Quantitative Modelling with Using Petri nets: A Case Study for the Treatment of Spinal Muscular Atrophy

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ABSTRACT

Randomness and uncertainty are deeply entangled with bioinformatics. Indeed, both concepts are inherited characteristics of biological systems that essentially affect interactions between biological components. Although there exist numerous stochastic and fuzzy methods dealing with these problems, it is not quite sure when which method can be used. In the present work, we model random timing of biomolecular events and uncertainty of biomolecular reaction rates in terms of stochastic Petri nets with fuzzy parameters. The approach is demonstrated through the case study of identification of optimal drug combinations for Spinal Muscular Atrophy. The model of the problem has been created in accordance with deterministic, pure stochastic and fuzzy stochastic approaches. Comparison of deterministic, pure stochastic and fuzzy stochastic approaches shows that all three approaches lead to significantly different results. Since fuzzy stochastic model leads to the best approximation of underlying biological network, it has been concluded that fuzzy stochastic model is the most appropriate modelling approach for the present case study.

Keywords: SMN2 expression, fuzzy stochastic Petri nets, quantitative modelling, simulation, validation.

ÖZ

Rastgelelik ve belirsizlik, biyolojik bileşenler arasındaki moleküler etkileşimlerin modellenmesinde dikkate alınması gereken biyolojik sistemlerin kalıtsal özellikleridir. Bu problemlerle ilgilenen çok sayıda stokastik ve bulanık yöntem bulunmasına rağmen, hangi yöntemin ne zaman kullanılacağı tam olarak belli değildir. Bu çalışmada, biyomoleküler olayların rasgtele zamanlamasını ve bulanık parametreli stokastik Petri ağları açısından biyomoleküler reaksiyon oranlarının belirsizliğini modelliyoruz. Yaklaşım Spinal Müsküler Atrofi için optimal ilaç kombinasyonlarının tanımlanması olgu çalışması için gösterilmiştir.Problemin modeli belirleyici, saf stokastik ve bulanık stokastik yaklaşımlara uygun olarak oluşturulmuştur. Deterministik, saf stokastik ve bulanık stokastik yaklaşımların karşılaştırılması, her üç yaklaşımın da temelde farklı sonuçlara yol açtığını göstermektedir. Bulanık stokastik model, biyolojik ağın en iyi yaklaşımına yol açtığından, mevcut olgu çalışması için bulanık stokastik modelin en uygun modelleme yaklaşımı olduğu sonucuna varılmıştır.

Anahtar Kelimeler: SMN2 gen ifadesi, bulanık stokastik modelleme, niceliksel modelleme, simülasyon, validasyon.

DEDICATION

To My Family

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
HDAC	Histone Deacetylase
mRNA	messenger Ribonucleic acid
P/T-net	Place Transition net
PTK-SMA1	Collection of tetracycline derivatives
qPCR	A quantitative polymerase chain reaction
SMA	Spinal Muscular Atrophy
SMN	Survival Motor Neuron
*SMN*1	Survival Motor Neuron 1
*SMN*2	Survival Motor Neuron 2
SMNΔ7	Dysfunctional Survival Motor Neuron
SPN	Stochastic Petri Net
TSA	Trichostatin A
VPA	Valproic Acid

Chapter 1

INTRODUCTION

Over the past two decades, there has been a growing interest in application of quantitative modelling of metabolic pathways, gene regulatory networks and signal transduction networks. Analysis of biological networks based on quantitative description of molecular interactions has proven to be an effective approach to predict the major molecular actors circumstancing biological processes. This modelling approach can particularly be used to predict potential drug candidates or their combinations. Once a drug candidate is in silico identified, then researchers perform wet lab experiments to investigate consistency of the recognized drug candidate. This stage is usually accomplished in tight collaboration with pharmacogeneticists. This is the way how potential drug candidates or their combinations are determined in line with target-based drug discovery.

A successful quantitative model is expected to be the closest approximation of the biological system, reproducing its structure and dynamic behavior to the desired level of detail. Important stages of quantitative modelling of biological systems are: (i) reproduction of the biological network on the base of rigorous study of biological databases and literature, (ii) creation of the quantitative model using techniques of computer science, (iii) validation of the model based on biological observations, (iv) in silico identification of potential drugs or drug combinations.

Randomness is an inherited feature of evolutionary biology and genetics. What we know is that Darwinian selection principles and combinatorial genetic lottery leading to fertilization rely on probabilistic laws. Intercellular mechanisms driving protein-to-protein interactions are characterized by a high degree of randomness. Molecular density, intrinsic random nature of phenomena and noise in an experiment are among factors triggering randomness in biological networks. A biological phenomena is subject to stochastic time delays due to external and internal conditions, e.g. availability of biological components, level of energy, temperature and pressure. This is why biological events occur randomly but not according to a predefined order. Research methods used for random processes include Chapman-Kolmogorov equation [1], stochastic differential equations [2], Gibson-Bruck algorithm [3], stochastic Pi-calculus [4], Gillespie algorithm [5], stochastic process algebra [6] and SPNs [7].

It is quite often that multiple wet lab experiments conducted under identical internal and external conditions lead to different observations. This is due to the inexactness of measurements and other parameters affecting biochemical reactions that are commonly summarized under the term “technical noise”. It is customary to use qualitative nature of linguistic approximation techniques to express biological as qualitative information. “Transcription of DNA sequence of a gene occurs faster than translation of its mRNA into protein” and “Cyclin D is almost disrupted by proteasome mediated “ubiquitination” are just two examples on how knowledge of biological nature is approximated by linguistic techniques. Expressed more precisely, imprecise and incomplete knowledge is often expressed by qualitative descriptions of parameters. Fuzzy logic is an effective way to model biological systems involving qualitative knowledge.

Mutations in the *SMN*1 gene result in the absence or insufficient production of SMN protein – the main cause of SMA. In Human genome, there is a copy of *SMN*1, namely *SMN*2, which could potentially be used for SMA treatment. But, unfortunately, *SMN*2 cannot fully compensate for the absence of *SMN*1. This is because *SMN*2 results in only 5-15% of the full length protein production, and 85-95% of the dysfunctional SMNΔ7 protein production. The idea of getting more SMN produced from *SMN*2 has attracted the researchers' interest. Numerous drug candidates tested for the treatment of SMA or at least decreasing its severity by increasing SMN levels by only 1.3- to 5-folds, were not enough to treat SMA.

In the present thesis, we predict the most efficient combinations of existing drug candidates/chemicals that theoretically result in maximum SMN concentrations produced from *SMN*2. We apply SPNs and fuzzy sets to create a quantitative model of SMN protein production network, then computationally validate the model to known biological observations, and finally conduct simulations to identify combinations of potential drugs that lead to the highest levels of SMN concentration. Based on computer simulations, we identify combinations of drug candidates which increase SMN levels up to 149.9-folds over untreated samples, which keeps hope for determination of prominent combination of potential drugs to be used for the treatment of SMA.

Chapter 2

MATERIALS AND METHODS

2.1 Petri nets

Petri nets were originally invented in the beginning of 1960s by Carl Adam Petri as expandable asynchronous computer architecture which does not require long wires. Several other sources claim that he developed Petri nets in August 1939 at early ages for the purpose of describing chemical processes [48]. Whichever may have been the truth, his invention was destined to live for very long life, expressed more precisely, live forever. Because of “easy to explain”, “easy to understand” and “easy to use” features, Petri nets have attracted many researchers’ attention and gained numerous applications from concurrent and asynchronous computer and flexible manufacturing systems to intriguing and biomolecular networks. Theoretical investigations expanded breadth and depth of the scope of knowledge in Petri nets. Nowadays, Petri nets have turned into a well-established theory and a popular modelling technique. Petri nets are the correlation between practical and theoretical areas. The relationship between the theoreticians and the practitioners is that the practitioners demonstrate the regularity of the model from the theoreticians, and the theorists can pass on the fact that the practitioners are close to reality.

Over the years, Petri nets have been enriched and expanded by adding various extensions and generalizations to facilitate development of appropriate models. Petri nets can be discrete to model discrete event systems, can be timed to learn dynamic

behaviour, can be coloured to represent large and cumbersome systems in a compact form, can be hierarchical to enable modular environment, can be continuous to keep track of changes in a well-grained mode, can be stochastic to cope with randomness, can be fuzzy to deal with uncertainty, etc. This is not the complete list of extensions and generalizations, but even this list makes impression about expressive power of Petri nets as a modelling tool. In what follows below, how Petri net types were used in the present research is briefly outlined.

A Petri net is a 5-tuple $R = (P, T, Pre, Post, m_0)$ such as

- $P = \{p_1, \dots, p_n\}$ is a finite and non-empty set of places;
- $T = \{t_1, \dots, t_m\}$ is a finite and non-empty set of transitions;
- $Pre: P \times T \rightarrow \mathbb{N}$ is weight function which assigns a natural number to arc from p to t if $(p, t) \in P \times T$ and 0 otherwise;
- $Post: T \times P \rightarrow \mathbb{N}$ is the weight function which assigns a natural number to arc from t to p if $(t, p) \in T \times P$ and 0 otherwise;
- $m_0: P \rightarrow \mathbb{N}$ is the initial marking.

A Petri net, which is also referred to as basic or regular Petri net or simply P/T-net, consists of nodes of two types: transitions and places. In this sense any Petri net can be represented by a bipartite graph. Arcs are between different typed nodes and run from places to transitions or vice versa, but not from place to place or from transition to transition.

Interpretation of places and transitions can change from application to application. It is typical to use input places for modelling preconditions, input data, input signals,

resources needed, conditions, input buffers, while output places for modelling post conditions, output data, resources released, output buffers. Actions are associated with transitions. A transition may simulate event, computation step, signal processor, task for job, clause in logic, processor, etc.

In Petri nets, information is represented by tokens and flow of information by relocation of the tokens. Flow of information is simulated by firing action. A transition t is said to be enabled if each input place p of t contains at least as much tokens as weight of the corresponding arc. A firing (or occurrence) of t removes tokens from its input places and adds tokens to its output places according to corresponding weight functions.

Over the years, basic Petri nets have been applied to discrete event systems, but these nets are not powerful enough to model dynamic systems with smoothly occurring sequence events. Continuous Petri nets are used instead to model structure and dynamics of systems where events occur continuously.

A continuous Petri net is a 5-tuple $R = (P, T, Pre, Post, m_0)$ such as

- $P = \{p_1, \dots, p_n\}$ is a finite and non-empty set of places;
- $T = \{t_1, \dots, t_m\}$ is a finite and non-empty set of transitions;
- $Pre : P \times T \rightarrow \mathbb{Q}^+$ is weight function which assigns a positive rational number to arc from p to t if $(p, t) \in P \times T$ and 0 otherwise;
- $Post : T \times P \rightarrow \mathbb{Q}^+$ is the weight function which assigns a positive rational number to arc from t to p if $(t, p) \in T \times P$ and 0 otherwise;
- m_0: $P \rightarrow \mathbb{N}$ is the initial marking.

In a continuous Petri net, tokens are turned into marks and we use rational numbers rather than natural numbers to represent amounts in places.

In dynamic systems time is an important parameter. Time Petri nets can be applied to monitor or screen deterministic time dependent behavior of dynamic systems.

A time Petri net is a 6-tuple (P, T, F, V, m_0, I) such as:

- (P, T, F, V, m_0) is a basic Petri net;
- $I: T \to \mathbb{Q}_0^+ \times (\mathbb{Q}_0^+ \cup \{\infty\})$ and for each $t \in T$ with $I(t) = (I_1(t), I_2(t))$ it holds that $I_1 \leq I_2$.

It is quite often that simply time Petri nets are not sufficient to describe dynamic system having random characteristics. In a stochastic Petri net time from enabling of a transition to its next occurrence is a random variable with negative exponential probability distribution function

$$F(t) = 1 - e^{-\lambda t} \text{ if } t \geq 0 \text{ and } F(t) = 0 \text{ otherwise, } \lambda > 0.$$

In the present research we use stochastic Petri nets for modelling SMN production network.

2.2 Fuzzy sets

Fuzzy logic has been originally invented by Zadeh in the beginning of 60s [8]. Since then this concept is extensively applied in scientific, engineering areas such as mathematics, artificial intelligence, sociology, robotics, mechatronics and medicine.

According to Zadeh's theory, a fuzzy set $\tilde{\zeta}$ defined on universal set X is represented by its membership function $\mu_{\tilde{\zeta}}: X \to [0,1]$. There exist several ways that one can define

fuzzy numbers. The rectangular, triangular and trapezoid types are the most frequently referred ones among them. In the current thesis we use triangular fuzzy sets, according to which crisp number c is represented by three numbers a, b and c such that $a \leq b \leq c$. a and c are respectively defined as the left and right borders of fuzzy interval and fuzzy interval $[a, c]$ itself is called the base, while b is called as the vertex of the fuzzy interval. Fuzzy number is monotonically increasing in the interval $[a, b]$ and its monotonically decreasing in interval $[b, c]$. Fuzzy number reaches its peak value at the point b. A triangular fuzzy number has the following general form:

$$\mu_{\zeta}(x) = \begin{cases} 0 & if \quad x \leq a \\ \dfrac{x-a}{b-a} & if \quad a \leq x \leq b \\ \dfrac{c-x}{c-b} & if \quad b \leq x \leq c \\ 0 & if \quad x \geq c \end{cases}$$

2.3 Software tools

Software tools used to conduct this research include Snoopy [9], Möbius [10], Stochastic Petri Net Package [12] and GreatSPN [13], while https://www.informatik.uni-hamburg.de/cgi-bin/TGI/tools/ collects links to 23 Petri net tools and software supporting SPNs. We used Snoopy software for stochastic modelling of SMN protein production networks and conduct simulations, which is available free of charge at https://www.informatik.uni-hamburg.de/TGI/PetriNets/tools/db/snoopy.html [11].

Most of Petri net software and tools cannot handle with fuzzy numbers. Once SPN model is created using one of the Petri net software such as Snoopy [9] it can be further fuzzified using Matlab or similar software.

Deterministic, stochastic and fuzzy stochastic models of SMN protein production network were compared in SPSS software, which is licensed to Eastern Mediterranean University.

Chapter 3

BIOLOGICAL CONTEXT

3.1 Spinal Muscular Atrophy

Motor neurons are neuronal cells in the central nervous system that control various downstream targets [14]. The degeneration and cell death of motor neurons may cause several diseases. Motor neuron diseases include Amyotrophic Lateral Sclerosis, Primary Lateral Sclerosis and SMA. SMA, which is on focus the in the present research, is divided into three clinical subgroups based on the disease severity. Type I SMA is the most severe type. Patients of this group have severe muscle weakness in the first months after birth. Patients cannot sit, stand or walk unaided. They usually die before the age of two years. Type II SMA patients have muscle weakness before 18 months of age. They usually cannot stand up or walk unaided. They usually die before the age of four years. Type III SMA is the mildest form. The patients are able to walk. They usually survive into adulthood [16].

SMA is caused by mutations in the *SMN*1 gene that results with the loss of the α-motor neurons of the spinal cord, which in turn leads to progressive atrophy of the limb and trunk muscles. SMA is one of the most common genetic causes of infant mortality, which affects 1 in 6,000-10,000 newborns. Let us take a look at how SMA is spread over the generations. Our bodies are made up of millions of cells. With the some expectations, nearly all cells have a structure called as the nucleus, which contains the chromosomes. SMN is involved in the cell pathway, including a well-characterized

role in the assembly of the spliceosome (a big complex molecule in nucleus) and biogenesis of ribonucleoproteins [15]. In the cells, there are usually two copies of each chromosome and there are two copies of each gene on each chromosome pair: one inherited from each parent. For instance, if both parents are carriers of SMA (one copy of *SMN*1 is faulty while another one is healthy), then their children can be normal in 25% of the cases, carrier of disease in 50% of the cases, affected /sick in 25% of the cases. Table 1 shows the chances of a child to be non-carrier, carrier and affected/sick in different families.

Table 1: Inheritance of SMA.

		P A R E N T 1		
		Non-carrier	Carrier	SMA
P A R E N T 2	Non-carrier	100% non-carrier	50% non-carrier 50% carrier	100% carrier
	Carrier	50% non-carrier 50% carrier	25% non-carrier 50% carrier 25% SMA	50% carrier 50% SMA
	SMA	100% carrier	50% carrier 50% SMA	100% SMA

Humans are the only species that contain a nearly identical copy of *SMN*1, which is the *SMN*2 gene. Unfortunately, *SMN*2 cannot fully compensate for the loss of *SMN*1 because *SMN*2 produces low levels of the full-length SMN protein and high levels of an aberrantly spliced SMNΔ7 protein. This alternative splicing event is caused by a silent ‘C’ to ‘T’ transition, which disrupts an exon splicing enhancer site, six nucleotides into SMN exon 7.

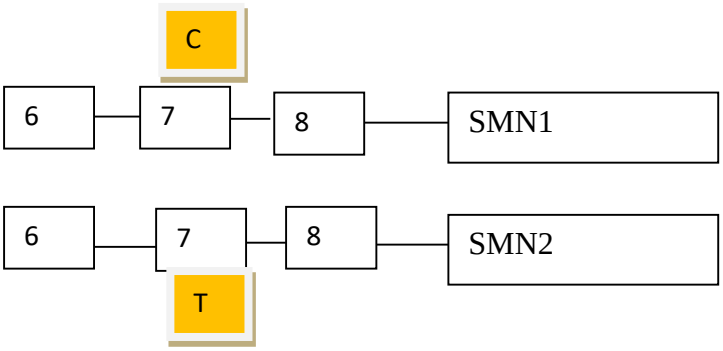

Figure 1: Difference between *SMN1*&*SMN2*.
*SMN*2 cannot fully compensate for the loss of *SMN*1 because *SMN*2 produces low levels of the full-length SMN protein and high levels of an aberrantly spliced SMNΔ7 protein. This alternative splicing event is caused by a silent 'C' to 'T' transition, which disrupts an exon splicing enhancer site, six nucleotides into SMN exon 7.

3.2 Treatment strategies

*SMN*2 is an important modifier of disease severity although *SMN*2 cannot directly compensate for the loss of *SMN*1. It is known that the number of *SMN*2 copies generally correlates with disease severity: disease severity decreases with increase of *SMN*2 copies [16]. The main idea behind the native *SMN*2 gene and its transcripts has been perceived as ideal candidates for therapeutic intervention.

There are several therapeutic approaches for the treatment of SMA or decreasing its severity. HDAC is known to inhibit *SMN2* transcription and therefore, increase of *SMN2* transcription through the inhibition of HDAC is proposed as a therapeutic method. Another therapeutic approach is based on the increase of *SMN*2 transcript via correcting alternative splicing which occurs after exon 7. Upregulating promoter activity of *SMN*2 and DNA demethylation are two more approaches to increase *SMN*2 activity. The present work combines all four approaches together to identify efficient combinations of potential drugs on the disease.

3.2.1 Inhibition of the inhibitor

One way to treat SMA is through the inhibition of HDAC activity that is known to suppress the *SMN*2 expression FDA approved drugs chemicals VPA, TSA, Dacinostat and Resveratrol which have earlier been studied in other diseases. VPA, TSA, Dacinostat and Resveratrol are the only HDAC inhibitors reported, so far, in the biological literature for which there are available qPCR and protein data on increased levels of SMN produced from *SMN*2.

Brichta *et al*. [17] observed 2- to 4-fold increase of SMN levels in fibroblast cultures derived from SMA patients treated with 0.5-–500 µM of VPA. VPA is a well-known drug that has regularly used in a long-term epilepsy treatment, and has recently been shown to yield therapeutic effects in mood disorders and migraine.

Avila *et al*. [18] observed that TSA treatment in SMA model mice results in 1.5- to 2-fold increase of SMN protein levels in the brain, liver, spinal cord and muscles 2 hours after the treatment.

Dayangaç-Erden *et al*. [19] noticed 1.3-fold increase in SMN protein levels relative to untreated cultures after treatment with 100 µM of Resveratrol. Resveratrol has been used as a drug for reducing cholesterol levels. Resveratrol is also known for its cancer preventive characteristics. Dayangaç-Erden *et al*. [19] reported about the effect of Resveratrol on the SMA patients. SMA type I cells were treated with Resveratrol to see its effect on *SMN*2 expression. Experiments showed that treatment with 100 milligrams Resveratrol increases SMN mRNA and protein levels produced from *SMN*2 by 1.2- to 1.3-fold.

Dacinostat is a new hydroxamate-based HDAC inhibitor with potential anticancer activity. With the exception on the effect of Dacinostat in Type II cells, it has been reported that Dacinostat increases *SMN*2 transcript and protein levels and promoted demethylation of the *SMN2* gene [20]. Low doses of Dacinostat are generally more potent than other hydroxamic acids. Dacinostat also shows fewer toxic effects to normal human cells. After results of viability using different concentrations of Dacinostat, it was observed that 32nM of this chemical increased SMN protein levels by 2.54 fold.

3.2.2 Regulating pre-mRNA splicing

While all the genetic information for functional SMN protein is present in the *SMN*2 gene, a translationally silent C to T change in *SMN*2 exon 7 results in exon skipping. This causes the production of a truncated, unstable SMNΔ7 protein. Hastings *et al*. [21] showed that treatment with the tetracycline derivative PTK-SMA1 in type III SMA mice promotes the inclusion of exon 7 into *SMN*2 mRNA during the splicing step, eliciting nearly 5-fold increase in SMN protein concentrations compared to untreated animals. Hastings *et al*. [21] reported that PTK-SMA1 is the only chemical identified to date that has been demonstrated to alter splicing by directly targeting the splicing reaction to promote a specific splicing pathway.

3.2.3 Upregulating promoter activity

Jarecki [22] suggested to enhance SMN transcription arising from *SMN*2 through the manipulation of the SMN2 promoter activity. It is reported in the same study that treatment with indole in patient-derived cells demonstrates direct effect on *SMN*2 promoter activity, increasing SMN transcription by 3-fold over the controls.

3.2.4 Targeting DNA methylation

Hauke *et al*. [23] demonstrated that *SMN*2 is subject to gene silencing by DNA methylation. In this sense, inhibition of *SMN*2 silencing conferred by DNA methylation represents a promising strategy for pharmacologic SMA therapy. AZA is a potential drug that positively affects SMN protein production by inhibiting methylation of *SMN*2 gene transcription factors. Hauke *et al*. [23] reported on 2-fold increase of SMN protein levels in SMA patients treated with AZA.

Chapter 4

QUANTATIVE MODELING WITH PETRI NETS

4.1 Related work

4.1.1 Stochastic Petri nets for modeling biological systems

SPN has a modeling power of standard PNs realizes fundamental results in stochastic molecular dynamics obtained by Gillespie [5]. SPNs can be used to represent structure and analyze inherited stochastic dynamics of biological systems. This is the reason why SPNs have attracted much of researchers' attention since 1990s. Many case studies of biological nature have been investigated over the last two decades. Below we review these applications.

Goss *et al*. [24] created model of plasmid *ColE*1 replication used SPNs. To the best of author's knowledge, this is the first case of application of SPNs for biological systems. Srivastava *et al*. [25] used SPNs to demonstrate that it is conceptually easy and simple to develop a model of *Escherichia coli* stress circuit, a gene regulatory network. Tsavachidou *et al*. [26] utilized stochastic activity network methodology to represent the key components of the female reproductive system. Bahi-Jaber *et al*. [28] applied colored SPNs to create and analyze complex stochastic epidemic models. Marwan et al. [27] used hierarchical structured SPNs to reconstruct the gene regulatory pathway controlling the commitment and sporulation on example of *Physarum polycephalum*. Mura *et al*. [29] developed model cell cycle in yeast using SPNs, and Napione *et al*. [30] created SPN model of signal transduction pathway for the angiogenesis process.

Lamprecht *et al*. [31] applied SPNs to develop model of Ca^{2+} release sites consisting of a number of intracellular Ca^{2+} channels that exhibit stochastic Ca^{2+} excitability, and Marwan *et al*. [27] used SPNs to investigate enteric bacteria phosphate regulation. Castaldi *et al*. [32] used SPNs to develop model of the tissue factor induced coagulation cascade, and Liu *et al*. [33] used fuzzy SPNs to develop a yeast polarization model having an infinite state space. Bashirov *et al*. [34] used SPNs to simulate, validate and analyze the p16-mediated pathway, disruption of which is among major causes of human cancers. Duranay *et al*. [35] created deterministic model of SMN production network for restricted set of SMA drug candidates and determined combination which increases up to 3.84-fold SMN protein levels.

4.1.2 Fuzziness in modeling biological systems

Because information on kinetic parameters is more vague than crisp, it is customary to represent corresponding information in the form of natural language based qualitative knowledge. Fuzzy logic is known to be a good method to cope with vagueness in biological systems. Here is a short review of biological models developed in terms of Petri nets with fuzzy sets:

Sokhansanj *et al*. [36] developed an algorithm that allows to create a model of intergenetic interactions introducing fuzzy sets. Gintrowski [37] modified this algorithm to essentially reduce the search time in gene network. In a quantitative model of a gene network suggested by Hamed [38] imperfect kinetic data is reproduced using the theory of fuzzy logic. Valette *et al*. [39] and Ding *et al*. [40] used fuzzy timed Petri nets and Tüysüz *et al*. [41] used fuzzy logic and SPNs for modeling manufacturing systems. Mehraei [42] exploited fuzzy stochastic hybrid Petri nets in modelling of mood disorder treatment. Liu *et al*. [33] combined SPNs and fuzzy logic to create a

quantitative model of biological systems in which reaction rates are fuzzy numbers. Bordon *et al*. [Bordon2018] use of fuzzy logic and Petri nets to deal with unknown or imprecise data arising in gene regulatory processes. Liu *et al*. [33] suggested a class of colored fuzzy Petri nets bringing together colored Petri nets and fuzzy sets. But so far, SPNs and fuzzy logic have not been widely used in biological systems. Perhaps, the present paper is one of the first attempts [42, 43] to represent reaction rates and concentrations of genes, mRNAs, proteins and their complexes with fuzzy numbers.

4.2 Developing and validating the model

SPN model of SMN protein production model consists of 7 discrete places (Dacinostat, TSA, Resveratrol, VPA, AZA, PTKSMA1 and Indole), 11 continuous places (HDAC_premRNA, HDAC, Methyl, TF_producer, TF, SMN2_gene, SMN2_premRNA, SMN2_mRNA, SMN2, SMNDelta7mRNA, SMNDelta7), 25 transitions (T1-T19, d1-d6), 2 read, 7 inhibitory and 32 regular arcs. Discrete places represent drug candidates, while continuous places stand for biological components whose concentration changes continuously over the time. Treatment by a drug candidate is modelled by an inhibitory arc directed from a discrete place to a transition. Treatment by a drug candidate is enabled if discrete place is empty and it is disabled otherwise. To model treatment by a combination of drug candidates we consequently keep empty related discrete places. A Boolean variable is associated to keep track of absence/presence of a drug treatment. In this model, transitions represent biological phenomena e.g., transcription, translation, binding, gene activation, methylation and degradation. Figure 2 illustrates Snoopy snapshot of the model. In this figure read arcs and inhibitor arcs are respectively represented by a black dot and hollow dot as arc head. Read arcs enable continuous expression of SMN2 gene and production of transcription factor, while inhibitory arcs simulate enabling/disabling a drug treatment.

We validate the model for TSA, VPA, Dacinostat and Resveratrol that inhibit HDAC, PTK-SMA1 which modulates pre-mRNA splicing, Indole which upregulates promoter activity and AZA which targets DNA methylation. To run application for a combination of drug candidates we initialize data by placing tokens in corresponding places. All parameters of biological components and biological phenomena used in deterministic, stochastic and fuzzy stochastic models of SMN protein production network are shown in Table 1, Table 2, Table 3 and Table 4.

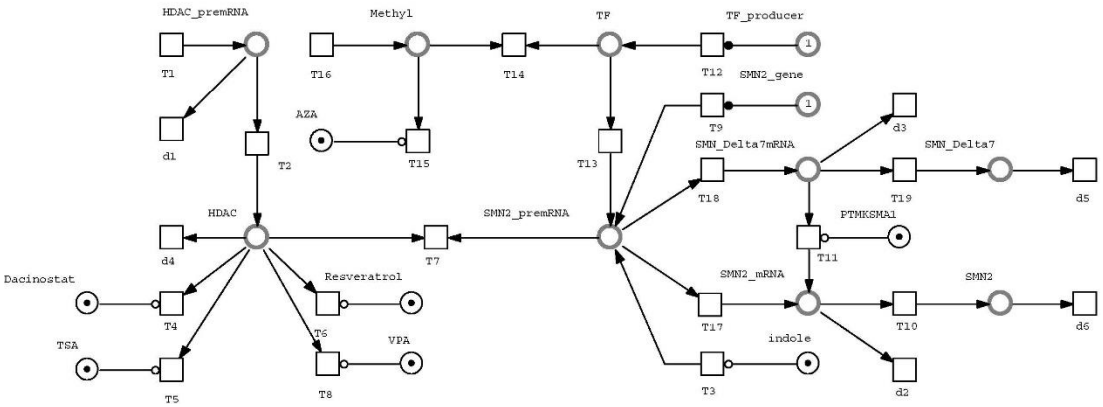

Figure 2: The complete model of SMN production network.

The model is computationally validated in accordance with knowledge derived from biological literature. We adjust rates of transitions T7, T10, T12, T13, T17, T18 and T19 so that to strike the balance between two protein types produced from SMN2, which is 85 percent SMNΔ7 and 15 percent SMN. To calibrate the model for each chemical, we turn rates of T3, T4, T5, T6, T8, T11 and T15 until reaching desired level of SMN concentration for treatment with specified drug candidate. We set the rates of transitions representing degradations to those used in [44, 45, 46, 47]. The reaction rates are calibrated in terms of stochastic replications by further averaging obtained results.

Table 2: Description of the model components.

Entity name	Entity type	Variable	Value	Initial value
VPA	Discrete	VPA	0	Boolean
Dacinostat	Discrete	Dacinostat	0	Boolean
TSA	Discrete	TSA	0	Boolean
Resveratrol	Discrete	Resveratrol	0	Boolean
PTMK-SMA1	Discrete	PTMK-SMA1	0	Boolean
AZA	Discrete	AZA	0	Boolean
Indole	Discrete	Indole	0	Boolean
HDAC	Continuous	HDAC	0	Double
HDAC mRNA	Continuous	HDAC_mRNA	0	Double
SMN2 Gene Transcription Factor Producer	Continuous Continuous	SMN2_gene T.F. producer	1 1	Double Double
SMN2 mRNA	Continuous	SMN2_mRNA	0	Double
SMN Protein	Continuous	SMN	0	Double
Methyl	Continuous	METHYL	0	Double
Transcription Factor SMNΔ7 mRNA	Continuous Continuous	T.F. delta7mRNA	0 0	Double Double
SMNΔ7 Protein	Continuous	SMNdelta7	0	Double

Table 3: Degradation transition rates.

Process name	Process type	Transition	Calculation of the rates
mRNA degradation	Continuous	d1-d3	mi*0.05
Protein degradation	Continuous	d4-d6	mi*0.01

Table 4: Biological processes in stochastic model.

Process name	Process type	Transition	Calculation of the rates
Transcription of HDAC	Stochastic	T1	2
Translation of HDAC	Stochastic	T2	HDAC_premRNA*0.1*0.147
Binding of SMN2 premRNA and Indole	Stochastic	T3	HDAC_premRNA*0.1*0.147
Binding of Dacinostat and HDAC	Stochastic	T4	HDAC*0.0108
Binding of TSA and HDAC	Stochastic	T5	HDAC*0.0058
Binding of resveratrol and HDAC	Stochastic	T6	HDAC*0.0028
Binding of HDAC and SMN2 premRNA	Stochastic	T7	HDAC*SMN2_premRNA*5
Binding of VPA and HDAC	Stochastic	T8	HDAC*0.012
Activation of *SMN2* Gene	Stochastic	T9	SMN2_gene*1
Translation of SMN Protein Binding of SMN2 premRNA and PTMK-SMA1	Stochastic Stochastic	T10 T11	SMN2mRNA*0.1 delta7mRNA*0.41
Activation of Transcription Factor	Stochastic	T12	TF_producer*1

Binding of Transcription Factor and SMN2 premRNA	Stochastic	T13	TF*1
Binding of Transcription Factor and Methyl (DNA Methylation)	Stochastic	T14	TF*METHYL*1
Binding AZA and Methyl	Stochastic	T15	methyl*0.22
Activation of Methyl	Stochastic	T16	3
Transcription of SMN2 premRNA	Stochastic	T17	SMN2_premRNA*0.15
Transcription of SMNΔ7 mRNA	Stochastic	T18	SMN2_premRNA*0.85
Translation of SMNΔ7 protein	Stochastic	T19	delta7mRNA*0.1

Table 5: Biological processes in fuzzy stochastic model.

Process name	**Transitions**	**Rate function *f(T, K)***	**Kinetic parameter K**
Transcription of HDAC	T1	K1	K1 = (1.9, 2, 2.1)
Translation of HDAC	T2	HDAC_premRNA*K2	K2 = (0.095, 0.1, 0.105)
Binding of SMN2 premRNA and Indole	T3	K3	K3 = (0.145, 0.147, 0.15)
Binding of Dacinostat and HDAC	T4	HDAC*K4	K4 = (0.0105, 0.0108, 0.011)
Binding of TSA and HDAC	T5	HDAC*K5	K5 = (0.0055, 0.0058, 0.0061)
Binding of resveratrol and HDAC	T6	HDAC*K6	K6 = (0.0025, 0.0028, 0.003)
Binding of HDAC and *SMN2* Gene	T7	HDAC*SMN2_premRNA*K7	K7 = (4.95, 5, 5.05)
Binding of VPA and HDAC	T8	HDAC*K8	K8 = (0.01, 0.012, 0.015)
Activation of *SMN2* Gene	T9	SMN2_gene*1	K9 = (0.95, 1, 1.05)
Translation of SMN Protein	T10	SMN2mRNA*K2	K2
Binding of SMN2 premRNA and PTMK-SMA1	T11	delta7mRNA*K10	K10 = (0.4, 0.41, 0.42)
Activation of Transcription Factor	T12	TF_producer*K9	K9
Binding of Transcription Factor and SMN2 premRNA	T13	TF*K9	K9
Binding of Transcription Factor and Methyl (DNA Methylation)	T14	TF*METHYL*K9	K9
Binding AZA and Methyl	T15	methyl*K11	K11 = (0.2, 0.22, 0.25)
Activation of Methyl	T16	K12	K12 = (2.95, 3, 3.05)
Transcription of SMN2 premRNA	T17	SMN2_premRNA*K13	K13 = (0.1, 0.15, 0.2)
Transcription of SMNΔ7 mRNA	T18	SMN2_premRNA*K14	K14 = (0.8, 0.85, 0.9)
Translation of SMNΔ7 protein	T19	delta7mRNA*K2	K2

Switch to the fuzzy stochastic model is done in the following way: After the model is validated, for each transition kinetic parameter in the hazard function is changed from a crisp value b to a fuzzy number(a, b, c). Then 38000 separate stochastic runs for a, b and c are performed. At the end of procedure average mean for each parameters with the confidence level of 95% and the accuracy of 10^{-2} were measured.

4.3 Simulation results and their analysis

For each of deterministic, pure stochastic and fuzzy stochastic models and for each of the seven drug candidates and their 120 possible combinations separate replications were performed and the sample mean was automatically calculated and thereby SMN concentration was measured. Let C_n be a set of all possible combinations of n drugs for $n = 2, \dots, 6$, where each $c \in C_n$ is recognised by fuzzy interval, (x_c, y_c). The following algorithm creates a set of effective combinations of n drugs, E_n.

algorithm create set of effective drug combinations
input: set of n-combinations, C_n
output: set of effective n-combinations, E_n
for n:= 2 to 6 do
set E_n = 0;
remove c with the maximum y_c from C_n and add it in E_n
set $y_{max} = y_c$ and $x_{min} = x_c$
while E_n does not contain all effective n-combinations
if $y_c > y_{max}$ then
remove c from C_n and add it in E_n
$y_{max} = y_c$
if $x_c < x_{min}$ then $x_{min} = x_c$
return E_n

This algorithm constructs the set of effective n-combinations having the property: the lower limit of any effective n-combination is greater than the upper limit of any other n-combination for all n. This algorithm allows us to separate potentially beneficial drug combinations from those that not having this property. Fuzzy intervals of any two effective n-combinations are either overlapping or one of them contains the other. For instance, this algorithm finds six 3-combinations that span the interval (16.1, 46) (see

Figure 3). Only 35 out of 120 possible n-combinations were founded to be effective. Simulation results for all n-combinations and effective ones conducted in deterministic, stochastic and fuzzy stochastic models are respectively shown in Table 5, Table 6 and Table 7.

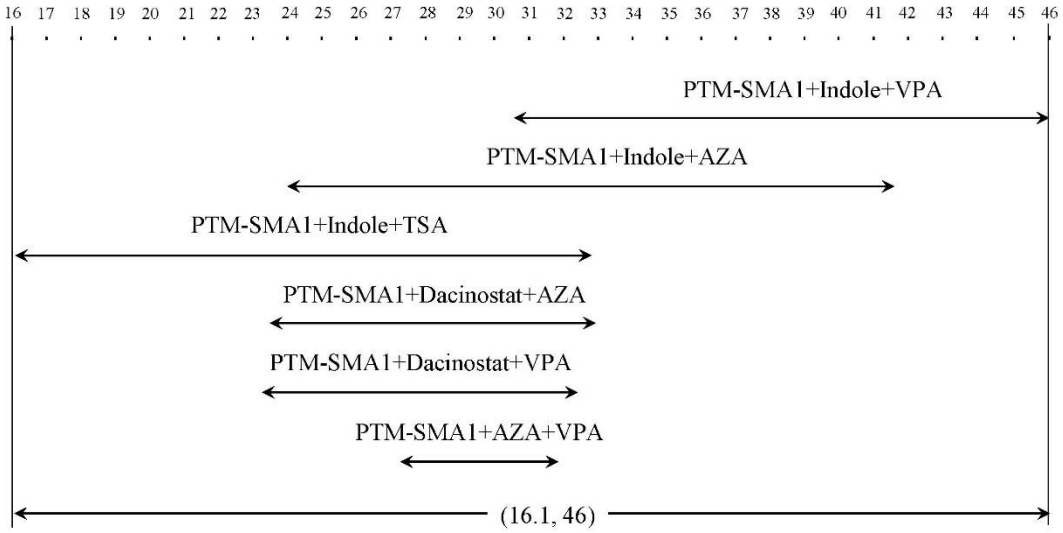

Figure 3: There are six effective 3-combinations out of 35 possible ones. Folds of SMN variation of effective 3-combinations span the interval (16.1, 46).

Table 6: Protein fold figures in deterministic and stochastic models.

Drug Name or Combination	**Protein Fold (deterministic)**	**Protein Fold (stochastic)**
Control	1	1
AZA	2	2.06
PTMK-SMA1	5	5.02
Indole	3	2.972
VPA	2.69	2.73
Dacinostat	2.542	2.589
TSA	1.702	1.785
Resveratrol	1.303	1.354
VPA+Dacinostat	4.106	4.639
VPA+AZA	5.044	5.605
VPA+Indole	7.024	7.124
VPA+PTKSMA1	13.442	14.263
VPA+TSA	3.3408	3.794
VPA+Resveratrol	2.972	3.256
Indole+AZA	11.893	7.62
Indole+PTKSMA1	15.03	15.367
Indole+Dacinostat	6.707	6.632
Indole+TSA	4.787	5.126
Indole+Resveratrol	3.8103	3.989

PTKSMA1+Dacinostat	12.716	13.278
PTKSMA1+AZA	9.926	11.007
PTKSMA1+TSA	8.509	9.071
PTKSMA1+Resveratrol	6.515	6.756
AZA+Dacinostat	4.795	5.228
AZA+TSA	3.306	3.81
AZA+Resveratrol	2.575	2.998
Dacinostat+TSA	3.198	3.514
Dacinostat+Resveratrol	2.833	3.065
TSA+Resveratrol	1.996	2.184
VPA+Dacinostat+TSA	4.738	5.668
VPA+Dacinostat+AZA	7.41	8.396
VPA+Dacinostat+Indole	9.918	10.201
VPA+Dacinostat+PTKSMA1	20.621	23.971
VPA+ Dacinostat+Resveratrol	4.391	5.146
VPA+ Resveratrol+TSA	3.614	4.264
VPA+ Resveratrol+Indole	7.619	8.065
VPA+ Resveratrol+ PTKSMA1	14.847	16.858
VPA+ Resveratrol+AZA	5.520	6.364
VPA+TSA+Indole	8.417	8.827
VPA+ TSA+ PTKSMA1	16.699	19.257
VPA+TSA+AZA	6.136	7.028
VPA+Indole+PTKSMA1	35.101	36.522
VPA+Indole+AZA	17.38	13.023
VPA+PTKSMA1+AZA	25.217	28.69
Dacinostat+TSA+ Resveratrol	3.475	4.035
Dacinostat+TSA+Indole	8.091	8.407
Dacinostat+TSA+ PTKSMA1	15.995	18.424
Dacinostat+TSA+AZA	5.902	6.799
Dacinostat+Resveratrol+Indole	7.328	7.664
Dacinostat+Resveratrol+ PTKSMA1	14.135	15.793
Dacinostat+Resveratrol+AZA	5.279	5.907
Dacinostat+ Indole+PTKSMA1	33.571	34.58
Dacinostat+Indole+AZA	16.979	6.769
Dacinostat+PTKSMA1+AZA	23.977	26.689
TSA+Resveratrol+ Indole	5.482	6.073
TSA+Resveratrol+PTKSMA1	9.974	11.397
TSA+Resveratrol+AZA	3.837	4.583
TSA+Indole+AZA	14.465	10.567
TSA+Indole+ PTKSMA1	23.936	25.822
TSA+AZA+PTKSMA1	16.532	19.614
Resveratrol+Indole+ PTKSMA1	19.015	20.683
Resveratrol+Indole+AZA	13.086	9.152
Resveratrol+PTKSMA1+AZA	12.874	14.996
Indole+PTKSMA1+AZA	59.523	39.111
VPA+Dacinostat+TSA+Resveratrol	4.998	6.131
VPA+Dacinostat+TSA+ AZA	8.373	9.78
VPA+Dacinostat+TSA+Indole	11.058	11.817
VPA+Dacinostat+TSA+PTKSMA1	23.696	29.199
VPA+Dacinostat+ Resveratrol+ AZA	7.83007	9.093
VPA+Dacinostat+ Resveratrol+ Indole	10.413	11.0831
VPA+Dacinostat+ Resveratrol+ PTKSMA1	21.945	26.455
VPA+Dacinostat+Indole+AZA	20.88	16.743
VPA+Dacinostat+Indole+PTKSMA1	49.557	52.891
VPA+Dacinostat+PTKSMA1+AZA	37.045	43.312
VPA+TSA+Resveratrol+AZA	6.586	7.778
VPA+TSA+Resveratrol+ PTKSMA1	18.071	22.011
VPA+TSA+Resveratrol+ Indole	8.922	9.658

VPA+Resveratrol+Indole+ PTKSMA1	38.095	41.023
VPA+Resveratrol+AZA+ PTKSMA1	27.611	32.733
VPA+Resveratrol+AZA+Indole	18.115	14.1
VPA+Indole+PTKSMA1+AZA	86.877	67.669
Dacinostat+TSA+Resveratrol+ PTKSMA1	17.376	21.012
Dacinostat+TSA+Resveratrol+Indole	8.646	9.358
Dacinostat+TSA+Resveratrol+AZA	6.357	7.636
Dacinostat+TSA+AZA+Indole	18.698	14.393
Dacinostat+TSA+ AZA+ PTKSMA1	29.515	35.135
Dacinostat+TSA+ PTKSMA1+Indole	40.452	43.963
Dacinostat+ Resveratrol+ AZA+Indole	17.742	13.666
Dacinostat+ Resveratrol+ AZA+ PTKSMA1	26.397	31.159
Dacinostat+ Resveratrol+ PTKSMA1+Indole	36.586	39.217
Dacinostat+ PTKSMA1+AZA+Indole	84.907	64.852
TSA+Resveratrol+ AZA+Indole	15.444	11.855
TSA+Resveratrol+ AZA+ PTKSMA1	19.184	23.894
TSA+Resveratrol+Indole+PTKSMA1	27.415	31.101
TSA+ PTKSMA1+AZA+Indole	72.328	54.683
Resveratrol+PTKSMA1+Indole+AZA	65.444	47.090
VPA-TSA-AZA-Indole	19.042	15.111
VPA-TSA-AZA-PTKSMA1	30.682	36.712
VPA-TSA-Indole-PTKSMA1	41.891	45.556
VPA+Dacinostat+TSA+Resveratrol+AZA	8.775	10.378
VPA+Dacinostat+TSA+Resveratrol+Indole	11.527	12.3
VPA+Dacinostat+TSA+Resveratrol+PTKSMA1	24.99	31.562
VPA+Dacinostat+TSA+Indole+AZA	22.214	18.28
VPA+Dacinostat+TSA+Indole+PTKSMA1	55.276	60.427
VPA+Dacinostat+TSA+PTKSMA1+AZA	41.874	50.427
VPA+ Dacinostat+Resveratrol+Indole+PTKSMA1	52.068	56.24
VPA+ Dacinostat+Resveratrol+Indole+AZA	21.468	17.618
VPA+ Dacinostat+Resveratrol+AZA+PTKSMA1	39.150	46.747
VPA+Dacinostat+Indole+PTKSMA1+AZA	104.399	86.02
VPA+TSA+Resveratrol+ Indole+PTKSMA1	44.773	49.285
VPA+TSA+Resveratrol+ PTKSMA1+AZA	33.044	40.492
VPA+TSA+Resveratrol +Indole+AZA	19.77	16.662
VPA+TSA+PTKSMA1+Indole+AZA	95.532	77.82
VPA+ Resveratrol+ PTKSMA1+Indole+AZA	90.895	72.717
Dacinostat+TSA+ Resveratrol +Indole+PTKSMA1	43.384	48.289
Dacinostat +TSA+ Resveratrol + PTKSMA1+AZA	31.901	39.057
Dacinostat +TSA+ Resveratrol + Indole+AZA	19.423	15.804
Dacinostat +TSA+ PTKSMA1+Indole+AZA	93.782	75.767
Dacinostat+ Resveratrol+ PTKSMA1+Indole+AZA	89.016	39.016
TSA+Resveratrol+ PTKSMA1+Indole+AZA	77.251	60.768
VPA+Dacinostat+TSA+Resveratrol+Indole+AZA	23.804	19.063
VPA+Dacinostat+TSA+Resveratrol+ Indole+PTKSMA1	57.601	63.841
VPA+Dacinostat+TSA+Indole+ PTKSMA1+AZA	111.086	94.647
VPA+Dacinostat+TSA+Resveratrol+ PTKSMA1+AZA	43.876	53.701
VPA+TSA+ Resveratrol+Indole+ PTKSMA1+AZA	98.842	82.862
Dacinostat+TSA+Resveratrol+Indole+ AZA+ PTKSMA1	97.15	80.664
VPA+Dacinostat+Resveratrol+ Indole+PTKSMA1+AZA	107.707	90.298
VPA+Dacinostat+TSA+Resveratrol+ Indole+PTKSMA1+AZA	113.799	98.142

Table 7: Protein fold figures in fuzzy stochastic model.

Drug candidate / combination of drug candidates	**SMN concentration (with fuzzy parameters)**	**Protein fold**
Control	(0.0398421,0.0722368,0.122658)	(1, 1)
AZA	(0.0626684,0.158737,0.352395)	(1.5,2.9)
PTMK-SMA1	**(0.282211,0.374737,0.553395)**	**(4.3,7.1)**
Indole	(0.0977895,0.226921,0.420842)	(2.4,3.4)
VPA	(0.101026,0.204684,0.382368)	(2.5,3.1)
Dacinostat	(0.104368,0.192211,0.314026)	(2.5,2.6)
TSA	(0.070437,0.135289,0.229421)	(1.8,1.9)
Resveratrol	(0.0530789,0.0996316,0.176)	(1.3,1.4)
VPA+Dacinostat	(0.183789,0.347395,0.571447)	(4.6,4.7)
VPA+AZA	(0.183763,0.414105,0.801974)	(4.6,6.5)
VPA+Indole	(0.268579,0.532079,0.881184)	(6.7,7.2)
VPA+PTKSMA1	**(0.697079,1.06079,1.60526)**	**(13.1,17.5)**
VPA+TSA	(0.143211,0.2805,0.488132)	(3.6,4)
VPA+Resveratrol	(0.118026,0.239895,0.430158)	(3,3.5)
Indole+AZA	(0.241184,0.571474,1.04516)	(6,8.5)
Indole+PTKSMA1	**(0.678974,1.14903,1.78834)**	**(14.6,17)**
Indole+Dacinostat	(0.273763,0.515605,0.783289)	(6.4,6.9)
Indole+TSA	(0.188974,0.382816,0.416184)	(3.4,4.7)
Indole+Resveratrol	(0.137684,0.297605,0.533237)	(3.5,4.3)
PTKSMA1+Dacinostat	**(0.723368,0.997421,1.32445)**	**(10.8,18.2)**
PTKSMA1+AZA	**(0.466053,0.810421,1.48471)**	**(11.7,12.1)**
PTKSMA1+TSA	(0.487263,0.680105,0.950711)	(7.7,12.2)
PTKSMA1+Resveratrol	(0.366711,0.507289,0.733447)	(6,9.2)
AZA+Dacinostat	(0.184921,0.388368,0.700895)	(4.6,5.7)
AZA+TSA	(0.123684,0.286211,0.55)	(3.1,4.5)
AZA+Resveratrol	(0.0909737,0.217816,0.451605)	(2.3,3.7)
Dacinostat+TSA	(0.144342,0.265237,0.420526)	(3.4,3.6)
Dacinostat+Resveratrol	(0.123605,0.227263,0.3695)	(3,3.1)
TSA+Resveratrol	(0.0871053,0.168289,0.280763)	(2.2,2.3)
VPA+Dacinostat+TSA	(0.236632,0.421579,0.671684)	(5.5,5.9)
VPA+Dacinostat+AZA	(0.321974,0.623921,1.04663)	(8.1,8.5)
VPA+Dacinostat+Indole	(0.437974,0.772158,1.45834)	(11,11.9)
VPA+Dacinostat+PTKSMA1	**(1.29237,1.79253,2.95082)**	**(23.3,32.4)**
VPA+ Dacinostat+Resveratrol	(0.206605,0.386,0.613237)	(5,5.2)
VPA+ Resveratrol+TSA	(0.164105,0.317474,0.539921)	(4.1,4.4)
VPA+ Resveratrol+Indole	(0.299368,0.599658,0.965263)	(7.5,7.9)
VPA+ Resveratrol+ PTKSMA1	(0.829526,1.25089,1.82858)	(14.9,20.8)
VPA+ Resveratrol+AZA	(0.216132,0.475868,0.864842)	(5.4,7)
VPA+TSA+Indole	(0.356579,0.667263,1.04105)	(8.5,8.9)
VPA+ TSA+ PTKSMA1	(1.00053,1.45629,2.06637)	(16.8,25.1)
VPA+TSA+AZA	(0.256395,0.542895,0.931816)	(6.4,7.6)
VPA+Indole+PTKSMA1	**(1.83468,2.76484,3.7435)**	**(30.5,46)**
VPA+Indole+AZA	(0.502289,0.981132,1.59061)	(12.6,13)
VPA+PTKSMA1+AZA	**(1.27189,2.15482,3.35466)**	**(27.3,31.9)**
Dacinostat+TSA+ Resveratrol	(0.168342,0.309342,0.464105)	(3.8,4.2)
Dacinostat+TSA+Indole	(0.363026,0.632605,0.938921)	(7.6,9.1)
Dacinostat+TSA+ PTKSMA1	(1.01929,1.39082,1.77234)	(14.4,25.6)
Dacinostat+TSA+AZA	(0.254421,0.512842,0.854368)	(6.4,7)
Dacinostat+Resveratrol+Indole	(0.316474,0.576237,0.869026)	(7.1,7.9)
Dacinostat+Resveratrol+ PTKSMA1	(0.843974,1.19045,1.54808)	(12.6,21.2)
Dacinostat+Resveratrol+AZA	(0.224553,0.448605,0.772763)	(5.6,6.3)
Dacinostat+ Indole+PTKSMA1	(1.89692,2.58679,3.31529)	(27,47.6)
Dacinostat+Indole+AZA	(0.504921,0.939026,1.47224)	(12,12.7)
Dacinostat+PTKSMA1+AZA	**(1.31074,2.0721,2.88245)**	**(23.5,32.9)**
TSA+Resveratrol+ Indole	(0.232947,0.446974,0.5065)	(4.1,5.8)

TSA+Resveratrol+PTKSMA1	(0.602974,0.8435,0.85)	(6.9,15.1)
TSA+Resveratrol+AZA	(0.154579,0.353895,0.446184)	(3.6,3.9)
TSA+Indole+AZA	(0.388579,0.792579,0.965)	(7.9,9.7)
TSA+Indole+ PTKSMA1	**(1.30716,1.95368,1.97)**	**(16.1,32.8)**
TSA+AZA+PTKSMA1	(0.882842,1.48968,1.55355)	(12.7,22.2)
Resveratrol+Indole+ PTKSMA1	(0.965974,1.54842,1.56)	(12.7,24.2)
Resveratrol+Indole+AZA	(0.309632,0.683184,0.824)	(6.7,7.8)
Resveratrol+PTKSMA1+AZA	(0.639868,1.12137,1.17113)	(9.2,16.1)
Indole+PTKSMA1+AZA	**(1.65682,2.93816,2.94)**	**(24,41.6)**
VPA+Dacinostat+TSA+Resveratrol	(0.2535,0.466842,0.606842)	(4.9,6.4)
VPA+Dacinostat+TSA+ AZA	(0.393658,0.733927,0.995947)	(8.1,9.9)
VPA+Dacinostat+TSA+Indole	(0.521421,0.874368,1.09182)	(8.9,13.1)
VPA+Dacinostat+TSA+PTKSMA1	(1.61763,2.19761,2.38984)	(19.5,40.5)
VPA+Dacinostat+ Resveratrol+ AZA	(0.348789,0.694774,0.932289)	(7.6,8.7)
VPA+Dacinostat+ Resveratrol+ Indole	(0.467237,0.822921,1.02476)	(8.3,11.7)
VPA+Dacinostat+ Resveratrol+ PTKSMA1	(1.44384,2.00645,2.152)	(17.5,36.2)
VPA+Dacinostat+Indole+AZA	(0.717395,1.24437,1.6505)	(13.5,19.9)
VPA+Dacinostat+Indole+PTKSMA1	(3.0425,3.94924,4.09034)	(33.3,76.4)
VPA+Dacinostat+PTKSMA1+AZA	(2.24297,3.25663,3.68353)	(30,56.3)
VPA+TSA+Resveratrol+AZA	(0.293447,0.793158,0.806711)	(6.6,7.4)
VPA+TSA+Resveratrol+ PTKSMA1	(1.13939,1.65105,1.82563)	(14.9,28.6)
VPA+TSA+Resveratrol+ Indole	(0.399079,0.721921,0.903263)	(7.4,10)
VPA+Resveratrol+Indole+ PTKSMA1	**(2.13611,3.06782,3.23418)**	**(26.4,53.6)**
VPA+Resveratrol+AZA+ PTKSMA1	(1.50487,2.44432,2.85724)	(23.3,37.8)
VPA+Resveratrol+AZA+Indole	(0.551868,1.06711,1.402)	(11.4,13.8)
VPA+Indole+PTKSMA1+AZA	**(3.48118,5.06432,5.61658)**	**(45.8,87.4)**
Dacinostat+TSA+Resveratrol+ PTKSMA1	(1.16208,1.56755,1.58)	(12.9,29.2)
Dacinostat+TSA+Resveratrol+Indole	(0.400421,0.695026,0.809816)	(6.6,10)
Dacinostat+TSA+Resveratrol+AZA	(0.298526,0.569342,0.721895)	(5.9,7.5)
Dacinostat+TSA+AZA+Indole	(0.620921,1.12289,1.37247)	(11.2,15.6)
Dacinostat+TSA+ AZA+ PTKSMA1	**(1.81934,2.63718,2.80297)**	**(22.8,45.7)**
Dacinostat+TSA+ PTKSMA1+Indole	**(2.54266,3.29211,3.34)**	**(27.2,63.8)**
Dacinostat+ Resveratrol+ AZA+Indole	(0.566053,1.03066,1.25911)	(10.3,14.2)
Dacinostat+ Resveratrol+ AZA+ PTKSMA1	(1.54476,2.31971,2.44126)	(19.9,38.8)
Dacinostat+ Resveratrol+ PTKSMA1+Indole	**(2.19368,2.94668,2.96)**	**(24.1,55.1)**
Dacinostat+ PTKSMA1+AZA+Indole	**(3.54045,4.89997,4.98655)**	**(40.6,88.9)**
TSA+Resveratrol+ AZA+Indole	(0.447763,0.882842,1.08992)	(8.9,11.2)
TSA+Resveratrol+ AZA+ PTKSMA1	(0.157211,1.79192,1.88676)	(3.9,15.4
TSA+Resveratrol+Indole+PTKSMA1	(0.226605,2.304,2.43)	(5.7,19.8)
TSA+ PTKSMA1+AZA+Indole	(0.391447,4.06421,4.12605)	(9.8,33.6)
Resveratrol+PTKSMA1+Indole+AZA	(0.310158,3.52687,3.53387)	(7.8,28.8)
VPA-TSA-AZA-Indole	(0.628132,2.76821,2.85)	(12.3,23.2)
VPA-TSA-AZA-PTKSMA1	**(1.78384,1.1515,3.18129)**	**(25.9,44.8)**
VPA-TSA-Indole-PTKSMA1	(2.48484,3.43655,3.57416)	(29.1,62.4)
VPA+Dacinostat+TSA+Resveratrol+AZA	(0.427395,0.780795,1.05824)	(8.6,10.7)
VPA+Dacinostat+TSA+Resveratrol+Indole	(0.551553,0.934421,1.15513)	(9.4,13.8)
VPA+Dacinostat+TSA+Resveratrol+PTKSMA1	(1.77745,2.383,2.57545)	(21,44.6)
VPA+Dacinostat+TSA+Indole+AZA	(0.822842,1.38026,1.80203)	(14.7,20.6)
VPA+Dacinostat+TSA+Indole+PTKSMA1	(3.62592,4.52205,4.7)	(38.3,91)
VPA+Dacinostat+TSA+PTKSMA1+AZA	**(2.72432,3.77921,4.67139)**	**(38.1,68.4)**
VPA+ Dacinostat+Resveratrol+Indole+ PTKSMA1	**(3.29897,4.20755,4.36826)**	**(35.6,82.8)**
VPA+ Dacinostat+Resveratrol+Indole+AZA	(0.765895,1.32855,1.71426)	(14,19.2)
VPA+ Dacinostat+Resveratrol+AZA+PTKSMA1	**(2.46945,3.49639,3.98684)**	**(32.5,62)**

VPA+Dacinostat+Indole+PTKSMA1+AZA	**(5.0055,6.47526,7.04818)**	**(57.5,125.6)**
VPA+TSA+Resveratrol+ Indole+PTKSMA1	**(2.77284,3.73742,3.88621)**	**(31.7,69.6)**
VPA+TSA+Resveratrol+ PTKSMA1+AZA	(2.01413,3.02179,3.5025)	(28.5,50.5)
VPA+TSA+Resveratrol +Indole+AZA	(0.669895,1.20445,1.57397)	(12.8,16.8)
VPA+TSA+PTKSMA1+Indole+AZA	**(4.33379,5.87832,6.39953)**	**(52.2,108.8)**
VPA+ Resveratrol+ PTKSMA1+ Indole+AZA	(3.89,5.48205,6.01101)	(49,97.6)
Dacinostat+TSA+ Resveratrol +Indole+PTKSMA1	**(2.82255,3.61439,3.8)**	**(31,70.8)**
Dacinostat +TSA+ Resveratrol + PTKSMA1+AZA	(2.05305,2.91111,3.09866)	(25.3,51.5)
Dacinostat +TSA+ Resveratrol + Indole+AZA	(0.6875,1.17937,1.46121)	(11.9,17.3)
Dacinostat +TSA+ PTKSMA1+Indole+AZA	**(4.41121,5.70256,5.90547)**	**(48.1,110.7)**
Dacinostat+ Resveratrol+ PTKSMA1+Indole+AZA	**(3.963,5.28974,5.4975)**	**(44.8,99.5)**
TSA+Resveratrol+ PTKSMA1+Indole+AZA	**(3.15203,4.54568,4.638)**	**(37.8,79.1)**
VPA+Dacinostat+TSA+Resveratrol+Indole+ AZA	(0.854158,1.43597,1.88129)	(15.3,21.4)
VPA+Dacinostat+TSA+Resveratrol+Indole + PTKSMA1	**(3.87884,4.79786,4.96605)**	**(40.5,97.3)**
VPA+Dacinostat+TSA+Indole+ PTKSMA1+AZA	**(5.7315,7.12171,7.71276)**	**(62.9,143.8)**
VPA+Dacinostat+TSA+Resveratrol+ PTKSMA1+AZA	**(2.96466,4.01726,4.55024)**	**(37.1,74.4)**
VPA+TSA+ Resveratrol+Indole+PTKSMA1+AZA	**(4.67176,6.21579,6.80258)**	**(55.5,117.3)**
Dacinostat+TSA+Resveratrol+Indole+AZA + PTKSMA1	**(4.75916,6.07034,6.27084)**	**(51.1,119.4)**
VPA+Dacinostat+Resveratrol+ Indole+PTKSMA1+AZA	**(5.33632,6.78187,7.35992)**	**(60,133.9)**
VPA+Dacinostat+TSA+Resveratrol+Indole +PTKSMA1+AZA	**(5.97258,7.38063,8.02611)**	**(65.4,149.9)**

Table 8: Protein fold figures for effective drug combinations in fuzzy stochastic model.

Drug Name or Combination	**SMN concentration (with fuzzy parameters)**	**Protein fold**
PTMK-SMA1	(0.282211,0.374737,0.55339)	(4.3,7.1)
PTKSMA1+Dacinostat	(0.723368,0.997421,1.32445)	(10.8,18.2)
PTKSMA1+AZA	(0.466053,0.810421,1.48471)	(11.7,12.1)
Indole+PTKSMA1	(0.678974,1.14903,1.78834)	(14.6,17)
VPA+PTKSMA1	(0.697079,1.06079,1.60526)	(13.1,17.5)
TSA+Indole+ PTKSMA1	(1.30716,1.95368,1.97)	(16.1,32.8)
Indole+PTKSMA1+AZA	(1.65682,2.93816,2.94)	(24,41.6)
VPA+Dacinostat+PTKSMA1	(1.29237,1.79253,2.95082)	(23.3,32.4)
Dacinostat+PTKSMA1+AZA	(1.31074,2.0721,2.88245)	(23.5,32.9)

VPA+PTKSMA1+AZA	(1.27189,2.15482,3.35466)	(27.3,31.9)
VPA+Indole+PTKSMA1	(1.83468,2.76484,3.7435)	(30.5,46)
Dacinostat+TSA+ AZA+ PTKSMA1	(1.81934,2.63718,2.80297)	(22.8,45.7)
Dacinostat+ Resveratrol+ PTKSMA1+Indole	(2.19368,2.94668,2.96)	(24.1,55.1)
VPA-TSA-AZA-PTKSMA1	(1.78384,1.1515,3.18129)	(25.9,44.8)
VPA+Resveratrol+Indole+ PTKSMA1	(2.13611,3.06782,3.23418)	(26.4,53.6)
Dacinostat+TSA+ PTKSMA1+Indole	(2.54266,3.29211,3.34)	(27.2,63.8)
Dacinostat+ PTKSMA1+AZA+Indole	(3.54045,4.89997,4.98655)	(40.6,88.9)
VPA+Indole+PTKSMA1+AZA	(3.48118,5.06432,5.61658)	(45.8,87.4)
VPA+TSA+Resveratrol+ Indole+PTKSMA1	(2.77284,3.73742,3.88621)	(31.7,69.6)
Dacinostat+TSA+ Resveratrol +Indole+PTKSMA1	(2.82255,3.61439,3.8)	(31,70.8)
VPA+ Dacinostat+Resveratrol+AZA+PTKSMA1	(2.46945,3.49639,3.98684)	(32.5,62)
VPA+ Dacinostat+Resveratrol+Indole+PTKSMA1	(3.29897,4.20755,4.36826)	(35.6,82.8)
TSA+Resveratrol+ PTKSMA1+Indole+AZA	(3.15203,4.54568,4.638)	(37.8,79.1)
VPA+Dacinostat+TSA+PTKSMA1+AZA	(2.72432,3.77921,4.67139)	(38.1,68.4)
Dacinostat +TSA+ PTKSMA1+Indole+AZA	(4.41121,5.70256,5.90547)	(48.1,110.7)
Dacinostat+ Resveratrol+ PTKSMA1+Indole+AZA	(3.963,5.28974,5.4975)	(44.8,99.5)
VPA+TSA+PTKSMA1+Indole+AZA	(4.33379,5.87832,6.39953)	(52.2,108.8)
VPA+Dacinostat+Indole+PTKSMA1+AZA	(5.0055,6.47526,7.04818)	(57.5,125.6)
VPA+Dacinostat+TSA+Resveratrol+Indole+ PTKSMA1	(3.87884,4.79786,4.96605)	(40.5,97.3)
VPA+Dacinostat+TSA+Resveratrol+PTKSMA1+AZA	(2.96466,4.01726,4.55024)	(37.1,74.4)
VPA+TSA+ Resveratrol+Indole+PTKSMA1+AZA	(4.67176,6.21579,6.80258)	(55.5,117.3)
Dacinostat+TSA+Resveratrol+Indole+AZA+ PTKSMA1	(4.75916,6.07034,6.27084)	(51.1,119.4)
VPA+Dacinostat+Resveratrol+ Indole+PTKSMA1+AZA	(5.33632,6.78187,7.35992)	(60,133.9)
VPA+Dacinostat+TSA+Indole+PTKSMA1+ AZA	(5.7315,7.12171,7.71276)	(62.9,143.8)
VPA+Dacinostat+TSA+Resveratrol+Indole+ PTKSMA1+AZA	(5.97258,7.38063,8.02611)	(65.4,149.9)

Comparison of simulation results showed that combination of seven drug candidates results in 149.9-fold increase over the control, which is the maximum increase of SMN concentration produced from *SMN*2. All seven chemicals examined in this research are compatible. However, they can cause serious side effects when used in combination. If this is the case then next combination of drug candidates can be examined for the same purpose. This is why no specific drug combinations were pointed at, but instead, a wide range of effective drug combinations were proposed. If

any combination causes unavoidable side effect there are still many other effective drug combinations that can be examined by pharmacogeneticsists.

Another interesting fact is that increase in number of drugs does not directly correlate with increase of SMN levels. As an example, it was that the most effective 5-combination PTK-SMA1&Dacinostat&Indole&VPA&AZA yields from 57.5- to 125.6-fold of SMN levels which is more than in case of many 6-combinations. The most effective 4-combination PTK-SMA1&Dacinostat&AZA&Indole is just another example which leads to from 40.6- to 88.9-fold increase of SMN levels. This is more than in case of five effective 5-combinations.

It was also observed that PTK-SMA1 is the most promising among seven chemicals as PTK-SMA1 is present in all 35 effective combinations. Indole is present in 23, AZA in 21, Dacinostat in 20, VPA in 19, TSA in 16 and Resveratrol in 14 effective combinations.

4.4 Comparison of modelling frameworks

To determine the most appropriate modelling framework it is necessary to find a way to compare simulation results obtained in deterministic, pure stochastic and fuzzy stochastic environments. Generally speaking, it is hard to select or determine the most suitable modelling environment based on simulation results. This is partially because simulation data may be not distributed according to a specific distribution rules. In this research, statistical methods were aapplied to compare samples obtained in three modelling environments.

Figures 4-8 graphically compare the simulation results for deterministic, pure stochastic, and fuzzy stochastic models. The solid curves superimposing on the data

for the fuzzy stochastic model were obtained by fitting interval (x, y) in the linear regime.

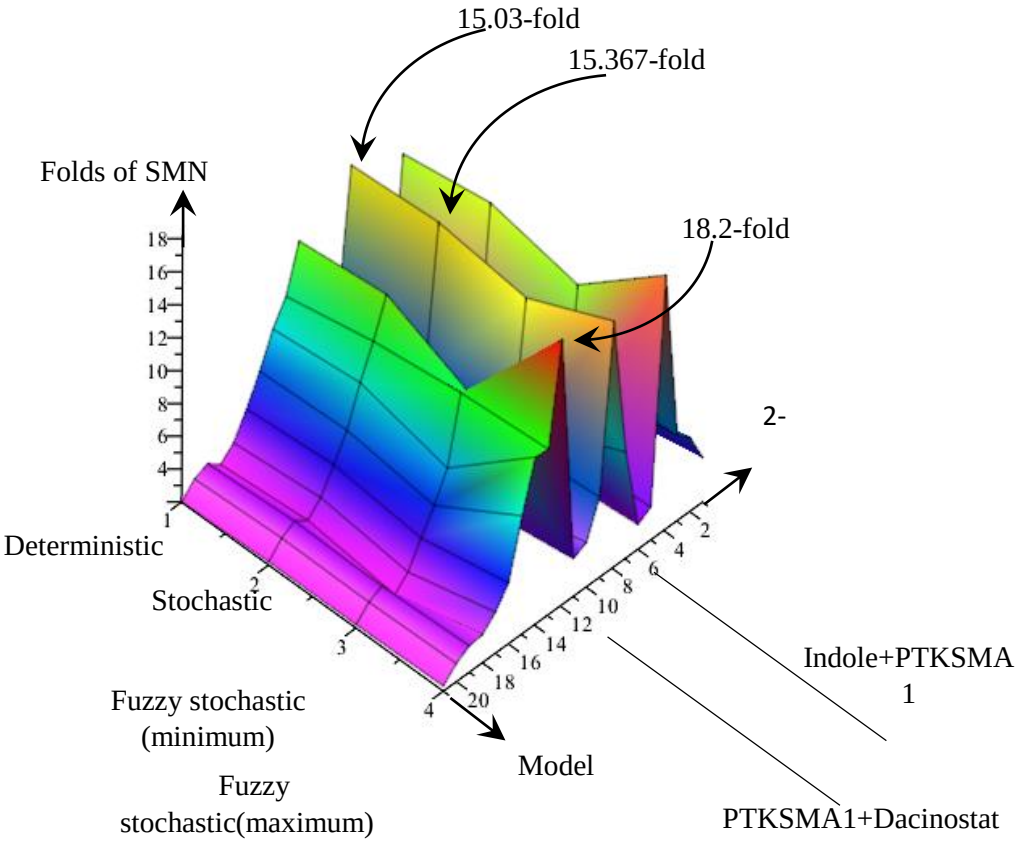

Figure 4: Distribution of the most effective 2-combinations of drug candidates in all three modelling environments.

For all n-combinations, both deterministic and stochastic simulations result in the same most effective drug combination while fuzzy stochastic case in general demonstrates different behavior. For instance, Indole&PTK-SMA1 is the most efficient 2-combination in deterministic and stochastic models, respectively resulting in 15.03- and 15.367-fold increase of SMN levels. But it turns out that PTK-SMA1&Dacinostat is the most efficient in fuzzy stochastic case, leading to 18.2-fold increase of SMN concentration (see Figure 4).

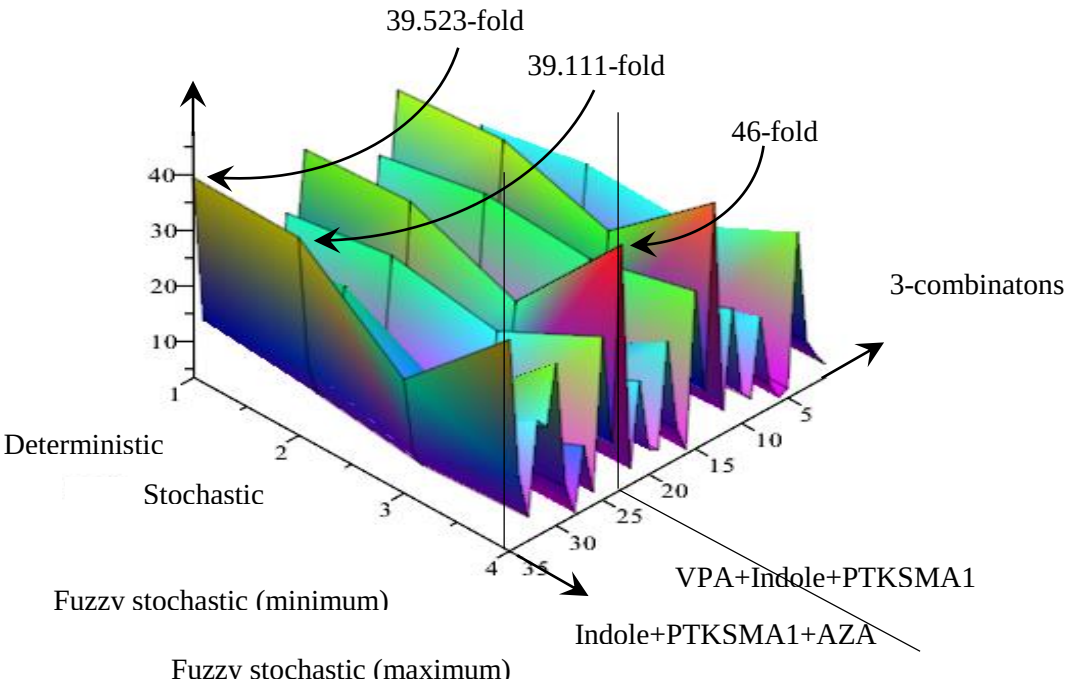

Figure 5: Distribution of the most effective 3-combinations of drug candidates in all modelling environments.

Similarly, deterministic and stochastic models agree that Indole&PTK-SMA1&AZA is the most efficient 3-combination, respectively resulting in 39,523- and 39.111-fold increase of SMN levels, with 46-fold over the control group VPA&Indole&PTK-SMA1 is the most efficient in fuzzy stochastic case (see Figure 5).

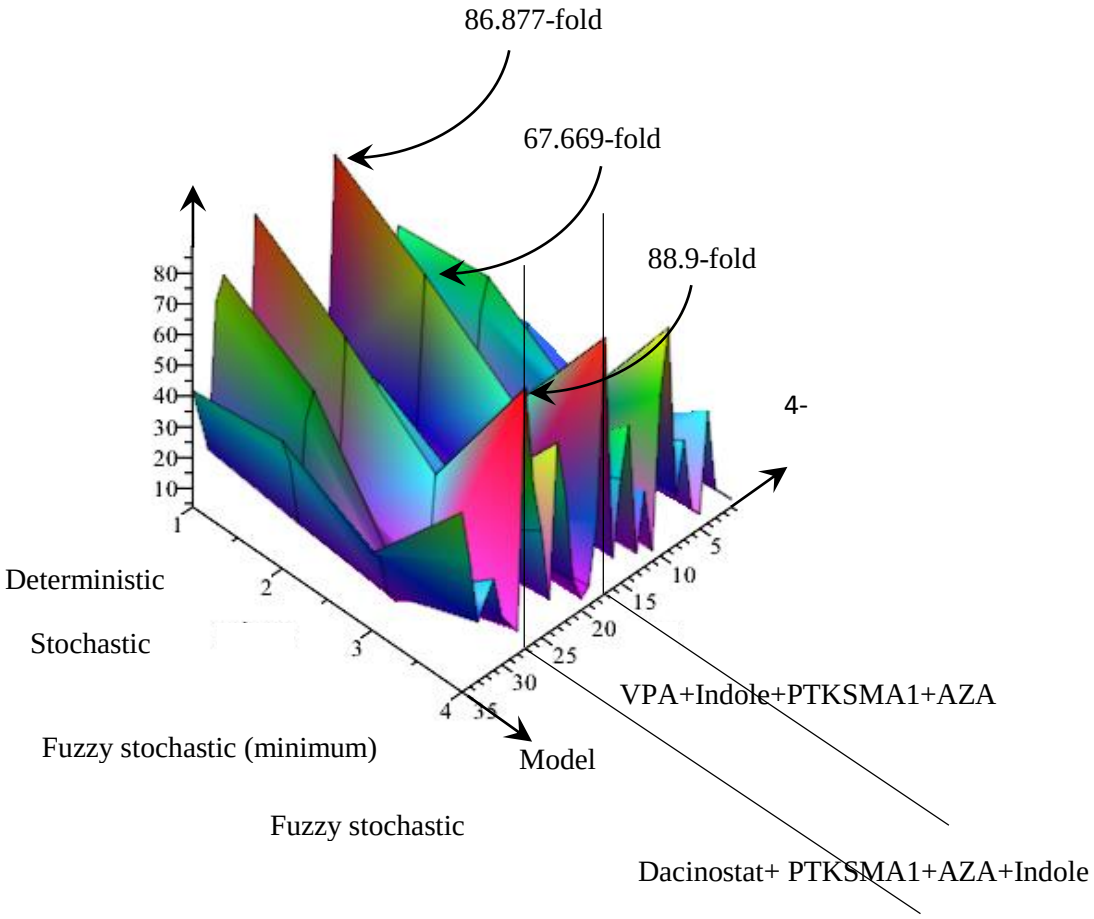

Figure 6: Distribution of the most effective 4-combinations of drug combinations in all modelling environments.

Statistical Package for Social Sciences determine if the deterministic, stochastic and fuzzy stochastic models agree or differ. For all three models, normality tests for all data sets were performed. It was found that none of the data sets were normally distributed. Then nonparametric statistical tests were conducted to pairwise compare data sets. These tests are based on the following hypotheses:

$$H_o: Median\ (x) = Median(y)$$

$$H_1: Median\ (x) \neq Median\ (y)$$

where x and y are variables created for deterministic, pure stochastic and fuzzy stochastic data sets such that $x \neq y$.

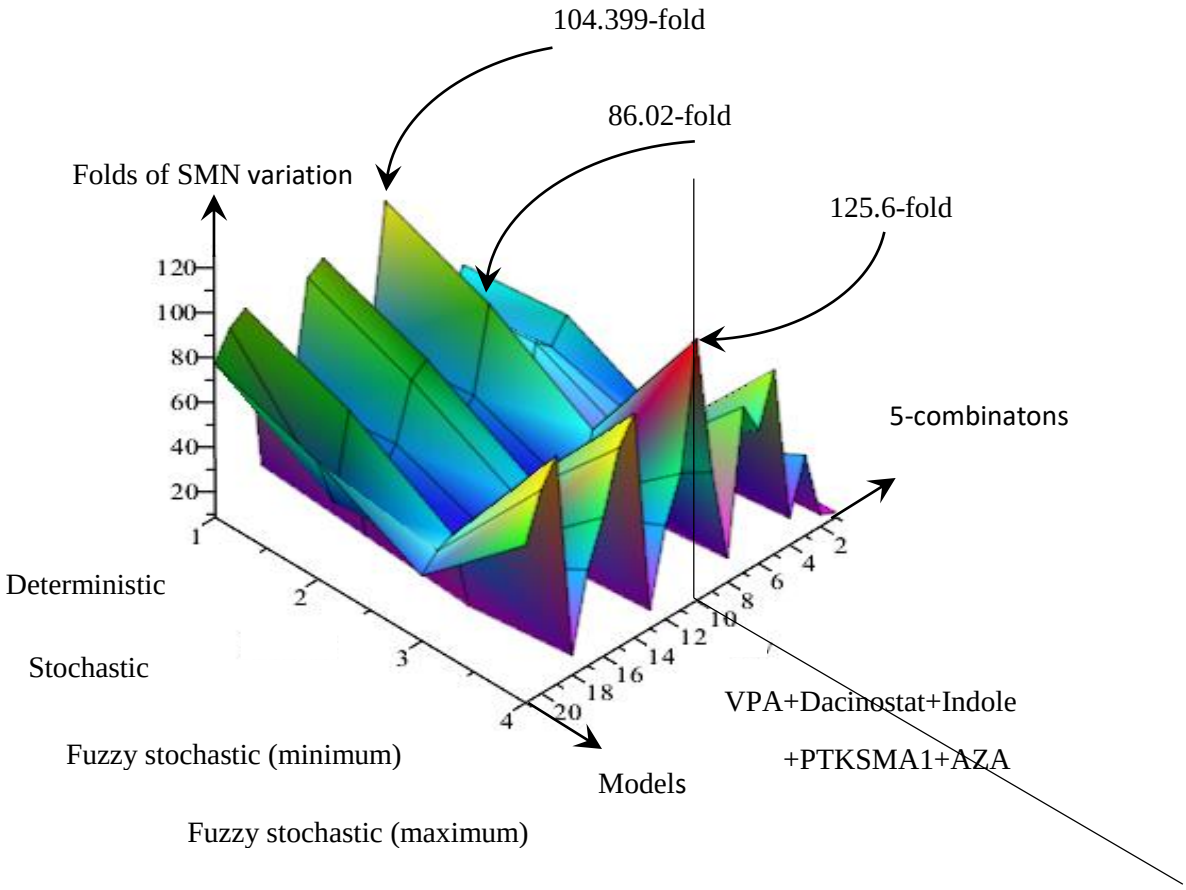

Figure 7: Distribution of the most effective 5-combinations of drug candidates in all modelling environments.

Friedman test was applied to compare deterministic, stochastic and fuzzy data sets which resulted with the rejection of the null hypotheses, H_0, with a $p < 0.001$. This indicates that medians of deterministic, pure stochastic and fuzzy stochastic data sets differ essentially. This is a bit surprising result, since simulation results for deterministic and pure stochastic models agree on the same the most effective combinations of drug candidates.

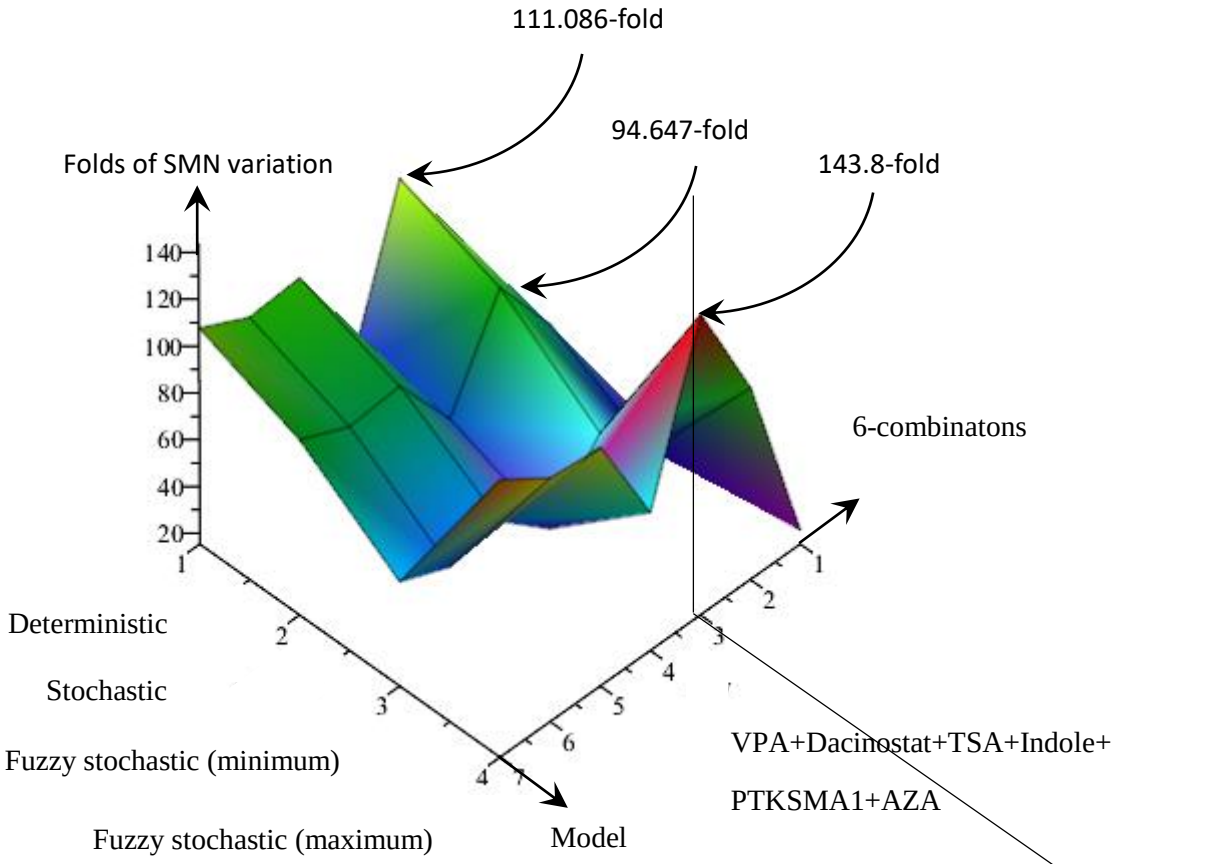

Figure 8: Distribution of the most effective 6-combinations of drug candidates in all modelling environments.

Wilcoxon Signed Rank Test was also conducted as a further post-hoc analysis, a paired difference test, which compares two related data sets on a single sample to assess whether data sets have the same distribution. Pairwise comparisons of data sets yielded a $p - value < 0.001$, which results the same outcome as Friedman test. Based on the results of statistical tests it was concluded that deterministic, stochastic and fuzzy stochastic models lead to substantially different results. Moreover, statistical analysis reveals that values in stochastic case are significantly higher compared to deterministic one, and that values in fuzzy stochastic case are essentially higher than in stochastic model. All these observations lead to the conclusion that fuzzy stochastic model is the

most adequate model for the case study as this model creates the closest approximation of underlying biological network.

Chapter 4

CONCLUSION

In the present thesis, based on rigorous mathematical foundations, the closest approximation of SMN protein production network was created and computer simulations were conducted to determine theoretically most effective combinations of known drug candidates. As a result of computer simulations, effective combinations of drug candidates that lead up to 149.9-fold increase of SMN protein produced from *SMN*2 gene were identified, though this figure for known drug candidates does not exceed 5-fold, thereby holding promise for beneficial effects on SMA patients.

Three modelling approaches for appropriateness to the present case study: deterministic, pure stochastic and fuzzy stochastic were compared. Statistical comparison of the data sets obtained for three approaches reveals that deterministic, pure stochastic and fuzzy stochastic modelling approaches lead in substantially different results which, together with the fact that fuzzy stochastic model successfully copes not only with randomness but also uncertainty, suggests that fuzzy stochastic model is the most appropriate choice for the present case study. The proposed approach can be easily adapted or extended to other biological networks.

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