Reviewer 2

1. Authors should cite Papadopoulos KP et al. Med. Engr. Physics 36:196 (2014) and compare their 5 ODEs/Logic Rules with the Papadopoulos coarse grained approach of 4 ODEs and 6 parameters.

*We kindly acknowledge the reviewer for directing us to the work by Papadopoulos KP et al. Papadopoulos and co-workers developed a coarse-grained model for thrombin generation that uses 4 Ordinary Differential Equations (ODEs) with 6 parameters. Similar to our work this study uses a reduced order approach in describing a complex biological system with few parameters. However we differ fundamentally in the model formulation as well as in the goals of the study. Firstly, our model is based on a hybrid approach. In each ODE describing a rate equation we combine traditional enzyme kinetic laws with a logical rule. The enzyme kinetic laws (like Michaelis-Menten) have a physiological basis and the logical rule represents the regulatory activity that controls the rate equation. The product of kinetic laws with logical rules encapsulates a complex interplay between substrates and their regulators that affect the rate equation. Secondly, we have modeled the dynamics of coagulation using the hybrid approach rather than thrombin generation modeled by Papadopoulos KP et al. i.e. our model captures greater mechanistic detail about coagulation. These details allowed predicting the roles of primary inhibitors like ATIII and Protein C. We were also able to use flux analysis to understand how the model balanced initiation, amplification and termination of thrombin generation in both normal and hemophilia modes.*

1. How is the particle swarm optimization different from a genetic algorithm?  What are the advantages of PSO over a more typical genetic algorithm?

*Particle Swarm Optimization (PSO) and Genetic Algorithm (GA) are both population based optimization algorithms. A Genetic Algorithm starts with a randomly generated ‘population’ that represents the various initial solution vectors of the fitness function (residual in our model). Based on pre-specified fitness selection methods ‘parents’ are chosen from these population members discarding rest of the population members. The parents are then used to generate ‘children’ through operations called ‘cross-over’ and ‘mutation’. The fittest children and parents are then used to generate parents for the next generation.*

*Particle Swarm Optimization like GA starts with a random population of particles that represent the solution vectors. The particles are then allowed to traverse in the search space of the fitness function (residual in our model) with certain velocities. The particle positions (which represent the solutions) are then updated based on a local experience and global experience. These operations are relatively very simple and computationally less expensive as compared to ‘cross-over’ and ‘mutation’ that are generally used in GA’s. Typical GA is primarily suited for binary value problems rather than continuous real-valued problems. The complexity of crossover and mutation operations in real valued problems increases with increasing precision of the numbers. In addition PSO never discards its solutions like GA. This leads to a greater diversity in solutions and thus allows us to search for a global optimum more effectively especially in case of multi-modal fitness functions.*

1. The authors know that APC is only present on endothelium in blood vessels and is not present in blood in tubes in clotting assays, thus PC=0% in Fig. 3.  The authors should comment if and how soluble thrombomodulin was used to obtain the data in Fig. 4.

*Physiological concentration of thrombomodulin is dependent on endothelial cell surface/volume ratio. Thus the concentration of Tm varies spatially. van’t Veer and co workers in a study to understand inhibitory mechanism of protein C on Tissue Factor induced thrombin generation varied the values of Tm from 0-10 nM. In these ranges Tm was found to activate protein C (PC) to activated protein C (APC). The effect of Tm observed in these ranges was found by van’t Veer et al. to be potentially physiologically relevant. Butenas and co-workers used thrombomodulin concentrations of 0.1 nM and 1 nM to activate the protein C pathway. The presence of thrombomodulin at these concentrations did not affect the maximum or total thrombin produced but affected the initiation phase causing a delayed amplification. Our hybrid model as shown in Figure 4 & Figure 5 has captured these effects.*

1. The most common test of thrombin generation conducted in many labs is to add increasing amounts of tissue factor (usually from 0.1 to 10 pM TF).  The authors present a Factor VIIa titration (Fig. 7) but it would also be very interesting to present a TF titration since that is the classic test of a models predictive accuracy. Predicting hemophilia is a fairly weak test of any model since severe deficits in FVIII or FIX are so immediately upstream of prothrombinase generation.

*We kindly agree with the reviewer’s comment that Tissue Factor (TF) is the most common initiator of the coagulation process. However we respectfully disagree that TF titration would be a better test as compared to prediction of hemophilia for our model. In our study we trained our model reproducing the experimental data from Butenas and co-workers. This study used VIIa-TF to initiate the coagulation process in the presence and absence of protein C pathway. Using the same set of parameters from our training data we were able to capture the coagulation dynamics under hemophilia A and hemophilia B conditions initiated using recombinant factor rFVIIa (rFVIIa) from a different group. We believe that this shows a surprisingly remarkable predictive ability and robustness of the model. We would also like to draw the reviewer’s attention to the predictions in panel A of Figure 7. We used different levels of trigger 1x-200x to initiate coagulation. Our model currently generalizes the various coagulation triggers or initiators like TF, VIIa-TF or rFVIIa. Thus the model would exhibit the same predictive ability based on various levels of TF.*

1. The authors could more clearly describe how a specific set of a large number of initial conditions (typically > 10-20 species concentrations in the large ODE models) are processed by the logic rules to give the regulatory term.  Since blood kinetics are mostly studied in the tube (no endothelium), Eqn 12 and 13 drop out to result in a 3 ODE model.  In this case there are only 3 regulatory terms (v-init, v-amp, and v-inh).  Can the author more clearly show how these 3 terms actually depend on various concentrations of actual species that experimentalists might control in the lab.

*We kindly acknowledge the reviewer’s comment that blood kinetics is studied in the tube and there is absence of endothelium. However Equations 12 and 13 do not drop out in our model in the presence of protein C pathway. These equations are concerned with the inhibitory role of activated protein C. To activate protein C pathway, Butenas and co-workers used soluble thrombomodulin (Tm) at 0.1 nM and 1 nM concentrations. At these concentrations soluble Tm was able to support protein C activation akin to activation of protein C by endothelium bound Tm in vivo.*

1. The language should be more uniform in the paper.  It seems the terms “regulatory term” and “control term” are used interchangeable.
2. The quantification of the regulatory role of the platelet is not well described in the paper.

Reviewer 3

1. Abbreviation TF should be expanded in line 59?.

*We thank the reviewer for pointing this discrepancy. In line 59, TF has been expanded to Tissue Factor. The line now reads as*

*‘Initially, thrombin is produced upon cleavage of prothrombin by fluid phase activated factor X (FXa), which itself has been activated by Tissue Factor/factor VII (TF/FVIIa) [10]’*

1. Fig. 2. In a figure body function f\_j(Z, k) should depend on \kappa according to Methods but does not on k. This should be reconciled.

*Change should be in the figure*

1. Authors should explain an advantage of their logic approach vs. simple Machaelis-Menten equation taking account several inhibitors (competitive, non-competitive, allosteric) and activators.

*We thank the reviewer for raising a very pertinent point. The hybrid approach uses a combination of logical rules represented by transfer functions along with traditional enzyme kinetic expressions. The use of logical rules is quite advantageous especially when there are several regulators and/or when the exact mechanisms behind regulatory activity are unknown.*

*Firstly, using transfer functions simplifies the regulatory logic when several inhibitors and activators are involved. For example, in our study νinit is a regulatory term that affects the rate of initiation. This term is influenced by the presence of coagulation activators like Tissue Factor (TF), factor VII (VIIa), factor XI, and factor XII and inhibitors like Tissue Factor Plasminogen Inhibitor (TFPI). Using Michaelis-Menten (MM) equation for each of these activators and inhibitors greatly increases the number of parameters (with each MM equation requiring at the least 3 parameters) that are involved in the regulatory logic. This considerably increases the computational burden in parameter estimation and model simulation. Secondly, unlike coagulation where the biochemistry is well defined, in many biological systems there is scarce information about the exact mechanism of enzyme activity (competitive, non-competitive, allosteric etc.). This causes an impediment in determining the exact form MM equation. Transfer functions do not necessarily require a detailed or exact knowledge of the enzyme mechanism. Consider again the example of νinit in our study. In Eq 16 we model this as a function of trigger and TFPI. Trigger here can be TF, TF/VIIa, rFVIIa or any other coagulation initiators. Further we use the min/max rule as shown in Equations 6-8 to quantify the cumulative effect of all the triggers and inhibitors (TFPI) on initiation. Using a logical rule allowed us to reduce the effect of different initiators to a simple rule. This can be especially a very useful and effective method when there is incomplete information. We used Hill like functions to describe the effect of initiators and inhibitors. Each function requires just 2 parameters unlike an MM equation, which requires a minimum of 3 parameters. Transfer functions also have the flexibility of form. As mentioned by us in our study (Page 20, Lines 331-334) they can be a Hill like function or otherwise. Thus the use of logical rules using transfer functions reduces the computational complexity and also helps in encoding unknown/less known regulatory logic.*

4. Detailed information on experimental assays should be added in caption of Figs. 3,4, and 5 or in the text.

*Detailed information on experimental assays has been added in the text. Please page 7, lines 134-148.*

1. The authors should show how they introduced time-delay parameter T\_D into the model (10)-(14) and clarify whether they solved ODEs or differential-delay equations.

*Not sure of this one*

1. Table of the final kinetic parameters obtained as a result of fitting procedure should be added to the text to allow readers to reproduce the results.

*To be added*

1. Table with the names of species x\_i and their initial concentration should be added.

*To be added*

1. Line 344: Commonly the maximum rate is defined as the product of catalytic rate, k, and enzyme concentration, e, or this is enzyme activity instead of author’s notation k\_{max} and activity \epsilon.

*Not sure of this one*

1. Eq. 9:  there should be f\_i(Z) rather than f\_i(x)

*fi(x) has been changed to fi(Zj) in equation 9. Please see the corresponding equation.*

1. Discussion seems to be reduced. Instead of the discussion of reduced model expansion, it would be better to compare briefly the results obtained in the author’s reduced approach with the results obtained in the unreduced models reviewed by authors in Introduction.

   Typos:

 Page 6, line 2 there should be “Michaelis-Menten “

*The typo has been corrected. Please see the change on Page 6, line 2.*