Stochastic Simulation of the Coagulation Cascade: A Petri Net Based Approach

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Abstract. In this paper we developed a Stochastic Petri Net (SPN) based model to introduce uncertainty to capture the variability of biological systems. The coagulation cascade, one of the most complex biochemical networks, has been widely analyzed in literature mostly with ordinary differential equations, outlining the general behavior but without pointing out the intrinsic variability of the system. Moreover, the computer simulation allows the assessment of the reactions over a broad range of conditions and provides a useful tool for the development and management of several observational studies, potentially customizable for each patient. We describe the SPN model for the Tissue Factor induced coagulation cascade, more intuitive and suitable than models hitherto appeared in the literature in terms of bioclinical manageability. The SPN has been simulated using Tau-Leaping Stochastic Simulation Algorithm, and in order to simulate a large number of models, to test different scenarios, we perform them using High Performance Computing. We analyze different settings for model representing the cases of "healthy" and "unhealthy" subjects, comparing their average behavior, their inter- and intra-variability in order to gain valuable biological insights.

Keywords: Systems Biology, Coagulation, Stochastic Simulation, Petri Nets.

1 Introduction

Petri Nets are a modeling tool widely used to represent biological systems, thanks to their intuitive graphical representation which helps to model and understand even systems with a complex structure. The graphical aspects of the Petri net are quite similar to biochemical network representation, and this gives superior communication ability to models and facilitates their design.

Moreover, modelling biological systems requires to take into account uncertainty in system behaviors, due to external interferences (drugs, environment, etc), inter-variability (personal features) and intra-variability (intrinsic noise and low number of

I. Caragiannis et al. (Eds.): Euro-Par 2012 Workshops, LNCS 7640, pp. 248–262, 2013.

molecules) of complex systems. Using the Petri Nets extension, Stochastic Petri Nets (SPNs), we can describe these random aspects (Goss and Peccoud, 1998; Srivastava, Peterson and Bentley, 2001).

A biological pathway which has recently attracted strong interest for its potential to augment bioclinical knowledge in several therapeutic domains, is the coagulation cascade (Chatterjee et al., 2010; Wajima, Isbister and Duffull, 2009). This model has been deeply investigated, resulting in complex networks where two sub-pathways, *Intrinsic* and *Extrinsic* interact in a *Common* pathway to produce active fibrin which affects the haemostasis process (Butenas and Mann, 2002). Both pathways are required for normal hemostasis and there are different feedback loops between the two pathways that amplify reactions to produce enough fibrin to be able to crosslink each other to form a fibrin clot.

Modulation of extrinsic (Tissue Factor (TF) Pathway) and intrinsic (Contact Pathway) pathways is affected either by several positive and negative feedback mechanisms, or by a set of natural proteins that restrict coagulation progression. In healthy physiological conditions constant generation of small amounts of coagulation active factors (active proteases) effects the constitutive heamostasis, while another biological mechanism, the fibrinolytic system, counterweighs the process. All together, coagulation, anticoagulation and fibrinolysis define a delicate physiological balance, named "Haemostatic Balance" (Gaffney, Edgell and Whitton, 1999); significant deviations from this equilibrium, hypercoagulability or hypocoagulability unbalance, can evolve in cardiovascular adverse events.

Most of the existing papers model coagulation as a system of ordinary differential equations (ODEs), considering single complex, partial pathways, cell based model, comprehensive humoral coagulation network, or with a PDEs approach to model rheological behavior of molecules, blood flow. As pointed out by Danforth et al. 2009, these approaches cannot express the variability of the coagulation pathway, which is widely validated both in wet lab experiments and in vivo system (Couris et al. 2006). This aspect has been considered by a few works using different techniques, ranging from sensitivity parameter analysis (Luan et al. 2007), ODEs with stochastic coefficients (Corlan and Ross 2011), stochastic differential equation of dissipative particle dynamics method (Filipovic, Kojic and Tsuda 2008) and probability distributions evaluation of reactions for sophisticated stochastic simulations (Lo et al., 2005). However, none of these stochastic approaches allows a detailed analysis of the coagulation system whose results can be matched with existing clinical tests, in particular the "Prothrombin Time" (PT) test.

In this paper the authors developed a model of the coagulation, represented by counterbalance of coagulation and anticoagulation proteins, based on a Petri Net modeling framework (Sec.2). This model offers an intuitive graphical representation of TF pathway with a manageable modularity. The intrinsic and extrinsic pathways have been evaluated as separate entities to match *in vitro* test results, but the *in vivo* analysis requires to consider both of them during the simulation. The presented model represents a new stochastic bioclinical approach of modelization of the extrinsic pathway, which implies the action of the intrinsic way in order to have model applicable to clinical analysis.

This work is focused on comparing the behaviour of a "healthy" subject with that of an "unhealthy" subject, starting from a Stochastic Petri Net (SPN) model and changing the initial marking to represent the effect of a prothrombotic event. The models will be analyzed using simulation: first we will use a deterministic approach to tune the model parameters, converting the SPN into a continuous Petri Net (CPN) and solving the related system of ODEs (Sec.3); second, we will perform the actual analysis with stochastic simulation, employing Gillespie's Stochastic Simulation Algorithm (Sec.4).

It is clear that the stochastic model is more effective than its deterministic counterpart in unearthing valuable biological insights, but its computational demands can become prohibitive even for moderate size networks. Approximate methods, along with the use of high performance computing (HPC) are shown in Sec.5 to bring the computational complexity of the stochastic approach within manageable limits.

2 Petri Nets and Biochemical Pathways

A Petri Net is a weighted, directed and bipartite graph, originally conceived for modeling systems with interacting concurrent components and now widely used to study complex model such as biochemical pathways. This paper employs notation used in (Heiner, Gilbert and Donaldson, 2008). In terms of a chemical reaction network, coagulation involves a sequence of highly connected concurrent processes with many simultaneous positive and negative feedback loops that specify the beginning, the progression and the amplification of whole system.

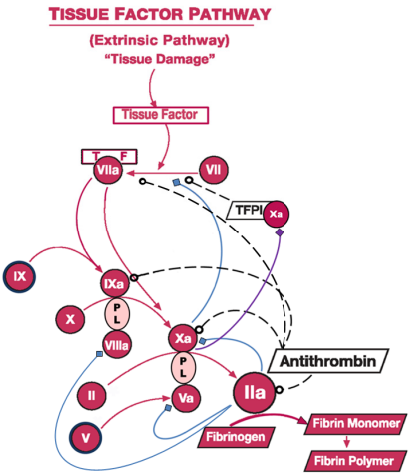
The graphical representation of the Petri net is quite similar to a biochemical network: places represent compounds (such as metabolites, enzymes, cofactors etc.) participating in a reaction, which is represented by a transition. The arcs which connect places and transitions will represent the stoichiometry of the reaction, while the tokens associated to a place will represent the amount of molecules of that particular reactant.

The most abstract representation of a biochemical network is qualitative and is minimally described by its topology by using a place-transition Petri Net (p-t PN, or qualitative Petri Nets, QPN).

Using this formalism, we have been able to build up a model of the coagulation pathway. In particular, the relevant role played in coagulation process by the Tissue Factor (Chu, 2011), also in cardiovascular diseases (Steffel, 2006; Mackman, 2004), has supported the idea of modeling the extrinsic pathway in details. We accurately reproduce the pathway, including all the positive and negative feedback circuits, as well as the exact number of molecules involved in the process.

The starting point in building the model is to define the initial marking and reaction rate constants, which we obtained from biological databases as Brenda (http://www.brenda-enzymes.org), model repositories as BioModel Database (http:// www.ebi.ac.uk/biomodels-main), integrated with other data found in PubMed (http://www.ncbi.nlm.nih.gov/pubmed). The initial marking represents in our model

the average value of the observed physiological range The reaction rates have been tuned with a trial and error approach (based on enzyme kinetic assumptions) to replicate the thrombin generation time given by a bioclinical PT test (Khanin et al., 1999), and reproduce a titration curve of the thrombin formation in a biochemical test (Butenas, van't Veer and Mann,1999). Another approach to approximate the reaction constants is given in (Shaw, Steggles and Wipat, 2006). In the initial marking (M_0), only 11 places have a non-zero number of tokens, which ranges from 75 to $3{,}01 \cdot 10^{8}$ molecules (the number has been deduced from the values reported in Fig.1), with a considered plasma volume of $1 \cdot 10^{-10}$ liters. The whole set of places, transitions and parameters (initial marking and rate constants) are given as supplementary material on the authors' website (www.nedd.unimib.it - Downloads - Supplementary Materials).



Procoagulant Proteins

Species	Molec. Weight (kDa)	Plasma Level (ug/ml)
Fibrinogen (I)	340	3000
Prothrombin (II)	72	90
Factor V	330	10
Factor VII	50	0.5
Factor VIII	295	0.1
Factor IX	57	5
Factor X	59	8
Tissue Factor	45	0.001
VIIa-TF Complex	95	0.0005

Anticoagulant Proteins

Species	Molec. Weight (kDa)	Plasma Level (ug/ml)
TFPI	38	0.001
Antithrombin III	58	290

Fig. 1. A graph representation of Tissue Factor Pathway (modification of image from http://www.enzymeresearch.co.uk) and species physiological characteristics. The pathway can be subdivided in a procoagulant subpathway (red arcs), positive feedbacks (blue arcs), negative feedbacks (purple arcs) and inhibitor subpathways (black dashed arcs).

Fig.2 shows the Tissue Factor pathway represented as a Petri Net model and the description of all the places. We use macro-nodes to give a neat and hierarchically structured representation of our model. Fig.3 shows in detail the two types of reactions described in the hierarchical PN: complex association/dissociation (AD#) and Michaelis-Menten enzyme reaction (MM#). The hierarchical structure of the model does not change any properties of the flat model (Breitling et al., 2008). Our network is composed, in total, by 26 places and 18 macro-nodes, which can be unfolded into a network of 35 places and 43 transitions. The standard semantics for qualitative Petri nets does not associate a time with transitions or the sojourn of tokens at places, and thus these descriptions are time-free. The qualitative analysis considers however all possible behavior of the time-independent system.

Timed information can be added to the qualitative description by using an extension of PN called Stochastic Petri Nets (SPN). The SPN description preserves the discrete state description, but in addition associates a probabilistically distributed firing rate (waiting time) with each reaction. All reactions which occur in the QPN can still occur in the SPN, but the probability that a reaction will happen within a period of time depends on the associated firing rate, which is defined as a negative exponential probability density function:

$$f_{X_t} = \lambda_t(m) \cdot e^{(-\lambda_t(m)\cdot\tau)} \quad , \tau \geq 0$$

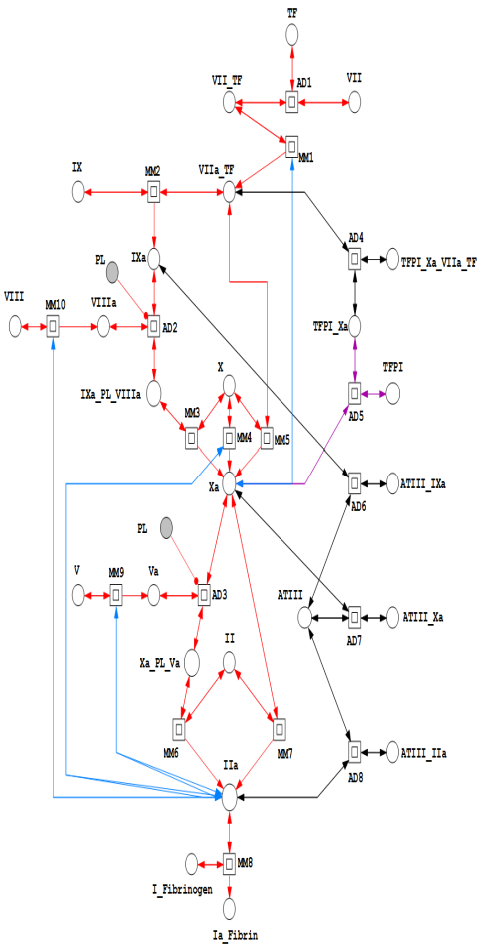
where the waiting time X_t is function of the transition rate $\lambda_t(m)$ (a *stochastic mass-action hazard function* as defined in (Heiner, Gilbert and Donaldson, 2008)) and the time τ. SPN are a specification language which enables both a deterministic solver based on ODE (through another specific called Continuous Petri Nets), and a stochastic solver based on Gillespie Stochastic Simulation Algorithm (SSA).

3 Deterministic Approach

The continuous model, represented by a Continuous Petri Net (CPN), replaces the discrete values of species with continuous values, and instantaneous firing of a transition is carried out like a continuous flow.

The semantics of a continuous Petri Net is defined by a system of ODEs, whereby one equation describes the continuous change over time of the token value in a given place, influenced by transitions which increase or decrease the token value. A Continuous PN contains all the information needed to generate the system of ODEs. Each place in CPN will determine an ODE based on its pre- and post-transitions: for example, from a simple example of Continuous PN we can generate an equation for each place by looking at the transitions directly connected to the places. The fast simulation time given by deterministic approach allows tuning the initial marking of the model in order to have a result that matches either the behavior shown in literature or in deterministic simulations. Also, this approach allows determining the average simulation time: as stochastic simulation can be time consuming, we need at least an approximation of the time span required to observe our target behaviour.

To confirm the reliability of our simulations, we compared the behavior of fibrin (Ia) and thrombin (IIa) in the mathematical model proposed by Khanin, Rakov and Kogan (1998), with the results given by the deterministic ODE solver on our model (Fig.4). The images show clearly that both products have the same trend as in Khanin's mathematical model. The deterministic approach is useful to compare the average behavior of our model to literature, but it cannot highlight other important characteristics unidentifiable in the average behavior, such as the biological systems variability. These problems will be overcome with the stochastic approach.



List of places	Description
TF	cofactor
VII	zymogen
VII_TF	complex
VIIa_TF	active complex
IX	zymogen
IXa	active enzyme
VIII	pro-cofactor
VIIIa	cofactor
PL	phospholipid
IXa_VIIIa_PL	active complex
X	zymogen
Xa	active enzyme
V	cofactor
Va	cofactor
Xa_Va_PL	active complex
II (Prothombin)	zymogen
IIa (Thrombin)	active enzyme
I (Fibrinogen)	precursor
Ia (Fibrin)	active precursor
TFPI	inhibitor
TFPI_Xa	inhibitor complex
TFPI_Xa_VIIa_TF	inhibitor complex
ATIII	inhibitor
ATIII_IXa	inhibitor complex
ATIII_Xa	inhibitor complex
ATIII_IIa	inhibitor complex

Fig. 2. High-level Petri Net model of Tissue Factor pathway, with biological description of the places

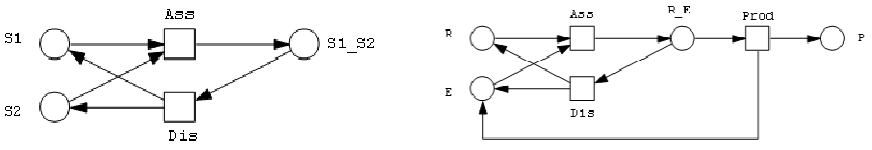


Fig. 3. Description of macro-nodes. (a) Representation of association/dissociation of a complex (AD# in high-level PN). (b) Representation of a Michaelis-Menten enzyme reaction (MM# in high-level PN).

4 Stochastic Approach

Deterministic simulation techniques assume that concentrations vary deterministically over time and that concentrations vary continuously and continually. However, these assumptions may not be valid for some important aspects of biological systems and this limitation can hamper capturing important properties of the system under study. The most important limitations of deterministic approach are the following ones:

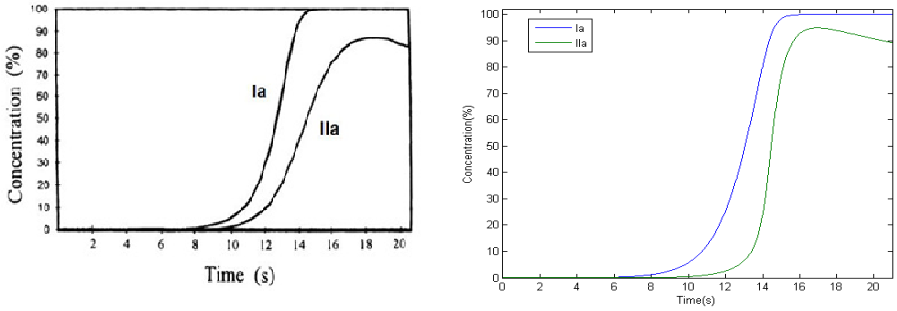
- Although efficient in terms of computational costs, it cannot accurate for systems that contain low-rate reaction, related to species occurring in small molecular quantities. If a system contains small quantities of a species, then representing this species in molecular reactions by its average values can introduce either significant bias in model predictions or can even change the model's qualitative properties.
- When studying the behavior of bi-stable or multi stable systems, deterministic modeling (whose behaviour depends only on initial conditions) may not adequately describe the distributions of system responses, limiting to average values can cause overlooking important features of the system dynamics.
- Deterministic approach ignores stochastic variation of the system under study, which is given by the inability to represent much information about the molecules (such as position, orientation and momentum). As stochasticity in systems biology has a very important biological meaning (Cevenini et al., 2010), it is interesting to investigate the stochastic variation of system trajectories.

Stochastic simulation approach is applied in order to study effects of random fluctuations in numbers of molecular species in systems biology models.

The most common stochastic simulation algorithm (SSA) is the "Direct Method" proposed by Gillespie (Gillespie, 1977); it explicitly simulates each reaction event in the system, therefore it correctly accounts for all stochastic fluctuations. Thus, the algorithm has time complexity approximately proportional to the overall number of particles and reactions in the system. Direct Method uses two random numbers per step to compute which will be the next reaction and when it will happen (the time step τ), as described in (Gillespie 1977).

This method has the best accuracy among all Stochastic Simulation Algorithms, but it has a prohibitive time complexity for a system with a high number of reactions and particles.

In order to accelerate simulation of the coagulation model, we used a faster SSA called 'Tau-leap method' (Gillespie, 2001). This is faster than Direct Method, because it avoids the simulation of every single reaction event. Instead, it "leaps" along the time axis in steps of length τ, which contains many single reaction events.



(a) (b)

Fig. 4. Behavior of Fibrin (Ia) and Thrombin (IIa) in (Khanin, Rakov and Kogan, 1998) (a), and in our model (b)

τ must fulfill a property called "Leap Condition", which means it has to be small enough that no significant change in the reaction rates occurs during [t, t+τ]. Note that τ, differently from direct method, is not given by the reaction system, but it is fixed and user defined. Choosing the correct value for τ is a problem much discussed in literature (Gillespie, 2001; Cao and Gillespie, 2006). A single step in Tau-Leap usually takes more time than a step in Direct Method, because computing a Poisson random number is expensive. However, Tau-Leap method allowed us to simulate the coagulation model much faster than the Direct Method, because when the particle number increases, many reactions are simulated at once within one time step.

5 Experimental Results

Our main work is focused on simulating different models of haemostasis, some with healthy behavior and some with unhealthy behavior, in order to test the behavioral changes under the influence of external stimuli. For each kind of model we perform many simulations, in order to represent the real physiological variability of coagulation observable in laboratory tests. We can consider the phenomenon of variability of this system from two points of view, in which different simulation represent:

Inter-variability: different subjects we assume to have the same initial marking, that lead them to have different patterns of coagulation;

Intra-variability: a single subject, with an average initial marking, who can show different trends because of the intrinsic variability.

In both cases, performing several simulations we can observe how the variability of biological system is reproducible by stochastic simulation. This approach allows us to evaluate whether the sample paths generated by simulation follows the real behavior of the system in normal and stressed conditions, allowing the validation of our models.

5.1 Modeling and Simulation Framework

In order to perform simulation on our models, we employed two different tools:

- *Snoopy* (Rohr, Marwan and Heiner, 2010) has been used as a tool to build the models as Stochastic Petri Nets, to convert them to Continuous Petri Nets and perform deterministic simulation of the associated system of ODEs. Snoopy also allows to perform stochastic simulations using Direct Method SSA, but since in this work we based on Tau-Leaping Method, we chose to employ another tool for these simulations.
- *Dizzy* (Ramsey, Orrel and Bolouri, 2005), a biochemical kinetics simulation software package, which includes a series of tools to perform stochastic simulations of the models, in particular Tau-Leaping Method SSA.

Fig. 5 shows the scheme we followed to simulate the models. The first step is building a SPN based on the notions on coagulation, then we have two ways to simulate it: use Snoopy and generate the ODE system (based on a CPN) and solve it to get a deterministic result; export the SPN information in a SBML document, and use Dizzy simulate the model using Direct Method or Tau-Leaping Method.

At first, the simulations have been performed on a Dell Studio XPS 7100 with AMD Phenom II X6 1035T 2,60 GHz (with 6 cores) and 8 GB RAM, in order to test a small number of results and the average time requirements. After fixing the parameters of both models using deterministic approach, we have analyzed the variability of the system with a stochastic method. We tested both Direct Method SSA and Tau-Leaping Method, in order to find the best algorithm in terms of accuracy and time complexity. Direct Method takes a prohibitive time because of the size of the model. A single simulation takes 350 hours to simulate 10 seconds of coagulation, and simulating the model up to 25 seconds (the minimal time required for analyze the behaviour of the model) might take many months to complete. Tau-Leaping Method allows a faster simulation, inducing only a minor loss in accuracy. It takes around 8 hours to simulate 25 seconds of coagulation, performing one simulation for each core (for a total of six simulations in 8 hours). Our focus is on computation of an ensemble of Tau-Leaping SSA realizations, and we need at least 100 simulation results to estimate the characteristics of a model with an acceptable statistical accuracy. As our six-core system can perform only six realizations at a time, the whole process would require more than 120 hours. Thus, we decided to execute the algorithm on the Bari INFN Computer Farm (based on Torque/Maui, and composed of ~3700 CPU/Core with a disk space of ~1.3PByte), which allowed us to parallelize the process and simulate all the 100 realizations simultaneously, requiring a total of 8 to 16 hours to complete them (depending on the model and on the clock rate of the available cores). The simulations were performed on a single cluster, using up to 100 nodes to simulate all the realization simultaneously. We developed a Unix bash script which automatically modify the original (healthy model) changing its parameters (initial marking), and submit the relative jobs to the FARM. The results have been collected in .csv documents, and processed using MATLAB in order to compute the trajectories and significant values such as peak times (in particular, their mean and standard deviation).

It is important to note that we parallelize only the ensemble of SSA realizations, but each single simulation is not split on multiple processors. Most of the attempts in literature parallelized across the simulations, trying to speed up the process using GPUs (Li and Petzold, 2009) and improving the random number generation. Attempts have been made to parallelize single simulations (e.g. see Dittamo and Cangelosi (2009)), but these algorithms work by splitting the set of reactions among blocks, and the structure of our Stochastic Petri Net cannot be easily partitioned because of the strong interconnections among the nodes.

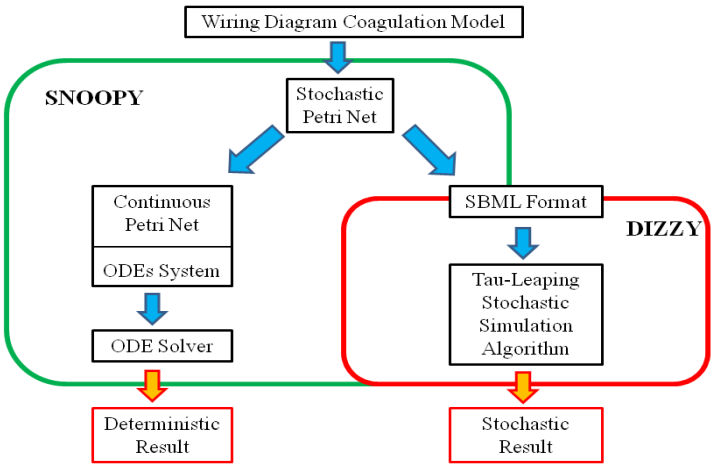


Fig. 5. Scheme of the simulation framework

5.2 Models Description

This work is focused on comparing the behaviour of a "healthy" subject with that of an "unhealthy" subject. We generate different models starting from the same Stochastic Petri Net shown in section 2, but changing the initial marking to represent the effect of a prothrombotic event. In particular, the unhealthy models have an higher initial number (from 10-fold to 1000-fold) of molecules in place representing the trigger factor of the extrinsic pathway, the Tissue Factor (TF place). This condition is clinically supported, reflecting the hypercoagulability state that arise locally during atherosclerotic plaque rupture (Reininger et al., 2010).

A second factor influencing the system is the VIIaTF complex which, as highlighted in literature (Monroe and Key, 2007), plays an important role in the thrombus formation process. A minimal amount is needed to start the coagulation cascade, but an excess of this complex due to prior cardiovascular inflammatory events can significantly affect the coagulation process. Therefore, we test the behaviour of our model with different amount of this factor, comparing a physiological amount with a pathological one, represented by a 2-fold and a 10-fold increase.

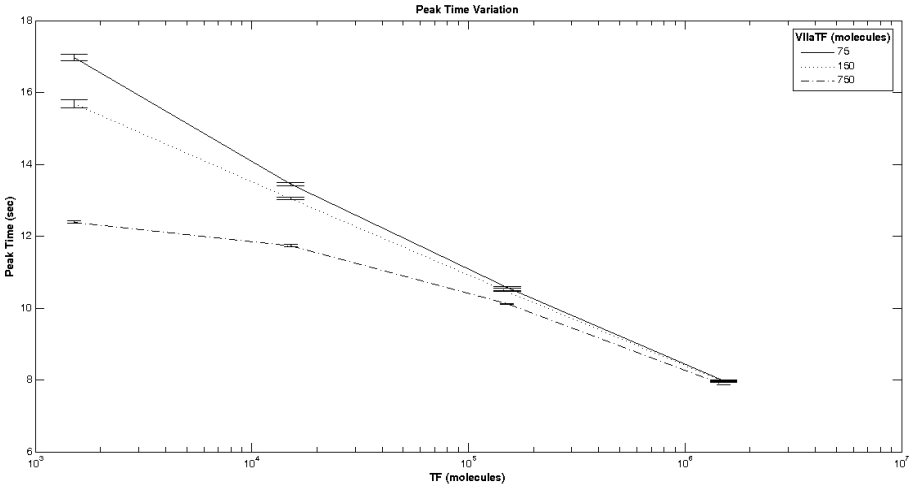


Fig. 6. Variation in mean and standard deviation of Thrombin (IIa) peak time

It is important to note that the reaction rate constants has not been modified from healthy to unhealthy models, because biological evidence proves that enzyme kinetics does not vary; nonetheless, the reaction firing rates change considerably between the two models because of the initial marking, promoting procoagulatory events.

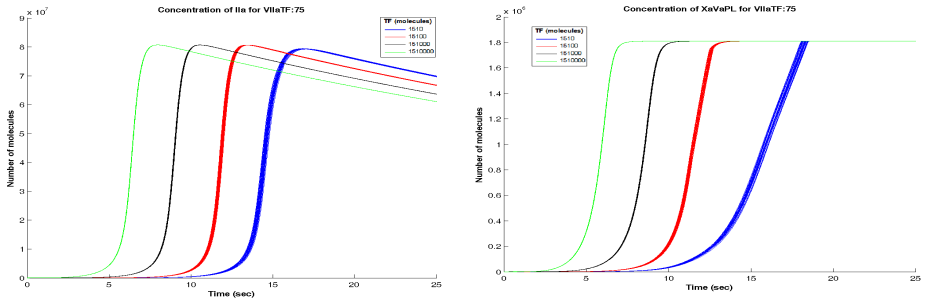
Table 1. Mean and standard deviation of peak value and peak time, in healthy and unhealthy models

	VIIaTF Normal		VIIaTF 2-Fold		VIIaTF 10-Fold	
Model	**Thrombin Mean Peak Time (sec)**	**Thrombin St.Dev. Peak Time (sec)**	**Thrombin Mean Peak Time (sec)**	**Thrombin St.Dev. Peak Time (sec)**	**Thrombin Mean Peak Time (sec)**	**Thrombin St.Dev. Peak Time (sec)**
Healthy	16,96	0,106	15,67	0,091	12,38	0,037
Unhealthy (TF 10-fold)	13,44	0,051	13,04	0,044	11,73	0,026
Unhealthy (TF 100-fold)	10,56	0,017	10,46	0,017	10,11	0,015
Unhealthy(TF 1000-fold)	7,97	0,008	7,94	0,008	7,86	0,008

5.3 Computational Results

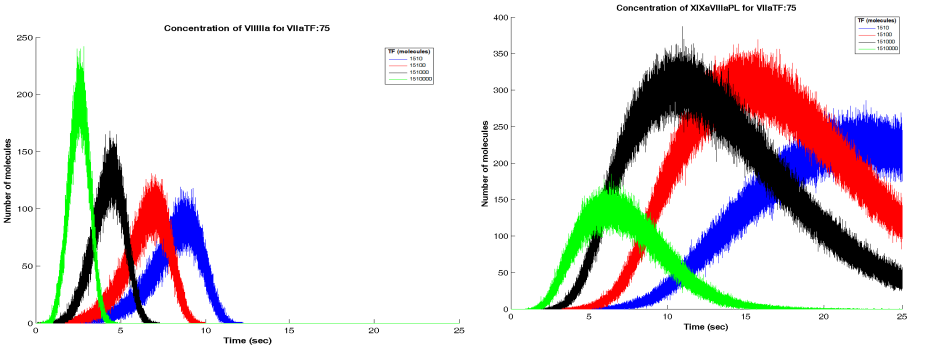
As we can see from Fig.7a, the amount of generated thrombin does not change from the healthy model to the unhealthy one, because the total availability of its precursor (prothrombin, II) is the same. Instead, we can see an anticipation of the growth in the unhealthy model, which is due only to the change of initial condition. This is observable even with a deterministic approach, but only from the stochastic results from in Table 1 we can notice how the unhealthy models show a lower intrapersonal variability compared to the healthy model, which shows an higher variability. A likely explanation of the lower variability in the unhealthy subject is given by the augmented amount of TF molecules, which leads to an higher rate for the procoagulant reactions. Since the rate of the inhibitory reactions is less affected, they will fire with a lower

frequency, thus reducing the noise on the main cascade. We can also see this effect just upstream of the activation of thrombin (Fig.7b), e.g. in XaVaPL complex (prothrombinase), which is the main responsible of the generation of prothrombin. This is consistent with the clinical evidence, where the biological variability is lower in patients with pro-thrombotic conditions, and higher in patients with pro-haemorragic phenotypes. The same effect is produced by an increased amount of factor VIIaTF, which also limits the influence of the increment of TF amount (high levels of TF do not affect a system with high levels of VIIaTF).



(a) (b)

Fig. 7. (a) Thrombin (IIa) molecules trend in healthy and unhealthy models; (b) The same intra-variability seen in prothrombinase (XaVaPL).



(a) (b)

Fig. 8. Trend in molecular number for VIII-IIa (a) and XIXaVIIIaPL (b), which shows a strong fluctuability

Besides the different variability among models (Fig. 7), there is another aspect which is captured only by the stochastic approach. The number of molecules involved in many reactions (Fig. 8) shows strong fluctuability during critical phases of the simulation, when the system is at the peak of its activity, a feature which is impossible to detect using a deterministic approach, and very challenging even in a wet-lab experiment. Although being a preliminary analysis, this result can motivate further analysis on how much specific molecules react when the system is at the peak of its activity.

6 Conclusion

In this paper we presented a SPN model for the extrinsic coagulation, with the purpose of simulating the behavior of the whole system. We employed Gillespie's Tau-Leaping SSA in order to reduce the simulation time while retaining a good approximation, and we developed models describing "healthy" and "unhealthy" subjects.

We proved that a stochastic approach allows to detect important features that deterministic models cannot discover: first, there are molecule types which appears in a very low amount (up to 10 molecules), which only the stochastic method allows to simulate without mathematical artifacts; second, we shown that an increase in the quantity of Tissue Factor or VIIaTF complex reduces the degree of variation of the system, which is confirmed by clinical evidence
where the biological variability is lower in of patients with pro-thrombotic conditions, and higher in patients with pro-haemorragic phenotypes; finally, only the stochastic simulation identified the high fluctuability in the system during critical phases, which will allow to analyze deeper how much the particles reacts when the system is at the peak of its activity.

This model, which better capture the true variability of this complex system, represent the coagulation in a more realistic manner compared to the deterministic models appeared in literature. This approach sets the background for the analysis of more detailed models, which will include pharmacological interactions. Future improvements will also include further development of the simulation framework, including the automatic stochastic simulation of models with a wide range of parameters, and the performance analysis of additional simulation software, in order to compare their CPU time and memory requirements.

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