MEL cells decreases the level of KLF-1 mRNA and BCL11A mRNA by 10% and 82%, respectively (Roosjen et al., 2014). Gene therapy with siCHD4 in CID cells also showed that KLF-1 and BCL11A gene expression were silenced by 70% and 40%, respectively (Amaya et al., 2013). Therefore, identifying new molecular targets is essential to improve efficacy of β-globin disorders gene therapies through induction of γ-globin gene expression.

2.2 Fuzzy Stochastic Petri net model

2.2.1 Fuzzy Stochastic Petri net model for drug-based treatments

The proposed model in this section is a Fuzzy Stochastic Hybrid Petri nets (FSHPN) model to describe fetal to adult hemoglobin switching network, and analyze it to identify potential treatments for β-globin gene disorders through γ-globin induction. Stochastic and Fuzzy properties were added to the model to deal with randomness and uncertain kinetic parameters, respectively. This model can quantitatively compare current drug-based treatments with predicted ones by comparing the simulation results, which were obtained by considering dynamic behaviors of proteins and multi-proteins which are responsible in reactivation of γ-globin gene expression.

The fundamental segment of the proposed model related to fetal to adult hemoglobin switching network was created based on extracted information from biological context in the literature (Sankaran et al., 2013). The proteins, which play major roles in the fetal to adult hemoglobin

switching network were assumed to be created by going through central dogma of molecular biology, that is all proteins were translated from their corresponding messenger RNAs (mRNAs), and the mRNAs were transcribed from their corresponding genes. In addition, the levels of mRNAs, proteins, and complexes in the model were kept low by considering natural degradation.

The proposed model consists of 7 discrete places, 27 continuous places, 60 transitions, and 97 arcs connecting places/transitions to other transitions/places. The discrete places stand for presence/absence of mutation in β-globin gene, and the drug-based treatments using Simvastatin and tBHQ, different dosages of ACY-957, ST-20, MS-275, and Erythroid Transcription Factors (ETF) inhibitor. The initial marking of these discrete places can be equal to 0 or 1. when there is no mutation in β-globin gene, the initial marking is 0, otherwise it is 1. In case of absence a certain drug-based treatment, the initial marking for that place is 1, otherwise it is 0. The continuous places in the model stand for level of genes, mRNAs, proteins, and multi-proteins as complexes. The initial marking of these continuous places is 0. The updated markings will accept fuzzy numbers from the fuzzy sets. The transitions in this model represent biological processes, such as transcription, translation, binding of proteins and multi-proteins, and degradation of mRNAs and proteins. All these transitions were considered as stochastic transitions. The details related to places, transitions, and arcs are illustrated in Tables 1-4.

2.2.2 Extended Fuzzy Stochastic Petri net model for gene-therapies

The FSHFPN model in the previous subsection was extended in the current section to analyze fetal-to-adult hemoglobin switching network to compare current gene therapies for β-globin gene disorders through γ-globin induction and identify novel molecular targets as a potential gene therapy to ameliorate β-globin gene disorders. The discrete places in the extended FSHFPNs model denote presence/absence of mutation in β-globin gene, and gene RNAi-mediate knockdown treatments with siMBD2, shMYB501, shBCL11A, siCHD4, and siRNA gene therapy by targeting BCL11A, FOG1, and HDAC1/2. The initial marking of presence or absence of β-globin gene was defined the same way in the previous subsection. When a certain RNAi-mediated knockdown treatment is absence, the initial marking for that place is 1, otherwise it is 0. The level of genes, mRNAs, proteins, and multi-proteins were considered using continuous places in the model with initial marking of 0. The updated markings can get fuzzy numbers and all transitions were considered as stochastic transitions as explained in the previous subsection. The proposed FSHFPN model includes 26 continuous, 9 discrete places, 62 stochastic transitions, 100 regular arcs, and 16 inhibitory arcs. The details related to places, transitions, and arcs in the extended FSHSPN model are illustrated in Tables 5-8. The snapshots of the proposed FSHFPN model are illustrated in Figure 3-4.

3 Results

3.1 Numerical Validation of the Models

3.1.1 Numerical validation for the drug-based model using experimental data

The proposed FSHPN model in this section was conducted using Snoopy Heiner et al. (2012). The simulation results were obtained by calculating the mean of 37500 simulation runs using the formula provided in Sandmann et al. (2008). All P-values in the simulation results were considered 0.05. The most challenging part of creating such mathematical modellings is deciding about the initial marking values and transition process rates (kinetic parameters). Unfortunately, this is usually the case that the exact kinetic parameters are either unknown or not accurate. It seems a cumbersome task to find the exact kinetic parameters based on web lab results since there are usually scarce and contradictory. To cover such uncertainties, fuzzy property was added to the model.

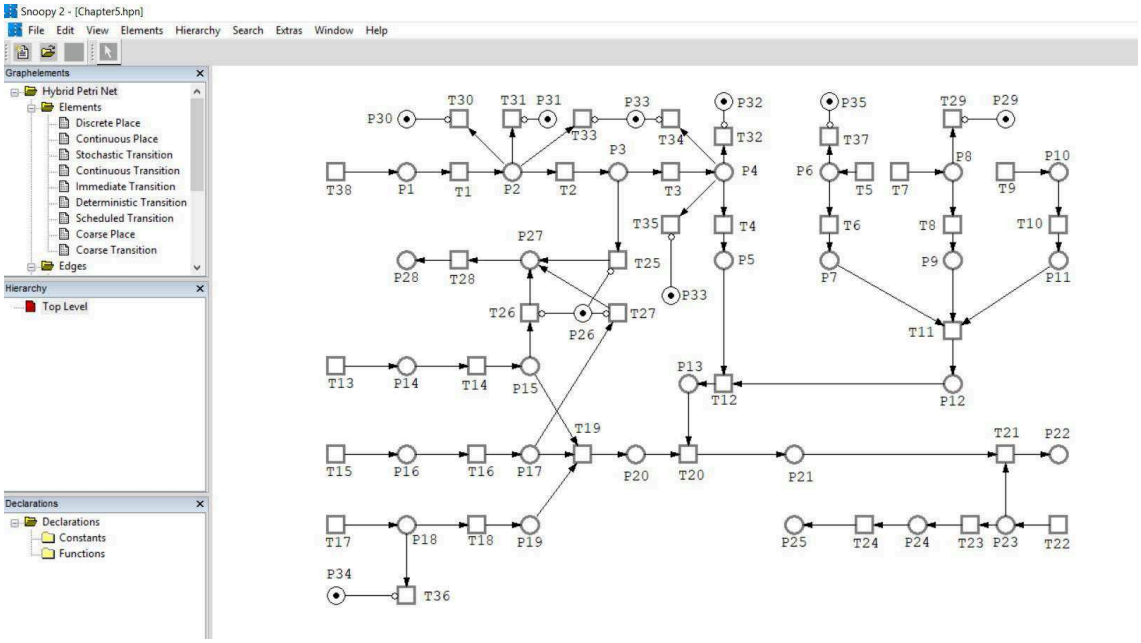

Figure 3: Snapshot of proposed SHFPN on Snoopy for the drug-based treatment

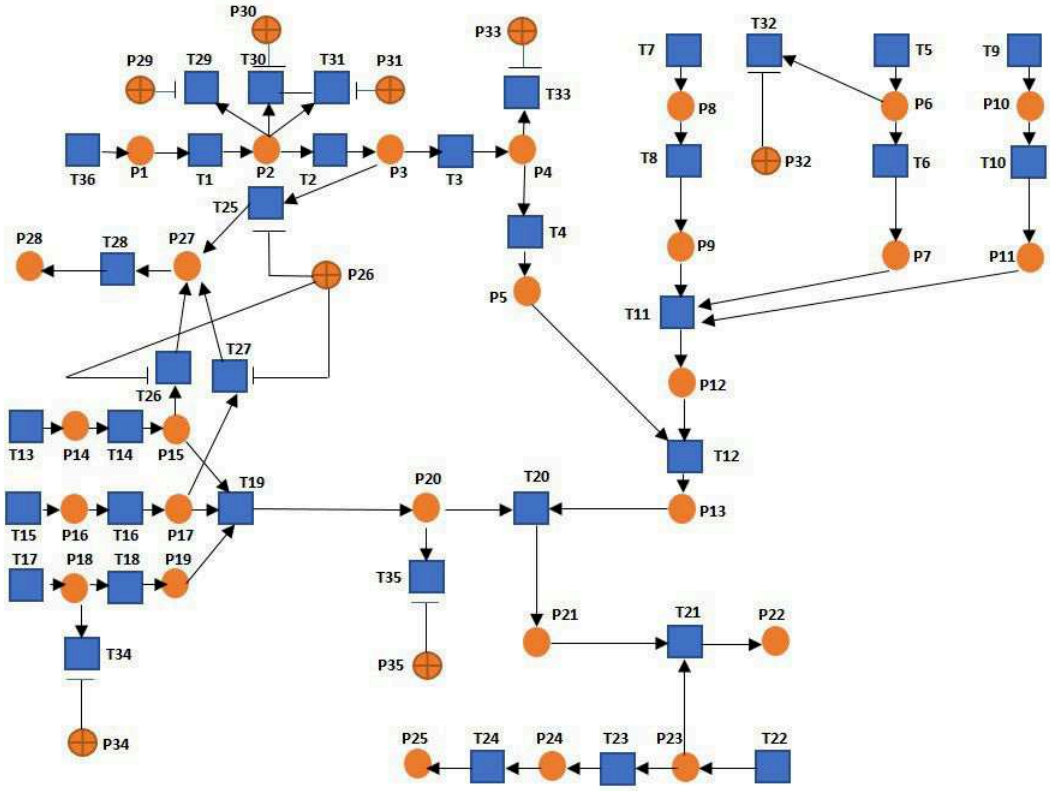

Figure 4: Drawing of proposed SHFPN model for the drug-based treatment

The estimated kinetic parameters were obtained using related works Mehraei et al. (2016). These kinetic parameters were carefully calibrated to validate the model of fetal-to-adult hemoglobin switching network and drug-based treatments with available and known qPCR data. The concentration level of each entities (y-axis) was plotted versus time (x-axis) called Petri time (pt). Each 5 pt corresponds to 3 months. Therefore, in the developmental stages of fetal-to-adult hemoglobin switching, fetal life starts at 15 pt and the child is born at 30 pt (9 months). The

validation of kinetic parameters was achieved by considering 100 pt in the simulation results, where the levels of all entities are at the stable steady-state. To obtain estimated kinetic parameters, the relation of β-globin mRNA level with γ-globin mRNA level during hemoglobin switching network was considered. The model was validated by reaching the closest approximation of the β-globin mRNA and γ-globin mRNA levels in Figure 5. The fetal-to-adult hemoglobin switching networks is illustrated in Figure 5, using simulation results related to only β-globin mRNA and γ-globin mRNA using Snoopy platform.

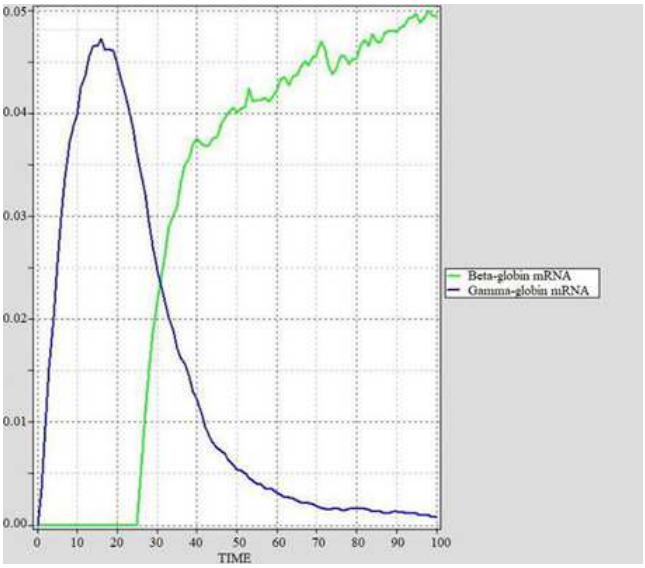

Figure 5: Snoopy Heiner et al. (2012) simulation results for β-globin and γ-globin wild type gene expression

The other part of validation of the model and its kinetic parameters is related to the amount of increasing/decreasing in the level of specific gene expressions when using certain drug-based treatments when β-globin gene is mutated. It was revealed when β-globin gene is mutated, the level of KLF-1 gene expression decreased approximately by 2.3-fold using both Simvastatin and tert-Butylhydroquinone (tBHQ) treatment in vitro experiments in primary human erythroid cells Macari et al. (2013). The simulation results at 100 pt in our proposed FSHPN model show that the level of KLF-1 mRNA decreases by 2.3-fold when kinetic parameter of transition 29 (T29) is equal to (0.15, 0.18, 0.20) fuzzy number. Binding of Simvastatin and tBHQ with KLF-1 mRNA in the proposed model leads to γ-globin gene expression induction, which agrees with results in Macari et al. (2013). Drug treatments of erythroid progenitors cultured from Sickle Cell Anemia and β-thalassemia patients revealed that MS-275 and ST-20 decrease the level of KLF-1 mRNA by 3- and 2.5-fold, respectively Dai et al. (2014). The simulation results at 100 pt show that the kinetic parameter of T30 and T31 should be considered as (0.3, 0.4, 0.5) and (0.35, 0.37, 0.40) fuzzy numbers to suppress KFL-1 mRNA level by 3- and 2.5-fold, respectively. Binding of these drugs with KLF-1 mRNA leads to γ-globin gene expression induction, which supports the experiment results conducted in Dai et al. (2014). The simulation results at 100 pt are illustrated in Figure 6 for KLF-1 mRNA level in untreated cell, and treated cell with Simvastatin and tBHQ, MS-275, and ST-20. In addition, ST-20 drug treatment of erythroid progenitors with mutated β-globin gene showed that HDAC1/2 mRNA level decreased by 6-fold. To reach this amount of suppression in HDAC1/2 mRNA, the kinetic parameter of T32 should be considered as (0.9, 1, 1.1) fuzzy number. The simulation results at 100 pt for HDAC1/2 gene expression in an untreated cell and treated cell with ST-20 are illustrated in Figure 7. Quantitative real-time PCR time course experiments with CD7 $1^{low}GlyA^{neg}$ cells differentiated for 5 days with 1μM ACY-957 showed that the level of BCL11A mRNA and SOX6 mRNA decrease by 1.4-fold and 2.3-fold using this drug-based treatment, respectively. To reach these number of decreased folds

in BCL11A mRNA and SOX6 mRNA, T33 and T34 should be considered as (0.35, 0.38, 0.40) and (0.20, 0.21, 0.22), respectively. The simulation results in our proposed model at 100 pt show that the biding of ACY-957 with BCL11A mRNA and SOX6 mRNA leads to induction of γ-globin gene expression. The simulation results at 100 pt for BCL11A and SOX6 mRNA levels in untreated cell and treated one are illustrated in Figure 8 and 9, respectively.

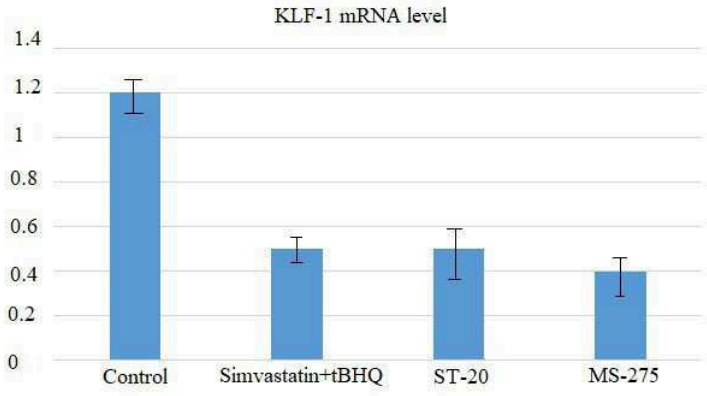

Figure 6: KLF-1 mRNA level simulation results in untreated cell versus treated cell with Simvastatin and tBHQ, ST-20, and MS-275

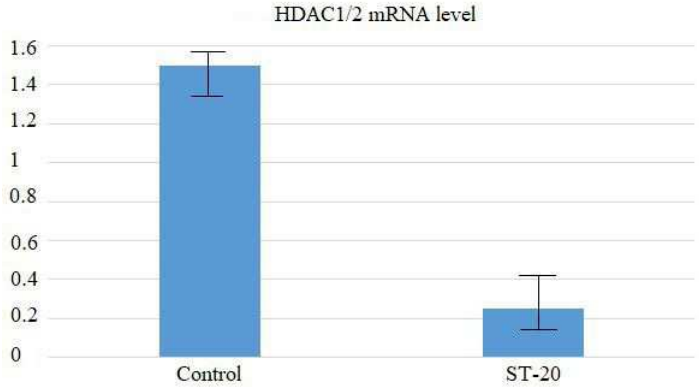

Figure 7: HDAC1/2 mRNA level simulation results in untreated cell versus treated cell with ST-20

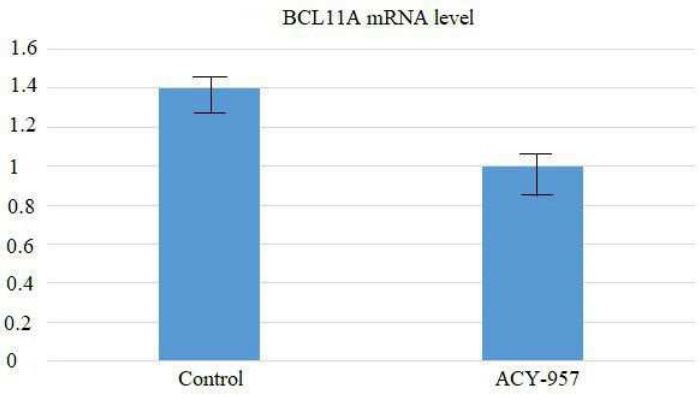

Figure 8: BCL11A mRNA level simulation results in untreated cell versus treated cell with ACY-957

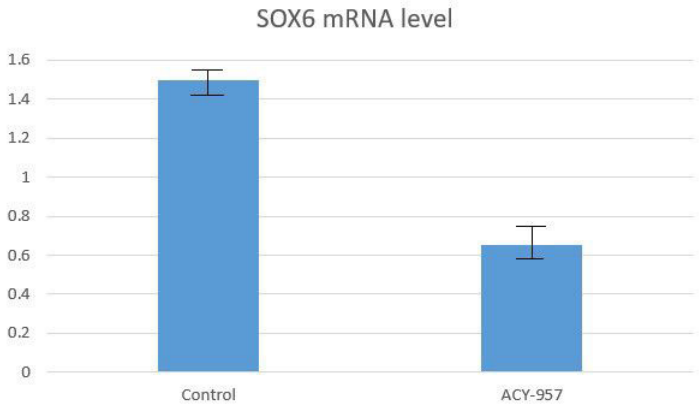

Figure 9: SOX6 mRNA level simulation results in untreated cell versus treated cell with ACY-957

3.1.2 Computational Validation of the Gene-therapy model with RT-qPCR data

The proposed SHFPN model in this section was conducted using Snoopy Heiner et al. (2012) with manually adding fuzzy parameters. The simulation results to be validated using known in vitro lab results were obtained by calculating the average mean of 37500 stochastic simulation runs using the formula provided in Sandmann et al. (2008) with P-value equal to 0.05. Since the wet lab results can be usually contradictory, fuzzy parameters were added to the model to deal with such vague or unknown kinetic parameters. The estimated kinetic parameters as membership 1 value of fuzzy numbers were obtained using related works Mehraei et al. (2016). The concentration level of each entities (y-axis) was plotted versus time (x-axis) called Petri time (pt). In the plots of simulation results, each 5 pt corresponds to 3 months. Therefore, in the developmental stages of fetal-to-adult hemoglobin switching network, fetal life starts at 15 pt and the child is born at 30 pt (9 months). The validation of kinetic parameters was achieved by considering 100 pt in the simulation results, where the levels of all entities are at the stable steady-state. The concentration levels of β-globin mRNA and γ-globin mRNA levels and their ratio comparing with each other in developmental stages of fetal-to-adult hemoglobin switching network were validated in the same way as in the previous section. The other part of validation of the model and its kinetic parameters is related to the amount of increasing/decreasing in the level of specific gene expressions including BCL11A, MBD2, CHD4, HDAC1/2, and FOG1 in fetal-to-adult hemoglobin switching network when using certain RNAi gene therapies when β-globin gene is mutated.

Experiments in chemical inducer dimerization (CID) dependent mouse bone marrow cells carrying β-globin yeast artificial chromosome (β-YAC) showed that siMBD2 treatment decreases the level of MBD2 mRNA by approximately 80% Gnanapragasam et al. (2011). The simulation results at 100 pt in our extended proposed FSHFPN model show that the level of MBD2 mRNA decreases by 80% when kinetic parameter of transition 29 (T29) is equal to (0.75, 0.76, 0.77) fuzzy number. Binding of MBD2 siRNA with MBD2 mRNA in the proposed model leads to γ-globin gene expression induction, which is in line with results in Gnanapragasam et al. (2011).

It was revealed that Myb is a critical regulator of KLF-1 and BCL11A Roosjen et al. (2014). Results of qRT-PCR of experiments with Myb500 and Myb501 short hairpin RNA as two shMyb constructs in transduction of murine erythroleukemic (MEL) cells show that KLF-1 and BCL11A mRNA levels reduce by 75% and 76%, respectively Gnanapragasam et al. (2011). The extended proposed model was validated in accordance with shMyb501 treatment. The simulation results at 100 pt show that the level of KLF-1 mRNA and BCL11A decreases by 4- and 4.2-fold when kinetic parameter of transition 30 (T30) is equal to (0.56, 0.58, 0.6) fuzzy number, respectively. Binding of shMyb501 with KLF-1 mRNA in the proposed model leads to γ-globin gene expression induction, which is a good fit to corresponding in vitro experiments in Gnanapragasam et

al. (2011).

It was shown that gene therapy with shBCL11A in MEL cells decreases the level of KLF-1 and BCL11A by 10% and 82%, respectively Roosjen et al. (2014). The simulation results at 100 pt show that the level of KLF-1 mRNA and BCL11A decreases by 1.1- and 5.6-fold when kinetic parameter of transition 31 (T31) is equal to (0.01, 0.02, 0.02) fuzzy number and transition 32 (T32) is equal to (1.4,1.5,1.6), respectively. Binding of shBCL11A with KLF-1 mRNA and BCL11A in the proposed model leads to γ-globin gene expression induction, which is in line with the wet lab results in Gnanapragasam et al. (2011).

It was revealed that CHD4 binds directly to KLF-1 and BCL11A and positively regulates them Amaya et al. (2013). In addition, it was reported that gene therapy with CHD4 siRNA in CID cells decreases the level of KLF-1 mRNA and BCL11A mRNA by 70% and 40%, respectively Amaya et al. (2013). The simulation results at 100 pt show that the level of KLF-1 mRNA and BCL11A decreases by 3.3- and 1.7-fold when kinetic parameter of transition 33 (T33) is equal to (0.01, 0.02, 0.02) fuzzy number and transition 34 (T34) is equal to (1.4,1.5,1.6), respectively. Binding of shBCL11A with KLF-1 mRNA and BCL11A in the proposed model leads to γ-globin gene expression induction, which is in line with the wet lab results in Gnanapragasam et al. (2011).

Simulation results by adding fuzzy numbers manually at 100 pt for KLF-1 gene expression in an untreated cell and a cell treated with shMyb501, shBCL11A, and siCHD4 is illustrated in Figure 10.

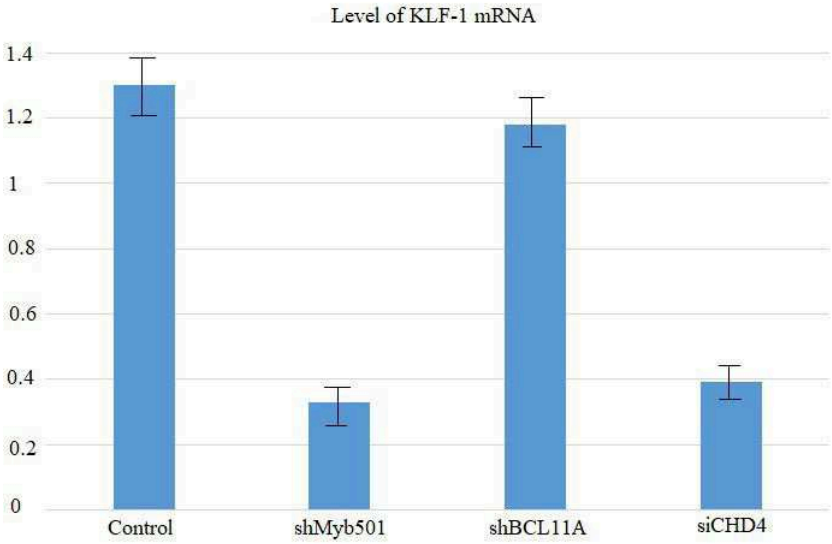

Figure 10: KLF-1 mRNA level simulation results in untreated cell versus treated cell with shMyb501, shBCL11A, and siCHD4

Simulation results by adding fuzzy numbers manually at 100 pt for MBD2 gene expression in an untreated cell and cell treated with siMBD2 is illustrated in Figure 11.

Finally, the simulation results by adding fuzzy numbers manually at 100 pt for BCL11A gene expression in an untreated cell and a cell treated with shMyb501, shBCL11A, and siCHD4 is illustrated in Figure 12.

3.2 Analysis of Simulation Results

3.2.1 Results for the Drug-based Model

It was shown that FSHPN can be used as a mathematical tool to model hemoglobin switching network and the effect of drug-based treatments on target entities. Moreover, it is possible to

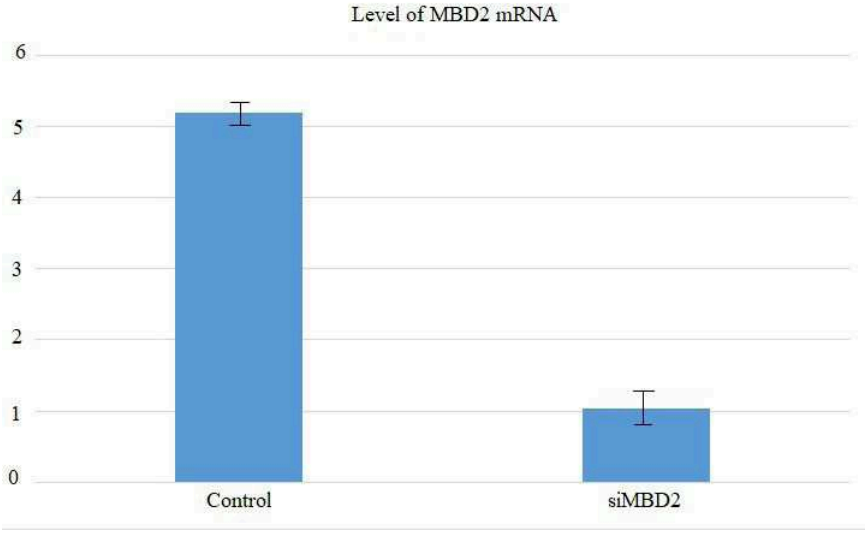

Figure 11: MBD2 mRNA level simulation results in untreated cell versus treated cell with siMBD2

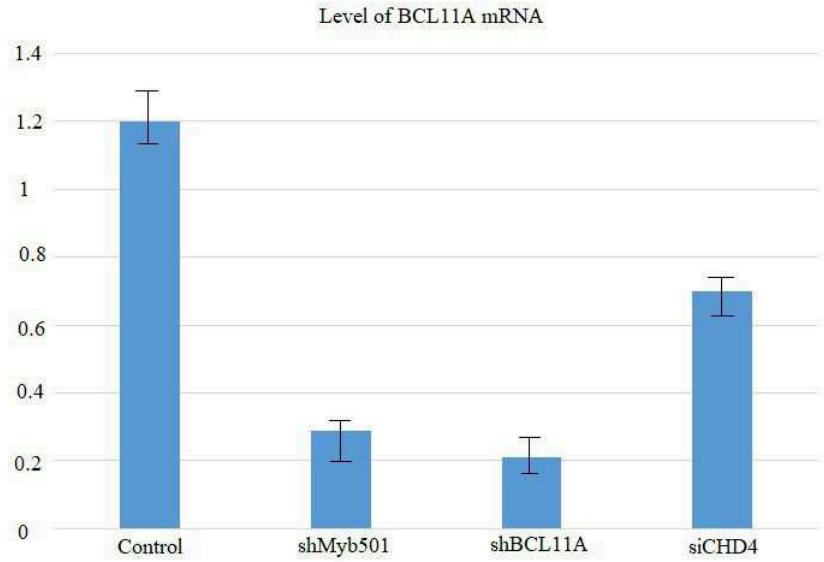

Figure 12: BCL11A mRNA level simulation results in untreated cell versus treated cell with shMyb501, shBCL11A, and siCHD4

analyze simulation results and compare the current treatments with each other. For three points of each fuzzy number (The number with membership 1, and boundary points), 37500 number of runs was applied and the mean point at 100 pt was measured for γ-globin mRNA level. The simulation results show that γ-globin gene expression was increased using combination of Simvastatin and tBHQ, MS-275, ST-20, and ACY-957 drug treatments by the number of folds of (2.5, 3.2, 3.4), (3.8, 4.2, 4.4), (2.4, 3, 3.2), (4, 4.4, 4.6), respectively. The summary of the simulation results is illustrated in Figure 13.

The simulation results in Figure 13 shows that the treatments with MS-275 and ACY-957 lead to more induction of γ-globin gene expression comparing to treatments with combination of Simvastatin and tBHQ, and treatment with ST-20. The simulation results of γ-globin mRNA level indicate that there is no significant increase of γ-globin mRNA level in the combination of Simvastatin and tBHQ comparing with ST-20 treatment. Moreover, these simulation results show that there is meaningful increase in γ-globin gene expression induction in treatment with

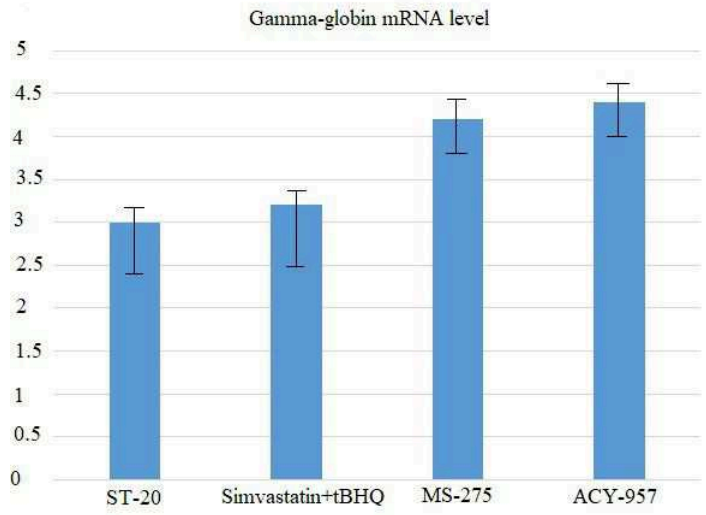

Figure 13: γ-globin gene expression levels treated with drug-based therapies using FSHFPN model

MS-275 comparing with ACY-957 treatment.

3.2.2 Results of In-Silico Simulations for the Gene-therapy Model

In the previous subsection, it was shown that FSHFPN model can be used as a mathematical tool to describe and analyze hemoglobin switching network to compare known drug-based treatments with each other. In this subsection, known gene therapies of beta globin gene disorders are compared with each other by considering the level of γ-globin gene expression. For three points of each triangle fuzzy number (The number with membership 1, and boundary points), 37500 number of runs was applied and the average mean at 100 pt was measured for γ-globin mRNA level. The simulation results show that γ-globin gene expression was increased using shMyb501, shBCL11A, siMBD2, and siCHD4 RNAi treatments by the number of folds of (2.5, 3.2, 3.4), (3.2, 3.8, 4.2), (1.6, 1.8, 2.1), (4.4, 4.8, 5.1), respectively. The summary of the simulation results is illustrated in Figure 14.

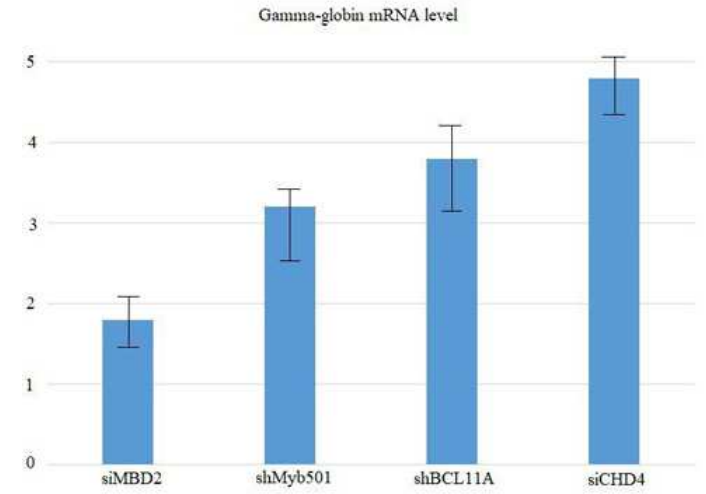

Figure 14: γ-globin gene expression levels treated with gene therapies using FSHFPN model

The simulation results in Figure 14 indicate that the treatments with CHD4 siRNA leads to more induction of γ-globin gene expression comparing to other known beta globin gene

disorders gene therapies. The simulation results of γ-globin mRNA level indicate that there is no significant increase of γ-globin mRNA level shBCL11A gene therapy comparing with shMyb501 treatment. Moreover, these simulation results show that there is meaningful increase in γ-globin gene expression induction in treatment with shMyb and shBCL11A comparing with siMBD2 treatment.

4 Conclusions

4.1 Fuzzy Stochastic Versus Deterministic Models

It is possible to model and approximately predict biological phenomena, such as developmental stage of fetal-to-adult hemoglobin switching Mehraei et al. (2016). However, environmental factors and essential noise of molecules can cause inevitable randomness in biological systems Meister et al. (2014). Randomness can significantly influence the behavior of biological systems, and it may dramatically affect the simulation results. Therefore, randomness should be considered as an important factor in modeling complex biological systems. To cover this problem, stochastic property can be added to the Petri net model Heiner et al. (2008). Moreover, most of kinetic parameters in complex biological networks are unknown or uncertain, and can't be measured or estimated accurately. Therefore, fuzzy sets can be used to address the problem of vagueness in measuring the accurate kinetic parameters Liu et al. (2016). Thus, fuzzy property can be added to the Petri net model to address this problem with unknown and uncertain kinetic parameters Liu et al. (2016).

4.1.1 Comparison for the drug-based model

As a case study, we can compare deterministic Hybrid Functional Petri nets (HFPN) Mehraei et al. (2016) with the proposed FSHPN in the previous section based on simulation results of γ-globin gene expression induction. The simulation results in deterministic HFPN showed that γ-globin mRNA level was increased with drug-based treatments by 3.1-fold with ST-20 treatment, 3.4-fold with Simvastatin and tBHQ treatment, 4.1-fold by MS-275 treatment, and 4.4-fold by ACY-957. These simulation results are illustrated in Figure 15.

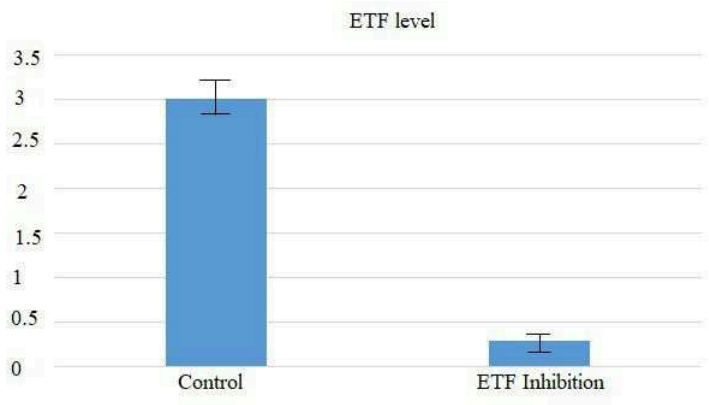

Figure 15: γ-globin gene expression levels treated with drug-based therapies using deterministic HFPN model

The simulation results of γ-globin mRNA level in Figure 15 shows that drug-based treatment with ACY-957 is the most effective one since it increases the level of γ-globin gene expression the most. However, it doesn't indicate whether the induction of γ-globin gene expression is significantly more than other drug treatments of not. The simulation results of γ-globin mRNA level in Figure 13 doesn't show significant difference between drug-based treatment of ACY-957

and MS-275. However, it shows that there is a significant difference between these drug treatments comparing with ST-20 and the combination of Simvastatin and tBHQ treatments with 95% confidence level. The confidence level can be measured based on the number of stochastic simulation run Sandmann et al. (2008). Therefore, adding fuzzy and stochastic properties to the model helps not only with the accuracy of the simulation results, but also it makes it possible to compare different treatments with each other by indicating the level of error.

It should be mentioned that deterministic models are not without advantages. Deterministic models can be used as a proper approximation of models with fuzzy and stochastic properties, and they can shed light on the way complex biological systems work with a single simulation run Mehraei et al. (2016). The simulation running time can take much longer in FSHPN comparing with HFPN models, and this makes it difficult to find the proper process rates for the model in the short amount of time. Therefore, deterministic models can be used as a primitive model to receive better understanding of a certain biological systems. Then, by using the simulation results obtained by such deterministic models, FSHPN can be created faster and more accurately to obtain trust worthy simulation results with certain confidence levels.

4.1.2 Comparison for the gene therapy model

As another case study, we can compare deterministic model of Hybrid Functional Petri nets (HFPN) proposed in Bashirov et al. (2017) with the proposed SHFPN with fuzzy parameters in the current study based on simulation results of γ-globin gene expression induction. The simulation results in deterministic HFPN showed that γ-globin mRNA level was increased with RNAi gene therapies by 1.9-fold with siMBD2 treatment, 3.4-fold with shMyb501 treatment, 4-fold by shBCL11A treatment, and 5-fold by siCHD4 treatment Bashirov et al. (2017). These simulation results are illustrated in Figure 16.

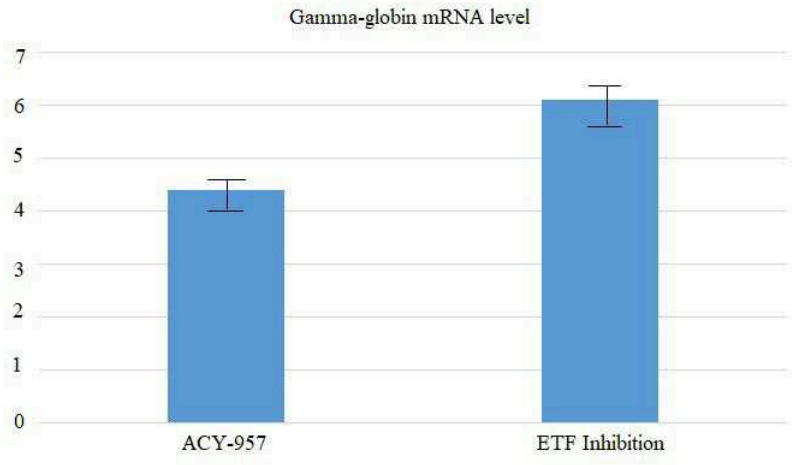

Figure 16: γ-globin gene expression levels treated with RNAi gene therapies using deterministic HFPN model

The simulation results of γ-globin gene expression in Figure 16 shows gene therapy with CHD4 siRNA is the most effective one since it increases the level of γ-globin gene expression the most. However, it does not indicate whether the induction of γ-globin gene expression is significantly more than other gene therapies or not. In Figure 14, the simulation results in SHFPN model with adding fuzzy parameters manually shows with 95% confidence that CHD4 siRNA gene therapy significantly increase the level of γ-globin mRNA comparing with other known gene therapies. The simulation results of γ-globin mRNA level in Figure 16 shows that shBCL11A treatment increases γ-globin gene expression more than shMyb501, but in Figure 15 it was shown that this difference in increasing the level of γ-globin mRNA is not significant and

it is not possible to conclude shBCL11A is a more efficient treatment for beta globin disorders comparing with shMyb501. Therefore, adding fuzzy and stochastic properties to the model helps not only with the accuracy of the simulation results, but also it makes it possible to compare different treatments with each other by indicating the level of error based on stochastic simulation runs Sandmann et al. (2008).

As mentioned in the previous subsection, deterministic models are not without advantages since they can be used as a proper approximation of models with fuzzy and stochastic properties, and they can shed light on the way complex biological systems work with a single simulation run Mehraei et al. (2016). The simulation running time can take much longer in FSHPN comparing with HFPN models, and this makes it difficult to find the proper process rates for the model in the short amount of time. Therefore, deterministic models can be used as a primitive model to receive better understanding of a certain biological systems. Then, by using the simulation results obtained by such deterministic models, FSHPN can be created faster and more accurately to obtain trust worthy simulation results with certain confidence levels.

4.2 Prediction of Potential Targets

4.2.1 Prediction of Novel Targets for Drug Therapy

It was shown that FSHPN can be used not only to model fetal-to-adult hemoglobin switching network, but also compare known drug-based treatments of β-thalassemia in term of γ-globin gene expression induction. In this section, a novel strategy will be introduced by predicting level of γ-globin mRNA by manipulating the inhibition rates of different mRNAs, proteins, and multi-proteins. The simulation results of the proposed FSHPN model revealed that inhibition of erythroid transcription factors (ETF), which are KLF-1, GATA1, FOG1 leads to meaningfully more γ-globin gene expression induction comparing with known drug-based treatments and other hypothetical predictions. As it was shown in Table 2, choosing (0.10, 0.12, 0.15) fuzzy number as kinetic parameter of ETF inhibitor leads to γ-globin gene expression induction. The level of erythroid transcription factors in untreated cells and treated cells with ETF inhibition is illustrated in Figure 17. The mean of 37500 number of stochastic runs with 95% confidence level was measured at 100 pt for γ-globin mRNA level. The simulation results showed that γ-globin mRNA level was increased by the number of folds of (5.7, 6.1, 6.3). Comparing the most effective drug-based treatment of β-thalassemia (ACY-957) based on the simulation results of the proposed FSHPN model with ETF inhibition as a novel drug-based treatment showed that ETF inhibition increases the induction of γ-globin gene expression significantly much more than ACY-957 treatment with 95% confidence interval. The mean of 37500 stochastic simulation results at 100 pt is illustrated for both ACY-957 and ETF inhibition in Figure 18. These results validate the results obtained in corresponding deterministic HFPN model Mehraei et al. (2016) with p-value less than 0.05. Therefore, ETF inhibition can be a novel strategy to treat β-globin disorders since it significantly leads to the highest γ-globin gene expression induction comparing to other known treatments.

4.2.2 Identifying the Most Efficient Molecular Targets for Gene Therapy

It was shown that FSHFPN can be used not only to model fetal-to-adult hemoglobin switching network, but also compare known gene therapies of β-globin disorders such as thalassemia by considering the level of γ-globin gene expression induction. In this section, it will be shown that the proposed FSHFPN can be analyzed and a novel strategy can be introduced by predicting level of γ-globin mRNA by manipulating the inhibition rates of different mRNAs, proteins, and multi-proteins. The simulation results of the proposed FSHFPN model revealed that inhibition of FOG1, HDAC1/2, and BCL11A gene expressions using the combination of siFOG1, siHDAC1/2, and siBCL11A gene therapies leads to meaningfully more γ-globin gene expression induction comparing with known gene therapies and other hypothetical predictions. As it was shown in

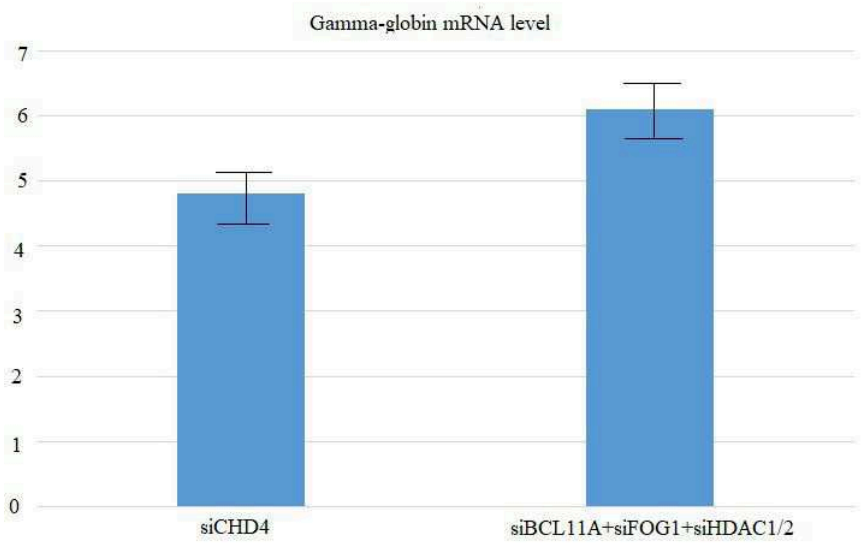

Figure 17: Erythroid Transcription Factors (ETF) level

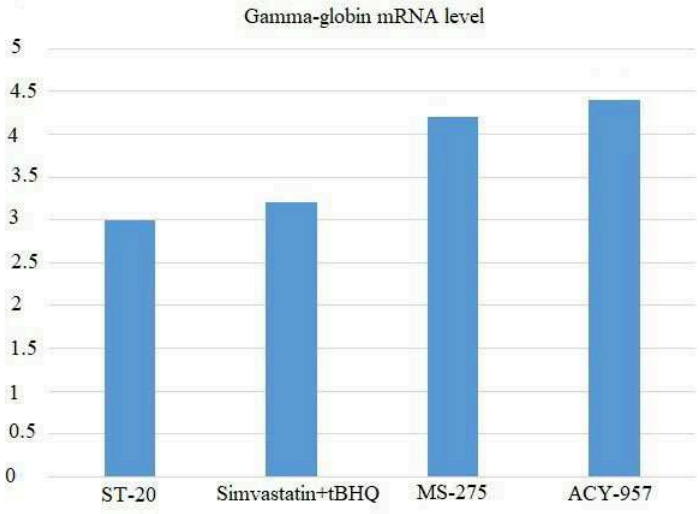

Figure 18: γ-globin gene expression levels treated with ACY-957 versus ETF inhibition

Table 2, choosing (0.01, 0.12, 0.15), (3.85, 4, 4.15), and (0.9,1,1.1) fuzzy numbers as kinetic parameters of siBCL11A, siFOG1, and siHDAC1/2 leads to γ-globin gene expression induction, respectively. The average mean of 37500 stochastic simulation runs at 100 pt with 95% confidence level shows that γ-globin gene level was increased by the number of folds of (5.7, 6.1, 6.5). Therefore, by comparing the most effective gene therapy of β-thalassemia (siCHD4) based on the simulation results of the proposed FSHFPN, it can be concluded that the proposed novel strategy significantly increases the induction of γ-globin gene expression much more than siCHD4 treatment with 95% confidence level. The mean of 37500 stochastic simulation results at 100 pt is illustrated for both siCHD4 gene therapy and the proposed novel strategy with combination of siFOG1, siHDAC1/2, and siBCL11A gene therapies in Figure 19. These results validate the results obtained in corresponding deterministic HFPN model Bashirov et al. (2017) with p-value less than 0.05. Therefore, combination of siFOG1, siHDAC1/2, and siBCL11A gene therapies can be a novel strategy to treat β-globin disorders since it significantly leads to the highest γ-globin gene expression induction comparing to other known gene therapies.

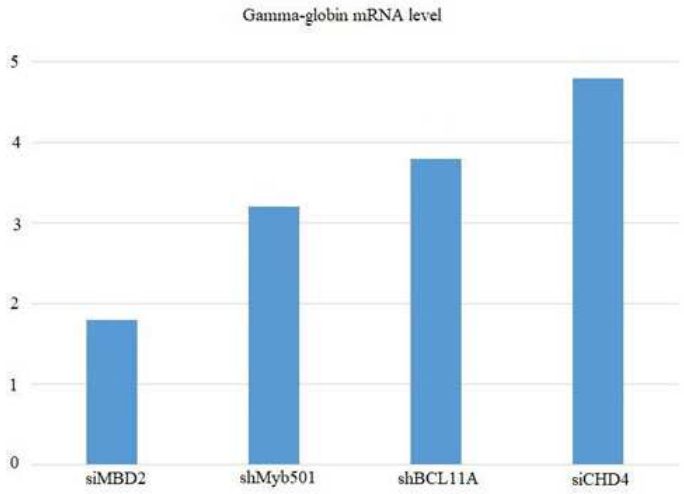

Figure 19: γ-globin gene expression levels treated with siCHD4 versus combination of siBCL11A, siFOG1, and siHDAC1/2 gene therapies

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Table 1: Places in the proposed FSHFPN

Place name	Variable	Type	Initial marking
c-Myb	P1	Continuous	0
KLF-1 mRNA	P2	Continuous	0
KLF-1	P3	Continuous	0
BCL11A mRNA	P4	Continuous	0
BCL11A	P5	Continuous	0
HDAC1/2 mRNA	P6	Continuous	0
HDAC1/2	P7	Continuous	0
MBD2 mRNA	P8	Continuous	0
MBD2	P9	Continuous	0
CHD3/4 mRNA	P10	Continuous	0
CHD3/4	P11	Continuous	0
NuRD	P12	Continuous	0
BCL11A_NuRD	P13	Continuous	0
GATA1 mRNA	P14	Continuous	0
GATA1	P15	Continuous	0
FOG1 mRNA	P16	Continuous	0
FOG1	P17	Continuous	0
SOX6 mRNA	P18	Continuous	0
SOX6	P19	Continuous	0
ETF	P20	Continuous	0
BCL11A_NuRD_ETF	P21	Continuous	0
γ-globin_BCL11A_NuRD_ETF	P22	Continuous	0
γ-globin gene	P23	Continuous	0
γ-globin mRNA	P24	Continuous	0
HbF	P25	Continuous	0
Mutation	P26	Discrete	0
β-globin mRNA	P27	Continuous	0
HbA	P28	Continuous	0
Simvastatin+tBHQ as KLF-1 mRNA suppressor	P29	Discrete	1
MS-275 as KLF-1 mRNA suppressor	P30	Discrete	1
ST-20 as KLF-1 mRNA suppressor	P31	Discrete	1
ST-20 as HDAC1/2 mRNA suppressor	P32	Discrete	1
ACY-957 as BCL11A mRNA suppressor	P33	Discrete	1
ACY-957 as SOX6 mRNA suppressor	P34	Discrete	1
ETFI (ETF inhibitor)	P35	Discrete	1

Table 2: Transitions in the proposed FSHFPN

Transition name	Variable	Rate function $f(T,K)$	Kinetic parameter K	Delay
Transcription of KLF-1 mRNA	T1	$K1 * P1$	$K1 = (0.9, 1, 1.1)$	0
Translation of KLF-1	T2	$K2 * P2$	$K2 = (0.05, 0.1, 0.15)$	0
Transcription of BCL11A mRNA	T3	$K1 * P3$	$K1$	0
Translation of BCL11A	T4	$K2 * P4$	$K2$	0
Transcription of HDAC1/2 mRNA	T5	$K1$	$K1$	0
Translation of HDAC1/2	T6	$K2 * P6$	$K2$	0
Transcription of MBD2 mRNA	T7	$K1$	$K1$	0
Translation of MBD2	T8	$K2 * P8$	$K2$	0
Transcription of CHD3/4 mRNA	T9	$K1$	$K1$	0
Translation of CHD3/4	T10	$K2 * P10$	$K2$	0
Binding of HDAC1/2,MBD2, and CHD3/4	T11	$K2 * P7 * P9 * P11$	$K2$	0
Binding of NuRD with BCL11A	T12	$K2 * P5 * P12$	$K2$	0
Transcription of GATA1 mRNA	T13	$K1$	$K1$	0
Translation of GATA1	T14	$K2 * P14$	$K2$	0
Transcription of FOG1 mRNA	T15	$K1$	$K1$	0
Translation of FOG1	T16	$K2 * P16$	$K2$	0
Transcription of SOX6 mRNA	T17	$K1$	$K1$	0
Translation of SOX6	T18	$K2 * P18$	$K2$	0
Binding of GATA1,FOG1, and SOX6	T19	$K2 * P15 * P17 * P19$	$K2$	0
Binding of ETF with BCL11A-NuRD	T20	$K2 * P13 * P20$	$K2$	0
Binding of γ-globin gene with ETF-BCL11A-NuRD	T21	$K2 * P21 * P23$	$K2$	0
Activation of γ-globin gene	T22	$K3$	$K3 = (0.009, 0.01, 0.011)$	0
Transcription of γ-globin mRNA	T23	$K2 * P23$	$K2$	0
Translation of HbF	T24	$K2 * P24$	$K2$	0
Activation of β-globin mRNA by KLF-1	T25	$K4 * P3$	$K4 = (0.001, 0.002, 0.003)$	35
Activation of β-globin mRNA by GATA1	T26	$K4 * P15$	$K4$	35
Activation of β-globin mRNA by FOG1	T27	$K4 * P17$	$K4$	35
Translation of HbA	T28	$K2 * P27$	$K2$	0
Binding of Simvastatin-tBHQ with KLF-1 mRNA	T29	$K5 * P2$	$K5 = (0.15, 0.18, 0.20)$	0
Binding of MS-275 with KLF-1 mRNA	T30	$K6 * P2$	$K6 = (0.3, 0.4, 0.5)$	0
Binding of ST-20 with KLF-1 mRNA	T31	$K7 * P2$	$K7 = (0.35, 0.37, 0.40)$	0
Binding of ST-20 with HDAC1/2 mRNA	T32	$K1 * P6$	$K1$	0
Binding of ACY-957 with BCL11A mRNA	T33	$K8 * P4$	$K8 = (0.35, 0.38, 0.40)$	0
Binding of SOX6 with BCL11A mRNA	T34	$K9 * P18$	$K9 = (0.20, 0.21, 0.22)$	0
Binding of ETF with Its inhibitor	T35	$K10 * P20$	$K10 = (0.10, 0.12, 0.15)$	0
Activation of c-Myb	T36	$K1 * P1$	$K1$	0

Table 3: Natural degradation transitions in the proposed FSHFPN

Degradation type	Variable	Rate function $f(D,K)$	Kinetic parameter K
mRNA degradation	$D1 - D10$	$Pi * K2$	$K2$
Protein degradation	$D11 - 24$	$Pi * K3$	$K3$

Table 4: Arcs in the proposed FSHFPN

Arc	Arc type
$A1 - A59$	Input process
$A60 - A87$	Output process
$A88 - A97$	Input inhibitor

Table 5: Places in the extended FSHFPN

Place name	Variable	Place type	Initial marking
C-Myb	P1	Continuous	0
KLF-1 mRNA	P2	Continuous	0
KLF-1	P3	Continuous	0
BCL11A mRNA	P4	Continuous	0
BCL11A	P5	Continuous	0
HDAC1/2 mRNA	P6	Continuous	0
HDAC1/2	P7	Continuous	0
MBD2 mRNA	P8	Continuous	0
MBD2	P9	Continuous	0
CHD4 mRNA	P10	Continuous	0
CHD4	P11	Continuous	0
NuRD	P12	Continuous	0
BCL11A_NuRD	P13	Continuous	0
GATA mRNA	P14	Continuous	0
GATA	P15	Continuous	0
FOG1 mRNA	P16	Continuous	0
FOG1	P17	Continuous	0
SOX6 mRNA	P18	Continuous	0
SOX6	P19	Continuous	0
ETF	P20	Continuous	0
BCL11A_NuRD_ETF	P21	Continuous	0
γ-globin_BCL11A_NuRD_ETF	P22	Continuous	0
γ-globin gene	P23	Continuous	0
γ-globin mRNA	P24	Continuous	0
HbF	P25	Continuous	0
Mutation	P26	Discrete	0
β-globin mRNA	P27	Continuous	0
HbA	P28	Discrete	0
MBD2 siRNA	P29	Discrete	1
shMyb501	P30	Discrete	1
shBCL11A	P31	Discrete	1
CHD4 siRNA	P32	Discrete	1
BCL11A siRNA	P33	Discrete	1
FOG1 siRNA	P34	Discrete	1
HDAC1/2 siRNA	P35	Discrete	1

Table 6: Transitions in the extended FSHFPN

Transition name	Variable	Rate function $f(T,K)$	Kinetic parameter K	Delay
Transcription of KLF-1 mRNA	T1	$K1 * P1$	$K1 = (0.9, 1, 1.1)$	0
Translation of KLF-1	T2	$K2 * P2$	$K2 = (0.05, 0.1, 0.15)$	0
Transcription of BCL11A mRNA	T3	$K1 * P3$	$K1$	0
Translation of BCL11A	T4	$K2 * P4$	$K2$	0
Transcription of HDAC1/2 mRNA	T5	$K1$	$K1$	0
Translation of HDAC1/2	T6	$K2 * P6$	$K2$	0
Transcription of MBD2 mRNA	T7	$K1$	$K1$	0
Translation of MBD2	T8	$K2 * P8$	$K2$	0
Transcription of CHD3/4 mRNA	T9	$K1$	$K1$	0
Translation of CHD3/4	T10	$K2 * P10$	$K2$	0
Binding of HDAC1/2,MBD2, and CHD3/4	T11	$K2 * P7 * P9 * P11$	$K2$	0
Binding of NuRD with BCL11A	T12	$K2 * P5 * P12$	$K2$	0
Transcription of GATA1 mRNA	T13	$K1$	$K1$	0
Translation of GATA1	T14	$K2 * P14$	$K2$	0
Transcription of FOG1 mRNA	T15	$K1$	$K1$	0
Translation of FOG1	T16	$K2 * P16$	$K2$	0
Transcription of SOX6 mRNA	T17	$K1$	$K1$	0
Translation of SOX6	T18	$K2 * P18$	$K2$	0
Binding of GATA1,FOG1, and SOX6	T19	$K2 * P15 * P17 * P19$	$K2$	0
Binding of ETF with BCL11A-NuRD	T20	$K2 * P13 * P20$	$K2$	0
Binding of γ-globin gene with ETF-BCL11A-NuRD	T21	$K2 * P21 * P23$	$K2$	0
Activation of γ-globin gene	T22	$K3$	$K3 = (0.009, 0.01, 0.011)$	0
Transcription of γ-globin mRNA	T23	$K2 * P23$	$K2$	0
Translation of HbF	T24	$K2 * P24$	$K2$	0
Activation of β-globin mRNA by KLF-1	T25	$K4 * P3$	$K4 = (0.001, 0.002, 0.003)$	35
Activation of β-globin mRNA by GATA1	T26	$K4 * P15$	$K4$	35
Activation of β-globin mRNA by FOG1	T27	$K4 * P17$	$K4$	35
Translation of HbA	T28	$K2 * P27$	$K2$	0
Binding of siMBD2 with MBD2 mRNA	T29	$K5 * P8$	$K5 = (0.75, 0.76, 0.77)$	0
Binding of shMyb501 with KLF-1 mRNA	T30	$K6 * P2$	$K6 = (0.56, 0.58, 0.6)$	0
Binding of shBCL11A with KLF-1 mRNA	T31	$K7 * P2$	$K7 = (0.01, 0.02, 0.03)$	0
Binding of shBCL11A with BCL11A mRNA	T32	$K8 * P4$	$K8 = (1.4, 1.5, 1.6)$	0
Binding of siCHD4 with KLF-1 mRNA	T33	$K9 * P2$	$K9 = (0.44, 0.45, 0.48)$	0
Binding of siCHD4 with BCL11A mRNA	T34	$K10 * P4$	$K10 = (0.20, 0.21, 0.22)$	0
Binding of siBCL11A with BCL11A mRNA	T35	$K11 * P4$	$K11 = (0.10, 0.12, 0.15)$	0
Binding of siFOG1 with FOG1 mRNA	T36	$K12 * P16$	$K12 = (3.85, 4, 4.15)$	0
Binding of siHDAC1/2 with HDAC1/2 mRNA	T37	$K1 * P6$	$K1$	0
Activation of c-Myb	T38	$K1 * P1$	$K1$	0

Table 7: Natural degradation transitions in the proposed FSHFPNs

Degradation type	Variable	Rate function $f(D,K)$	Kinetic parameter K
mRNA degradation	$D1 - D10$	$Pi * K2$	$K2$
Protein degradation	$D11 - 24$	$Pi * K3$	$K3$

Table 8: Arcs in the proposed FSHFPN

Arc	Arc type
$A1 - A70$	Input process
$A71 - A100$	Output process
$A101 - A116$	Input inhibitor