A Hybrid-System Model of the Coagulation Cascade

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Abstract

The process of human blood clotting involves a complex interaction of continuous-time/continuous-state processes and discrete-event/discrete-state phenomena, where the former comprise the various chemical rate equations and the latter comprise both threshold-limited behaviors and binary states (presence/absence of a chemical). Whereas previous blood-clotting models used only continuous dynamics and perforce addressed only portions of the coagulation cascade, we capture both continuous and discrete aspects by modeling it as a hybrid dynamical system. The model was implemented as a hybrid Petri net, a graphical modeling language that extends ordinary Petri nets to cover continuous quantities and continuous-time flows. The primary focus is simulation; specifically, we are interested in (1) fidelity of simulation to the actual clotting process in terms of clotting factor concentrations and elapsed time; (2) simulating known clotting pathologies; and (3) providing fine-grained predictions which may be used to refine clinical understanding of blood clotting. Finally, we examine the sensitivity of a critical purely-continuous subsystem to rate-constant perturbation.

1 INTRODUCTION

The process of blood clotting in mammals is complicated, involving the interaction of more than a dozen coagulation factors as well as a number of proteins from the kinin-kallikrein system and protein inhibitors. Attempts to model coagulation mathematically therefore usually focus on a smaller subset of interactions, perhaps one of the so-called pathways or just a portion of one of them (cf. [1], [2], [3], [4], [5], [6] and [7]). Such models generally consist of a set of coupled, usually nonlinear, differential equations governing the time evolution of protein concentrations. Although on one level of analysis all of the processes in blood clotting comprise discrete events (e.g., cleavage and formation of chemical bonds), at the scale of interest it is concentrations that matter, and these exhibit continuous dynamics.

Continuous systems are nevertheless inadequate to model the entire coagulation cascade, because (1) certain events are better modeled as "switched" [8] (e.g., reactions that take place if and only if calcium is present, of zinc ions exceed a threshold [9], [10]); (2) the continuous reactions of the cascade take place on very different time scales, and replacing fast ones with switches avoids the numerical difficulties in simulating all of them at once; and (3) the current state of clinical knowledge is not sufficient to provide differential equations for all the interactions of the cascade, yet qualitative knowledge can nevertheless often be incorporated in the form of discrete events toggled (possibly) by thresholds (e.g., the thrombin concentration that is required for appreciable conversion of factor XIII into its activated form).

We therefore model the coagulation cascade as a hybrid system (HS), i.e. one consisting of interacting continuous and discrete dynamics. (See [11] for a thorough introduction.) We require the model to capture faithfully, and over a wide range of parameter settings, the major qualitative aspects of human blood clotting, but also that it be perspicuous and easily modifiable, to accommodate the biologist and clinician. In light of these constraints, the model was implemented using hybrid Petri nets (HPNs), a graphical modeling formalism for modeling hybrid systems. Classical Petri nets are a well known computational formalism for the modeling of discrete-event dynamical systems, with constructs for sequential and concurrent process execution, for resource consumption and production, and for inhibition. HPNs extend classical Petri nets from the domain of purely discrete phenomena to the domain of hybrid dynamics, by supplementing the traditional discrete-event architecture with continuously varying events and states (see Chapter 3 of [12] for a formal and detailed description of HPNs).[1]

We show here that the model can reproduce certain gross features from the clinical literature (time-to-clotting and the time course of thrombin, the most

[1] Our current implementation is based on the VISUAL OBJECT NET++ platform, a dedicated HPN modeling and simulation environment [13], which includes a graphical language offering a suite of object-oriented programming (OOP) features: hierarchical organization, inheritance and object reuse. We plan to make the software publicly available, but presently interested parties should contact the authors.

important coagulant) of (1) normal clotting, as well as of the coagulation disorders (2) hæmophilia A and (3) factor-V Leiden. More generally, the model provides a platform for *in silico* investigation of blood clotting, which is not presently fully understood. This includes the relevance of certain reactions, the nature of various clotting pathologies, and the effects of pharmacological interventions on those disorders. More broadly still, the model demonstrates the utility of using hybrid systems, and in particular hybrid Petri nets, to model cascade-like biological processes where both discrete and continuous dynamics play a role. In virtue of its ability to incorporate both types of dynamics, the model is able to support robust analysis and prediction in cases where parts of a complex process may be known precisely (with differential equations) while other aspects may have qualitative descriptions only (through punctuated phase changes, discrete transitions, and threshold behaviors). This ability to reason effectively with representations of multiple granularities addresses a central requirement in modeling complex biological processes.

2 MODEL IMPLEMENTATION

Since the complete HPN is extremely large, it is omitted here, but the full model appears in Chapter 4 of [12], along with the blood factors and their initial concentrations (Table 2.1). Yet in spite of its size, the overall model comprises multiple instances of only five basic modules, with different parameters for different pathways, factors, and enzymes. This motivates the use of an *object-oriented* HPN modeling language; that is, one with constructs for information hiding and object reuse. Two of the basic modules have purely continuous dynamics (blood factor activation and factor-factor binding); the other three have hybrid dynamics (the initiation of blood clotting in the intrinsic clotting pathway, the formation of a fibrin clot, and switched activation).

Blood-factor activation. Fig. 1A depicts one of the basic aspects of the coagulation cascade (see Fig. 3), the enzyme-induced transformation of a blood factor (which may be either a serine protease or a glycoprotein) from its inactive (zymogen) form to its active configuration. The places IN_1, IN_2 and OUT represent the concentration (nM) of various blood factors: IN_1 is the zymogen and OUT is its activated form; IN_2 is the catalyzing enzyme; IN_1:IN_2 is an intermediate macromolecule. The places labeled with k's are the rate constants of classic enzyme kinetics: on-, off-, and catalytic rates. This reaction can also be written as a set of differential equations; square brackets are used to indicate concentrations:

$$\frac{d[\mathrm{IN}_1]}{dt} = k_{\mathrm{off}}[\mathrm{IN}_1{:}\mathrm{IN}_2] - k_{\mathrm{on}}[\mathrm{IN}_1][\mathrm{IN}_2] \tag{1}$$

$$\begin{aligned}\frac{d[\mathrm{IN}_2]}{dt} =& k_{\mathrm{off}}[\mathrm{IN}_1{:}\mathrm{IN}_2] - k_{\mathrm{on}}[\mathrm{IN}_1][\mathrm{IN}_2] \\ &+ k_{\mathrm{cat}}[\mathrm{IN}_1{:}\mathrm{IN}_2]\end{aligned} \tag{2}$$

$$\begin{aligned}\frac{d[\mathrm{IN}_1{:}\mathrm{IN}_2]}{dt} =& k_{\mathrm{on}}[\mathrm{IN}_1][\mathrm{IN}_2] - k_{\mathrm{off}}[\mathrm{IN}_1{:}\mathrm{IN}_2] \\ &- k_{\mathrm{cat}}[\mathrm{IN}_1{:}\mathrm{IN}_2]\end{aligned} \tag{3}$$

$$\frac{d[\mathrm{OUT}]}{dt} = k_{\mathrm{cat}}[\mathrm{IN}_1{:}\mathrm{IN}_2]. \tag{4}$$

Of course, since the variables IN_1, IN_2, and OUT participate in other reactions, Eqs. 1, 2, and 4 do not completely define the dynamics of any of these variables; the complete governing equations may contain additional additive terms from other reactions. That is why these three places have shaped outer rings in Fig. 1A: it indicates that they are "published," i.e. available to interact with other objects and hence other reactions.

Fig. 1A also includes a discrete switch, *sw*, that can turn off the binding and catalytic reactions. Most instantiations of this module in the model lack the switch and are therefore purely continuous, but several of the activation reactions of the clotting cascade do require free calcium ions. In the corresponding hybrid modules, if the *sw* place holds a token (or more than one), then the reactions may take place, otherwise they may not.

Factor-factor binding. The second recurring reaction is the binding of two blood factors (Fig. 1B). As in the activation reaction (Fig. 1A), the rates constants k_i are connected to transitions via "test arcs" (dashed lines), reflecting the fact that these quantities are unchanged by the reaction. The governing equations are simply:

$$\frac{d[\mathrm{IN}_1]}{dt} = k_{\mathrm{off}}[\mathrm{OUT}] - k_{\mathrm{on}}[\mathrm{IN}_1][\mathrm{IN}_2] \tag{5}$$

$$\frac{d[\mathrm{IN}_2]}{dt} = k_{\mathrm{off}}[\mathrm{OUT}] - k_{\mathrm{on}}[\mathrm{IN}_1][\mathrm{IN}_2] \tag{6}$$

$$\frac{d[\mathrm{OUT}]}{dt} = k_{\mathrm{on}}[\mathrm{IN}_1][\mathrm{IN}_2] - k_{\mathrm{off}}[\mathrm{OUT}] \tag{7}$$

Both of the continuous modules appears in many instantiations throughout the model, differing from each other only in their rate constants and their interconnections with the rest of the network. The differential equations that they model were drawn from [3], and are summarized in Table 5.1 of [12].

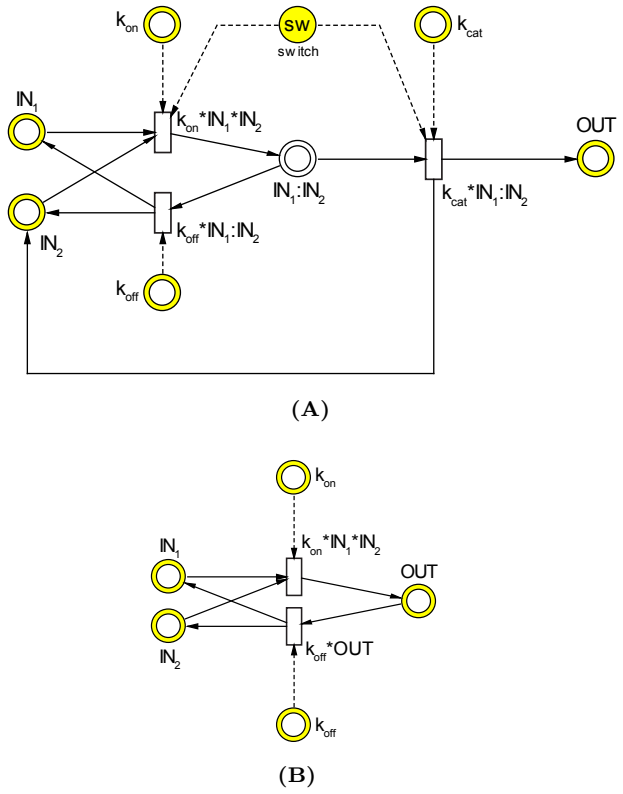


Figure 1: Continuous time/state Petri net modules. (A) The activation module, which models the activation of a zymogen (IN_1) into its active configuration (OUT) by a catalyst (IN_2). In versions with the discrete switch *sw*, the module is hybrid, and models the switching on/off of a reaction by the presence/absence of a variable. (B) The binding module, which models the (reversible) binding of two components, IN_1 and IN_2, into the macromolecule OUT.

The intrinsic pathway. The INIT-INTRINSIC module, shown in Fig. 2A, models the initiation of blood clotting via the intrinsic pathway. Details of this process were drawn from [10] and [9]; see also Table 1; we outline the main features here. The pathway begins with the exposure of a negatively charged surface and rising zinc concentrations, and ultimately activates factor XIa, through which it interacts with the rest of the cascade (see Fig. 3). Zinc-ion time evolution is modeled by a first-order differential equation (exponential growth up to an asymptote); various threshold concentrations trigger various intermediate reactions. The presence of the negatively charged surface, on the other hand, acts as a binary switch on solid-phase XII activation.

Notice that factor XIIa can activate kallikrein through either a "slow" or "fast" transition, where the former corresponds to activation of free prekallikrein and the latter to activation of prekallikrein bound to the surface of HMWK. The speeds of these reactions, fast and slow, are modeled by assigning appropriate time delays to the discrete transitions. Negative and positive feedback loops are also modeled: Activated kallikrein enables the fluid-phase activation of factor XII, which in turn activates more kallikrein. Conversely, a sufficient concentration factor XIIa inhibits its own activation via the serpin C1-inhibitor; sufficient quantities of factor XIa directly inhibit its own further activation by factor XIIa; and high zinc concentrations shits of the binding of high molecular weight kininogen to prekallikrein.

The fibrin module. Fig. 2B shows the FIBRIN module, which models the final portion of the blood clotting pathway: essentially the feedforward path from thrombin (IIa) to the formation of a clot. (Thrombin also takes part in numerous feedback reactions, which are modeled with the continuous modules lately discussed.) Data for this module were drawn from [14], [15], [16], [17], [18], and [19]; see Table 1 for details. Key features are the thrombin-thresholded intermediate reactions, modeled with test arcs; the fast and slow parallel pathways for cleavage of the Arg37-Gly38 bond of factor XIII (the fast cleavage requires lower levels of thrombin but additionally the presence of fibrin or fibrinogen); and the toggling of reactions by the binary presence/absence of calcium ions. Notice in particular that in addition to enabling certain reactions, calcium ions block the cleavage of the Lys513-Ser514 bond of factor XIII and therefore its inactivation. Negative feedback appears in the self-inhibiting production of fibrinopeptide B, and in the inhibitory effect of cross-linking of FXIIIa* and the fibrin polymers on the promoter action of fibrin and fibrinogen on factor XIII activation. The pathway terminates when cross-linking fraction reaches unity.

The complete cascade The portions of the coagulation cascade, Fig. 3, not modeled by the these two HPNs (Fig. 2) can be completely described in terms of the two continuous modules, factor-factor binding (Fig. 1B) and factor activation (Fig. 1A), and the latter's switched cousin (Fig. 1A). These include portions of the cascade not included in Fig. 3 for clarity, mostly the bindings of proteins to lipid substrates. The full model is depicted in Chapter 4 of [12].

Conceptually, the cascade is initiated through both the intrinsic and extrinsic pathways, the latter through the release of tissue factor (TF) and its binding to (lipid-bound) factor VII. Factor VII may be activated by factor Xa, but this requires completion of either the intrinsic or extrinsic pathways, both of which require the activation of factor VII itself. Thus it is generally accepted [3] that some very small quantity of factor VIIa (0.1 nM) exists in the blood stream prior to vascular injury. This does not imply spontaneous activation of the coagulation system since without tissue

Parameter	Type	Description	Value	Source
$[Zn^{2+}]$	arcweight	thresh. for HMWK:PK binding	0.3μM	[10]
$[Zn^{2+}]$	arcweight	thresh. for PK activation on cells	5μM	[9]
$[Zn^{2+}]$	arcweight	thresh. inhibition of HMWK:PK binding	10μM	[10], [9]
$[Zn^{2+}]$	arcweight	thresh. for FXII fluid-phase activation	10μM	[10]
$[Zn^{2+}]$	arcweight	thresh. for FXI:HMWK binding	10μM	[10]
Zinc flow rate	continuous transition (differential equation)	rate of zinc ion accumulation	$[\dot{Zn}] = 20 - [Zn]$	extrapolated from intrin. pathway time data
[IIa]	arcweight	thresh. for fast cleavage of Arg37-Gly38 bond	1.56nM	interpolated from [18]
[IIa]	arcweight	thresh. for slow cleavage of Arg37-Gly38 bond	90nM	interpolated from [18]
[IIa]	arcweight	thresh. for release of fibrinopeptide A	2.48nM	interpolated from [18]
[IIa]	arcweight	thresh. for release of fibrinopeptide B	3.28nM	interpolated from [18]
[XIII]	arcweight/delay	amount of fXIII cleaved per unit time:		
		fast cleavage	1nM/3s	interpolated from [18]
		slow cleavage	0.33nM/3s	interpolated from [18]
cross-linking	arcweight	% of cross-linking at which promoter effect of fibrin on Arg37-Gly38 cleavage is inhibited	40%	[14]

Table 1: Parameter values and types used in the HPN modules of Fig. 2A,B

factor the rest of the pathway cannot proceed.

Factor X activation marks the meeting point of the extrinsic and intrinsic pathways, after which the cascade proceeds through a series of feedforward and feedback bindings and activations (which include inhibitions) to the most important blood protein, activated factor II (called thrombin, or IIa). Thrombin then initiates fibrin production along the lines described above.

3 RESULTS

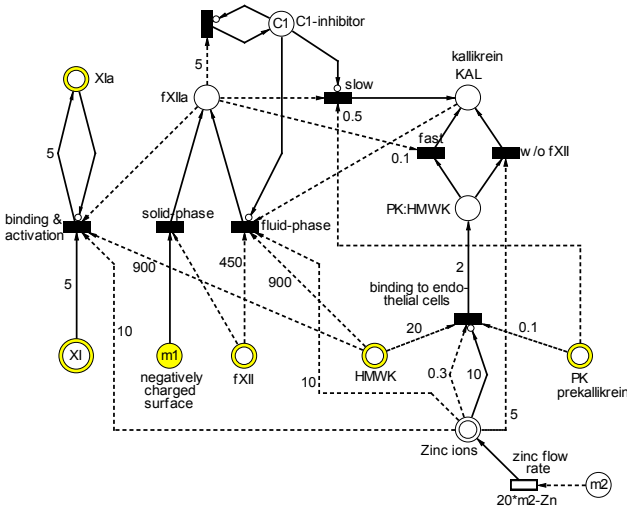
We choose to examine thrombin because it is the most important enzyme product of the coagulation cascade: it participates in far more reactions than any of the other factors, including both feedforward and feedback regulation, and is essential for normal blood clotting. Time to thrombin activation is consequently one of the major parameters measured in clinical tests. To evaluate the baseline performance of our model, we set the initial concentrations as shown in Table 2.1 of [12] and compared the time course of thrombin production in our computational simulation with results reported in the clinical literature [3, 8]. Fig. 4A shows the concentration of thrombin (in black) produced under normal conditions upon triggering of the clotting cascade. The value of thrombin is shown in nM, as a function of time, in seconds. Consistent with previously reported clinical studies, thrombin concentration using the model peaks at about 160 nM around 100 seconds (cf. [20]). A complete clot (i.e. 100% cross-linking) occurs after 105 seconds, which is again a reasonable figure.

Hæmophilia A, a common hæmoraphic disorder, results from deficiency of coagulation factor VIII. We therefore simulate it by setting the initial value of the continuous-HPN place VIII to 0.035 nM, which corresponds to the borderline between mild and moderate hæmophilia. The result also appears in Fig. 4A, in red: thrombin concentration peaks later (at 120 seconds) and much lower (at just over 6 nM), which is congruent with clinical observations. The maximum cross-linking achieved is 40%, which again is consistent with the disease pathology.

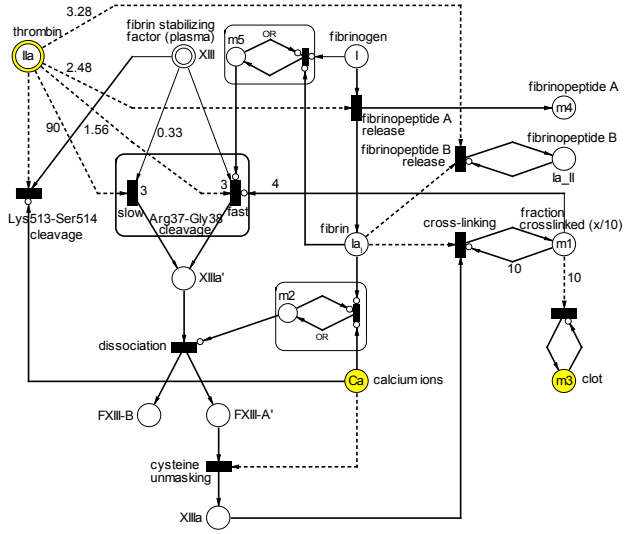
Factor-V Leiden is a coagulation disorder characterized by a condition called activated protein C resistance (APCR), in which a genetic mutation in the factor-V gene renders the resulting factor-V protein resistant to inactivation by activated protein C (APC). Factor V is a procoagulant, so the consequence of its slower rate of inactivation is generally a thrombophilic (propensity to clot) state. More sophisticated ways of simulating factor-V Leiden are available within the present model—we demonstrate one such in the context of a control schemes in [21]—but for now, we choose simply to remove the module by which APC inactivates factor V (see Section 2). As expected, the result (Fig. 4B) is an increase in the amount of thrombin produced, congruent with clinical observations (cf., e.g., [22]). This simulation of factor-V Leiden does not predict a shorter time to clot, and this, too, is in agreement with clinical results.

4 SENSITIVITY ANALYSIS

There are three distinct questions that can be addressed by analyzing the sensitivity of the model to its parameters. (1) The numerical values of these parameters were determined empirically, by chemical experiment, and are consequently subject to error; which errors have the biggest effect on model fidelity? (2) Suppose we wanted to change the course of coagulation by changing a reaction rate; where shall we get the most bang for our buck? (3) If a blood-clotting



(A)



(B)

Figure 2: Hybrid Petri net modules. (A) The initiation of the intrinsic pathway. (B) The final stages of blood clotting: the activation of factors I and XIII, and the formation of a clot. The semantics of hybrid Petri nets can be found in Chapter 3 of [12].

disease alters certain rate constants (as e.g. factor-V Leiden does), how dramatic will the effect be on the concentrations of blood proteins? For simplicity, we restrict the sensitivity analysis to a large portion of the cascade which *can* be described entirely by a set of ordinary differential equations; however, the sensitivity analysis can (in principle) and should be extended to the HPNs, as well (cf. for example [23]). The results must be interpreted accordingly.

We follow the technique employed on another model of blood clotting, [24], where a complete description can be found. Briefly, we derive (analytically, *con-*

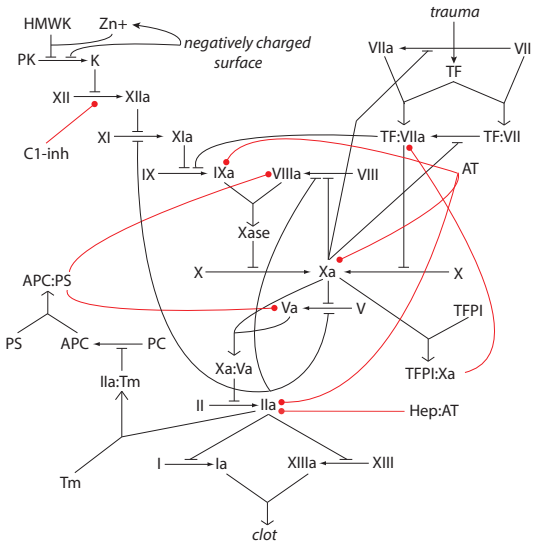
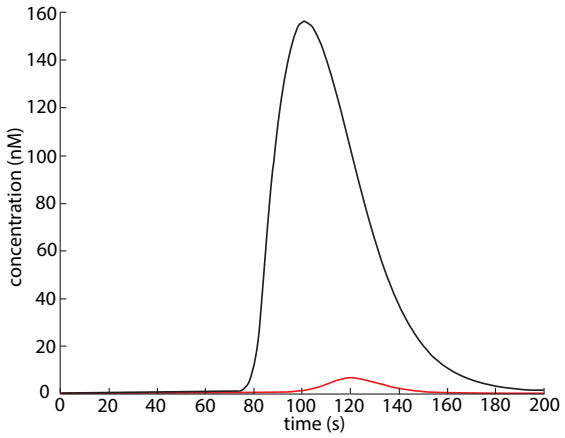


Figure 3: The coagulation cascade. Converging arrows represent bindings, flat-ended lines terminating on triangle-headed arrows label activations, and red lines denote inhibitory reactions, which may be either bindings or (de)activations. To minimize clutter, lipid bindings and other minor reactions have been omitted.

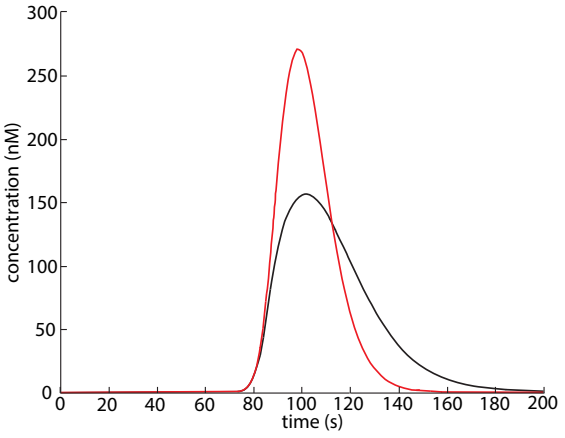
tra [24]) the differential equation governing the time evolution of the sensitivities, $\sigma_{ij}(t) := \partial x_i/\partial k_j$, for all states x_i and parameters k_j, and then simulate it numerically in tandem with the ODEs for the state variables. A Euclidean norm is taken across time and (normalized) states to compute an *overall* sensitivity for each parameter. Since we are interested in the sensitivity of the system not only along the orthodox trajectory predicted by our model, but for nearby trajectories as well, we simulate the ODEs for a range of different parameter values: on each of 200 trials we independently draw each k_j from a uniform distribution between 50% to 150% of its nominal value and re-simulate the two systems of differential equations. The resulting overall sensitivity vectors $\hat{\sigma}$ are normalized at each trial into a $[0, 1]$ range, and averaged over trials.

Most sensitive parameters. Table 2 shows the 25 most sensitive rate constants, ranked according to average normalized sensitivity $\bar{\sigma}_j$. We note that 200 trials appears to be sufficient for the $\bar{\sigma}_j$ to converge.

How does this compare to the sensitivity analysis of [24], which models more or less the same portions of the cascade, but including a few more equations for platelet binding? First of all, these platelet reactions account for about half their list of most "fragile" (sensitive) reactions—which is somewhat curious, since platelet disorders of coagulation are not common. Second, the reactions involved in Xa:Va activation of



(A)



(B)

Figure 4: Simulations of the time course of thrombin concentrations (factor IIa) in nM. (A) Moderate-to-seve hæmophilia (red) and normal clotting (black). (B) factor-V Leiden (red) and normal clotting (black).

thrombin are on average more sensitive in our model than the reactions for activation of factor X by the TF:VIIa complex; whereas the converse is true in [24]. In both, however, these reactions (and their related platelet reactions in [24]) dominate the list.

Sensitivity and prothrombin time. One of the (two) most common clinical tests for a clotting problem is the prothrombin-time (PT) test, a measure of the time required for TF-initiated coagulation of blood plasma. Blood is drawn into a test tube containing citrate, which soaks up the free calcium ions and thus prevents the main clotting reactions; is centrifuged to separate out the blood cells; and is then activated by addition of an excess of calcium and tissue factor. Clotting time is measured from the addition of TF to the first visible signs of clot formation in the

Rank	Reaction	mean ± std
1	Xa:Va:II ⇀ Xa:Va:mIIa	0.87 ± 0.17
2	Xa:Va + II_L ⇀ Xa:Va:II	0.72 ± 0.20
3	TF:VIIa:X ⇀ TF:VIIa:Xa	0.71 ± 0.21
4	TF + $VIIa_L$ ⇀ TF:VIIa	0.65 ± 0.20
5	Xa_L + Va_L ⇀ Xa:Va	0.65 ± 0.20
6	Xa:Va + II_L ↼ Xa:Va:II	0.62 ± 0.19
7	V:mIIa ⇀ Va_L + $mIIa_L$	0.56 ± 0.017
8	Xa_L + Va_L ↼ Xa:Va	0.53 ± 0.16
9	X_L + TF:VIIa ⇀ TF:VIIa:X	0.52 ± 0.18
10	X_L + TF:VIIa ↼ TF:VIIa:X	0.51 ± 0.17
11	XIa:IX ⇀ IXa_L + XIa	0.48 ± 0.15
12	PC_L + IIa:Tm ⇀ IIa:Tm:PC	0.47 ± 0.14
13	XI:IIa ⇀ XIa + IIa	0.46 ± 0.15
14	IXa_L + $VIIIa_L$ ⇀ IXa:VIIIa	0.46 ± 0.014
15	XI + IIa ⇀ XI:IIa	0.46 ± 0.15
16	V_L + $mIIa_L$ ⇀ V:mIIa	0.43 ± 0.13
17	X_L + IXa:VIIIa ⇀ IXa:VIIIa:X	0.43 ± 0.13
18	$VIIIa_L$ + APC:PS ⇀ APC:PS:VIIIa	0.41 ± 0.13
19	IIa:Tm:PC ⇀ APC_L + IIa:Tm	0.40 ± 0.13
20	VIII:mIIa ⇀ $VIIIa_L$ + $mIIa_L$	0.39 ± 0.12
21	XI + IIa ↼ XI:IIa	0.38 ± 0.13
22	V_L + $mIIa_L$ ↼ V:mIIa	0.38 ± 0.12
23	IX_L + XIa ⇀ XIa:IX	0.37 ± 0.11
24	IXa:VIIIa:X ⇀ Xa_L + IXa:VIIIa	0.36 ± 0.12
25	APC:PS:VIIIa ⇀ $VIIIai_L$ + APC:PS	0.35 ± 0.10

Table 2: Averaged, normalized sensitivities (see text) for the 25 most influential rate constants, and their associated reactions. No. 6 is a part of a binding reaction; no. 7, part of an activation.

tube, i.e. when about 10 nM thrombin (factor IIa) has been produced [25]. The healthy range for this time is considered to be 12-15 seconds.

The PT test can be simulated in our model by initializing clotting with a saturating amount of tissue factor (20 nM, vs. 0.005 nM in the simulations above). Yet this yields a clot time around 30 seconds. If we assume the *structure* of the model is correct, then this discrepancy must be caused by parameter errors—which is plausible, since literature values for rate constants vary by upwards of orders of magnitude [26] (or cf. the rate constants of [3] and [24], and their references).

Can this discrepancy be eliminated by small changes in parameters? No explicit solution is possible, but the sensitivity analysis allows a form of gradient ascent: we compute the sensitivity of thrombin over the first p seconds to changes in the rate constants, i.e. $\partial[\mathrm{IIa}]/\partial k_j$, and then change only the most sensitive parameters, moving them in the direction of greater thrombin. True gradient ascent would require the recalculation of the thrombin sensitivity at every step, but this requires prohibitively expensive numerical solution (see [12] for details), so instead we average

$$s_j^{[IIa]} = k_j \left(\frac{1}{x_i^*} \frac{\partial[\mathrm{IIa}]}{\partial k_j} \right) \Bigg|_{t=t^*}, \qquad (8)$$

Max Shift (%)	Min Params
60	12
50	19
40	70

Table 3: Minimum number of parameters required to shift in order to achieve a PT time of 13 seconds, for three different limits on the maximum parameter shift.

the thrombin sensitivity at the time of the clot (13 s), over 200 numerical simulations of the governing ODEs, each with randomly perturbed parameters, as above. Also note that although we have again neglected the discrete parts of the network, we can do so with confidence, since (1) the PT test ignores the intrinsic pathway, and (2) we can estimate clotting time from thrombin alone under the assumption of healthy functioning of fibrin and factor XIII.

How few parameters can we alter and still effect the desired PT, given an upper limit on those parameter shifts? Table 3 shows the results for three different upper limits.

Two interesting features of these results should be noted. First, entertaining parameter variances of the reasonable (in light of the dispersion of literature values) figure of 60% enables the model to match clinical PT times with the shifting of just 12 (out of over 100) parameters. This shows that very few parameters can do a lot of work. Contrariwise, forcing all rate constants to live within 40% of their nominal values precipitously increases this minimum number of rate constants, up to about two-thirds of them. Thus the relationship between the maximum shift and minimum number of parameters is evidently nonlinear; and in particular the latter moves from a small minority of the total number of parameters to a sizable majority as the maximum shift is drawn down under the 50% mark. We may likewise ask the reverse question: how small we can make the maximum parameter shift if we let *all* of the parameters vary? The answer is 38%—which, considering that 40% shifts require the movement of about 40 fewer rate constants, and 50% shifts require alteration of 90 fewer, indicates how very little effect these remaining constants have on thrombin levels—at least at $t = 13$ seconds.

5 DISCUSSION AND CONCLUSIONS

The simulations of factor-V Leiden and hæmophilia A were consistent with clinical results, and to that extent vindicate both the present model and its parameters as well as the methodology, i.e. a HS approach. Of course, consistency is not tantamount to quantitative identity, but here the obstacle lies not with the model so much as the state of the clinical data. As [27] has pointed out, most thrombin curves can be fit by an equation with a mere *four parameters*, so these data vastly underdetermine the parameters of the model. Including clot time does not substantially decrease the number of free parameters.

We interpret our simulations, then, as having demonstrated a method for incorporating qualitative or otherwise discrete information into a single model, which model is *consistent* with clinical findings; but we do not claim the model to be error-free. On the other hand, the model also provides a platform for testing clinical hypotheses, quantitative or qualitative, about the coagulation cascade, since consistency with known data is a necessary (if not sufficient) criterion for the correctness of those hypotheses. This provides a method for model refinement, as well. Furthermore, inasmuch as this kind of model can indeed capture all the relevant details of coagulation, the analysis techniques demonstrated in this paper will apply equally well to a "final," error-free model.

Finally, we saw that (restricting our view to the continuous subsystem) relative changes in protein concentrations are most sensitive to changes in the rate constants involving activation of thrombin by the Xa:Va complex and activation of Xa by the TF:VIIa complex. We also saw that these sensitivities could be exploited to change the model in the most parsimonious way in order to match the results of the PT test: shifting a mere 12 rate constants by as little as 60% sufficed to achieve a reasonable 15 seconds for this test.

On the other hand, it turns out that using these parameters in the original clotting simulations of this study vitiates their agreement with clinical results. That suggests that something more subtle than rate constant alterations is responsible for the discrepancy in PT-test results.

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