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ORIGINAL ARTICLE

Using a Systems Pharmacology Model of the Blood Coagulation Network to Predict the Effects of Various Therapies on Biomarkers

S Nayak[1], D Lee[2], S Patel-Hett[3], DD Pittman[3], SW Martin[1], AC Heatherington[2], P Vicini[4] and F Hua[2]*

A number of therapeutics have been developed or are under development aiming to modulate the coagulation network to treat various diseases. We used a systems model to better understand the effect of modulating various components on blood coagulation. A computational model of the coagulation network was built to match in-house *in vitro* thrombin generation and activated Partial Thromboplastin Time (aPTT) data with various concentrations of recombinant factor VIIa (FVIIa) or factor Xa added to normal human plasma or factor VIII-deficient plasma. Sensitivity analysis applied to the model revealed that lag time, peak thrombin concentration, area under the curve (AUC) of the thrombin generation profile, and aPTT show different sensitivity to changes in coagulation factors' concentrations and type of plasma used (normal or factor VIII-deficient). We also used the model to explore how variability in concentrations of the proteins in coagulation network can impact the response to FVIIa treatment.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? ☑ The coagulation network is comprised of a complex interplay of various procoagulant and anticoagulant factors. Several systems models of the network have been published, which have focused mostly on thrombin generation in normal plasma. • WHAT QUESTION DID THIS STUDY ADDRESS? ☑ This study used a validated computational model to predict the behavior of TGA and aPTT in response to modulations of the coagulation network. • WHAT THIS STUDY ADDS TO OUR KNOWLEDGE ☑ This work provides a coagulation network model that includes several clinically relevant biomarkers and has been validated with an extensive dataset. Using this improved model, this study highlights the differences in changes in lag time, peak thrombin, and AUC of the TGA and aPTT to modulation of various components in the coagulation network. The model also identifies potential biomarkers that can predict variability in FVIIa treatment effect. • HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS ☑ Our analysis can be helpful in selecting the appropriate biomarkers to understand the effect of therapy in hemostatic diseases. This work also demonstrated how we can use the model to identify biomarkers for predicting responses to therapies.

Coagulation is one of the steps involved in hemostasis, i.e., the cessation of blood loss from a damaged vessel. With the advantage of easy access to the blood, the proteins involved in the coagulation cascade have been extensively studied and well characterized.[1,2] The coagulation system includes both pro- and anticoagulant proteins and can be activated through either the intrinsic or the extrinsic pathway. Disruption of the balance between pro- and anticoagulant components can lead to various diseases, e.g., decreased procoagulant capability due to genetic or acquired loss of procoagulant factors can result in hemorrhagic diseases, such as hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency), hemophilia C (factor XI deficiency), parahemophilia (factor V deficiency), and hypoprothrombinemia (prothrombin deficiency).[3–7] On the other hand, decreased anticoagulant capability can result in thrombotic diseases such as antithrombin III (ATIII) deficiency, protein C deficiency, protein S deficiency (deep vein thrombosis), and factor V^{Leiden} thrombophilia).[8–11] Many therapies have been developed to treat coagulation system disorders, such as factor VIII (FVIII) and factor IX (FIX) replacement therapies for hemophilia A and B patients, respectively[12,13]; FVIIa (NovoSeven), prothrombin complex concentrate (PCC), and FEIBA as bypassing agents for the treatment of hemophilia A or B patients with inhibitors[14-16]; warfarin, heparins, and more recently with more targeted therapy such as thrombin inhibitor (dabigatran) or factor Xa (FXa) inhibitor (rivaroxaban and apixaban) for preventing venous thromboembolic disease (VTE).[17,18] Since the coagulation system is highly complex and nonlinear, with multiple feedback loops, quantitative understanding of the system using mathematical models can help support many aspects of drug development, such as new target identification,[19] better understanding of the mechanism of action of therapeutics, biomarker response and their variability, dosing regimen optimization, and prediction of therapeutic effects in different diseases.[19,20]

[1]Pharmacometrics, Global Innovative Pharma Business (GIPB), Pfizer Inc., Cambridge, Massachusetts, USA; [2]Quantitative Clinical Sciences, PharmaTherapeutics Clinical R&D, Pfizer Inc., Cambridge, Massachusetts, USA; [3]Rare Disease Research Unit Pfizer Inc., Cambridge, Massachusetts, USA; [4]Pharmacokinetics, Dynamics and Metabolism, New Biological Entities, Pfizer Inc., Cambridge, Massachusetts, USA. *Correspondence: F Hua (fei.hua@pfizer.com)
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There is increasing evidence for the thrombin generation assay as a useful biomarker for monitoring clinical effects of coagulation modulating therapies such as replacement therapy with FVIII or bypass therapy with FVIIa.[21,22] APTT is also widely used in the clinical setting to diagnose coagulation disorders and is a biomarker modulated by various therapies such as FVIII or FIX replacement therapy and FVIIa bypass agent. Therefore, a single coagulation model that accounts for both the endpoints, thrombin generation and aPTT, can be broadly useful, as earlier models have focused on each of these endpoints separately.[23–25] In this study, we attempt to bridge this gap by modifying and combining existing models from the literature to include both intrinsic and extrinsic pathways. We then use global parameter optimization to simultaneously fit in-house thrombin generation assay (TGA) and aPTT data with FVIIa or FXa spiked into normal human plasma (NHP) and factor VIII-deficient plasma (8DP). Finally, we used the model to understand differences in TGA and aPTT responses when various modulations or therapies are applied *in silico* and to identify if normal intersubject variability in protein concentrations could explain the observed variability in TGA response to FVIIa therapy.

METHODS

Experimental materials and methods

Please refer to the **Supplementary Materials** for experimental details about the TGA and the aPTT assay performed to generate calibration data for the model.

In silico methods

Development of the coagulation network model. A mechanistic model of the blood coagulation network was constructed including both intrinsic and extrinsic pathways and ending at fibrin formation. **Figure 1** shows the schematic of the network described in our model. The detailed description of model construction and model structure, as well as a complete list of reactions used in the model, is listed in the Supplementary Information (**Supplementary Information & Table of Reactions**), along with the rate parameters and nonzero initial conditions for proteins. The model assumed a well-mixed system for *in vitro* experiments. The resulting system of ordinary differential equations was simulated using MATLAB's (MathWorks, Natick, MA; v. 2012b 64-bit) SimBiology (v. 4.2) toolbox (see Supplementary Information: Coagulation_Systems_Model_Nayak_et_al.sbproj).

Simulation of TGA and aPTT assay. To optimize the model parameters, we performed the aPTT and TGA experiments *in silico*. TGA experiments were simulated by fixing the tissue factor (TF) level at 1 pM to stimulate extrinsic pathway activation and contact activator (CA) level at zero to inactivate the intrinsic pathway. All the proteins were diluted 1.7-fold and simulations were run for 60 minutes, mimicking the experimental conditions. TGA results were calculated in the model as the sum of thrombin and meizothrombin according to the formula: TGA = thrombin + 1.2 * meizothrombin.[26] Lag time, peak thrombin, and endogenous thrombin potential (ETP) (area under the curve, AUC) were derived from the TGA time profile. Lag time was calculated as the time required for the TGA assay value to reach 1/6th of the peak value (peak thrombin).[27] The aPTT experiment was simulated *in silico* in a two-step manner, first the preincubation was modeled by setting CA levels to 300 nM and only allowing reactions from CA to FIXa to occur (other reaction rates were set to zero) for the first 3 minutes. Afterwards, reaction rates were set back to their original nonzero values to simulate addition of $CaCl_2$. aPTT was measured as the time required for the area under the fibrin concentration–time curve to reach a value of 1,500 nM·s, after the initial 3-minute incubation time.[28]

Additionally, in order to simulate FVIII (or FIX) deficient plasma, initial level of FVIII (or FIX) was set to zero in the simulations.

Parameter optimization. First, we employed a global numerical optimization technique to fit TGA experimental data. The least-square-based objective function summarized the results from 28 experimental conditions, and the optimization algorithm minimized the difference in thrombin generation between experimental results and model predictions. The combined objective function can be represented as:

$$Error = \sum_{expt=1}^{expt=28} Weight_{expt} \sum_{time=1}^{time=end} \sqrt{\left(TGA_{expt,time} - TGA_{Model,time}\right)^2}$$

In our optimization calculations, we tried different weighting schemes (e.g., weighting after normalization by the number of data points in each experiment, weighting according to the relative error, adaptive weighting during optimization inversely proportional to error in the previous iteration, or inversely proportional to the model simulation value in the previous iteration, etc.), but kept all the weights equal to 1 for the final optimization, since it provided the best fitting results by visual inspection. The 28 different experimental conditions can be divided into four groups as follows:

- TGA in normal human plasma with seven different concentrations of FVIIa [0–4,000 nM] added
- TGA in normal human plasma with seven different concentrations of FXa [0–1 nM] added
- TGA in FVIII-deficient plasma with seven different concentrations of FVIIa [0–4,000 nM] added
- TGA in FVIII-deficient plasma with seven different concentrations of FXa [0–1 nM] added

The combined objective function was minimized using the simulated annealing global optimization method implemented in MATLAB (v. 2.12b). We allowed all the reaction rates to change within a 2-fold range from their nominal literature values during the optimization procedure. However, since the initial protein concentrations are well established and show consistent values among various published models,[26,28,29] we did not change them during optimization.

Next, we used the best set obtained by fitting TGA data to simulate aPTT experiments. Two sets of experimental conditions were available for model fitting:

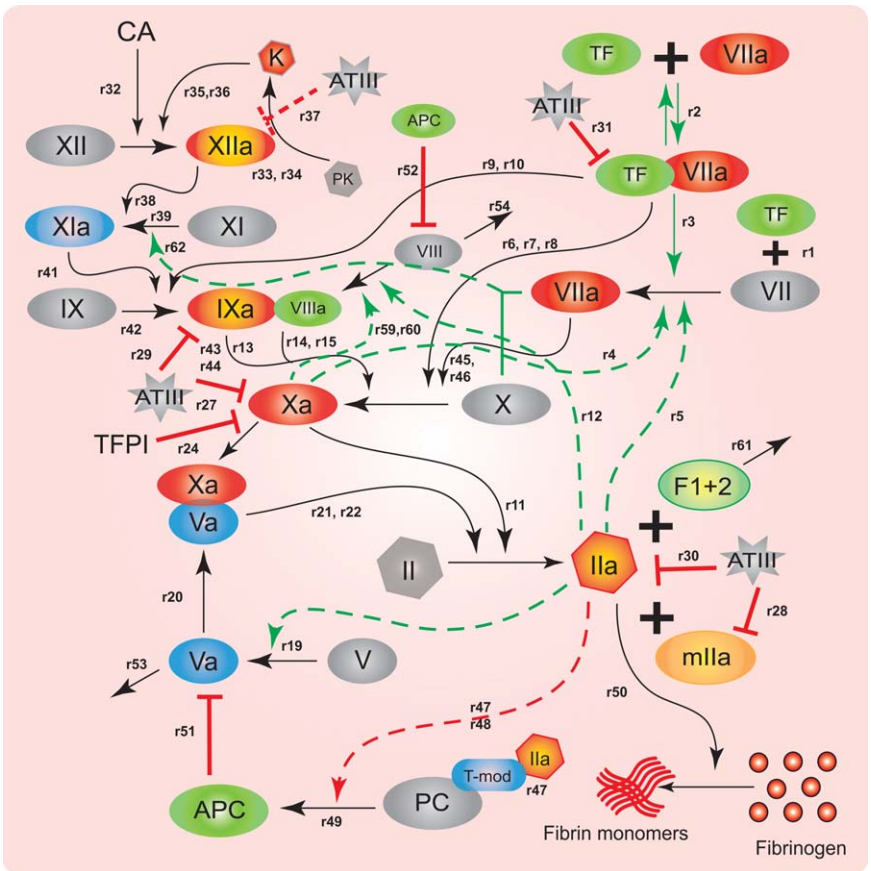

Figure 1 A schematic of the coagulation network. The green broken lines represent positive feedback mechanisms; red broken lines represent mechanisms that inhibit formation of thrombin, a key product of the coagulation network that catalyzes the formation of blood clots, and the solid red lines represent the activation of anticoagulant proteins in the system. The numbers alongside the arrows show the reaction number in the model. Not all the reactions in the model are shown here, to lessen the complexity of the figure. APC, active protein C; PC, protein C; TF, Tissue Factor; TFPI, Tissue Factor Pathway Inhibitor; CA, Contact Activators; F1 + 2, prothrombin fragment 1 + 2; mIIa, meizo-thrombin; Tmod, thrombomodulin; IIa, thrombin; PK, pre-Kallikrein; K, Kallikrein; ATIII, anti-thrombin III; XII, factor XII (FXIII); XI, factor XI (FXI); X, factor X (FX); IX, factor IX (FIX); VIII, factor VIII (FVIII); VII, factor VII (FVII); V, factor V (FV). For these factors the a after factor name indicates its active form.

- adding different levels of FVIIa [0.0444–444 nM] to FVIII-deficient plasma
- adding FXa [0.001–100 nM] to normal human plasma

In order to match the dose-dependent decrease of aPTT with increasing concentrations of FVIIa, we added the following additional reaction in the network as an attempt to model the interaction of the extrinsic and intrinsic pathways (see **Supplementary Table S1** for the full list of reactions in the model).

$$\text{FVIIa\_FX} + \text{FXIa} \rightarrow \text{FVIIa} + \text{FXa} + \text{FXIa}$$

This added reaction parsimoniously represents the combined effect on FX conversion of the extrinsic (FVIIa) and intrinsic (FXIa) pathway. Only the rate constant of this irreversible reaction was adjusted to match experimental aPTT results. We also confirmed that addition of this reaction did not affect our earlier results regarding fitting to the TGA data. This reaction was included for all the simulations shown in this article.

Simulation for the effect of population variability in protein concentrations on TGA responses to FVIIa treatment. To simulate TGA response to FVIIa treatment in hemophilia A subjects, the FVIII concentration was set to zero to mimic hemophilia A condition, and 20 nM FVIIa was added to the TGA simulation. To simulate population variability in protein concentrations, the initial concentrations of FVII, FX, FIX, FII, FV, TFPI, FXII, FXI, PK, Tmod, and fibrinogen were all randomly chosen within a range of ±25% from their nominal value. TGA responses to 20 nM of FVIIa for a total of 100 hemophilia subjects with randomly selected initial concentrations were recorded.

Local sensitivity analysis. Local sensitivity analysis was performed on TGA parameters using overall fully normalized sensitivity. Overall fully normalized sensitivity was chosen since it captures the effect of varying initial amounts of species, is calculated over the entire time period of simulation, and provides the best representation of the overall effect exerted. Such a parameter has been used previously for sensitivity analysis both in signaling cascades and in the

coagulation network.[25] Overall fully normalized sensitivity was calculated as:

$$S = \int_0^T \left(\frac{X(0)}{IIa(t)}\right) \frac{\partial [IIa(t)]}{\partial X}$$

where *IIa* represents thrombin, *X* is the protein/coagulation factor with respect to which sensitivity is being calculated, and T is the final time for the simulation (i.e., 3,600 seconds).

RESULTS

Model optimization by fitting to TGA and aPTT data

Figure 2 shows a comparison between experimental data and simulation results for TGA after parameter optimization. The experimental TGA data for different initial levels of FVIIa and FXa in normal human plasma (NHP) and FVIII-deficient plasma (8DP) are shown in the left-most column (circles) (**Figure 2**, **panels a,f,k,p**). The second column from the left (**panels b,g,l,q**) shows the corresponding optimized simulation results (lines). Panels in the right three columns of **Figure 2** (**panels c,h,m,r; d,i,n,s; e,j,o,t**) show the comparison between TGA parameters derived experimentally (gray lines) and from simulation results (red lines) for lag time, AUC (ETP), and peak thrombin. The FVIIa or FXa dose-dependent TGA profiles from simulations match well with the *in vitro* experimental data. In a few cases, e.g., simulated AUC with various concentrations of FXa added to 8DP (**panel s**), is consistently lower than the experimental value, but the dose-dependent trend is well captured.

Figure 2, **panel u**, shows a comparison between the aPTT values obtained from the experiments (gray bars) and the values obtained from simulations (red bars) for different levels of FVIIa added to FVIII-deficient plasma, whereas **Figure 2**, **panel v**, shows the same comparison for different levels of FXa added to normal human plasma. The model is able to match experimental values and predict the dose-dependent reduction in aPTT in both cases.

Effects of varying initial levels of proteins on TGA

Therapeutic intervention in the coagulation cascade often involves modulating the levels of inactive factors (e.g., FVIII or FIX replacement therapy for hemophilia A and B patients) or active factors (e.g., addition of FVIIa, procoagulation agent such as bypassing agent for hemophilia patients with inhibitors)[30–32] or inhibitors of anticoagulants (e.g., anti-TFPI[33]). Using the optimized model, we systematically tested the effect of varying the initial concentrations of various proteins (active and inactive) on the TGA profile in NHP or 8DP.

Zymogens or inhibitor proteins were varied by two orders of magnitude ($0.1\times - 10\times$) one at a time from the nominal value. TGA was then simulated in the model with perturbed protein levels and fold changes from nominal values ($Y_{new}/Y_{nominal}$) in TGA parameters were plotted (**Figure 3**). As expected, FXI and FXII are predicted to have no significant effect on any TGA parameters in NHP or in 8DP. Procoagulants such as TF and anticoagulants such as ATIII and TFPI significantly affect all three measures of TGA response in both NHP and 8DP. Decreasing ATIII has the largest effect on AUC and peak; however, its impact on lag time is minimal. Similarly, decreasing PC in NHP has a larger effect on AUC and peak, but the effect on lag time is much smaller. Modulation in FVII in 8DP seems to have a greater effect on lag time, but the effect on peak thrombin and AUC seems to be muted. Based on the magnitude of change and the number of proteins that can change it, lag time is the most tightly regulated among the three parameters and therefore hardest to change by therapeutic intervention *in silico*, whereas peak thrombin values are most easily modulated, especially in 8DP. Full TGA profiles on varying zymogens are shown in **Supplementary Figures S1–S3**.

Our results also point to the subtle differences in FVIII-deficient plasma (hemophilia A) and normal plasma, e.g., changing FX levels leads to 0–50% change in all three measures of TGA in normal plasma, but corresponding changes in 8DP are much lower for peak thrombin and AUC, suggesting that modulating FX may not have as big an effect in hemophilia as expected from normal plasma. FV modulation, however, shows a trend pointing in the opposite direction.

The effects of active proteins on TGA parameters in NHP and 8DP are shown in **Figure 4**. Since most of the active proteins have zero value as initial concentrations, we could not use ($0.1\times - 10\times$) nominal levels in this analysis. Thus, we picked the same concentrations 0, 0.05, 5, or 120 nM for all the active proteins, to cover both physiological and super-physiological values while allowing a comparison of sensitivity among them. Different from their corresponding zymogens, FXIa and FXIIa showed a considerable effect on all the parameters of TGA. Similarly, opposite from FV, increasing FVa has little effect on peak thrombin and AUC, but decreases lag time. FVIIa also has a smaller effect on peak and AUC, but exerts a larger effect on lag time. Interestingly, TGA from NHP seems to be more sensitive to FVIIa level increases compared with TGA from 8DP.

The effect of varying protein initial concentrations in FIX-deficient plasma is almost the same as in 8DP; therefore, the results are shown in **Supplementary Figures S4–S5.**

Effects of varying initial levels of proteins on aPTT

Similar to the analysis for TGA, we also used the model to study the effects of varying the initial concentrations of zymogens or their active forms on aPTT (**Figure 5**). The initial concentration of zymogens and inhibitor proteins were varied by two orders of magnitude ($0.1\times - 10\times$) from their nominal levels (**Figure 5**, **panel a**) and active proteins were added at 0.05, 5, or 120 nM concentrations (**Figure 5**, **panel b**) to match previous simulations for TGA response. The dashed red and blue lines indicate aPTT values when all the protein concentrations are at their nominal values for NHP and 8DP. As seen from **Figure 5** (**panel a**), as expected, proteins in the intrinsic pathway and common pathway (FIX, FXI, FXII, and FX) have a larger effect on aPTT than FV and FVII, for both NHP and 8DP. Among inhibitors, ATIII affects aPTT in 8DP and NHP, but TFPI and PC have no effects in either type of plasma. Overall change

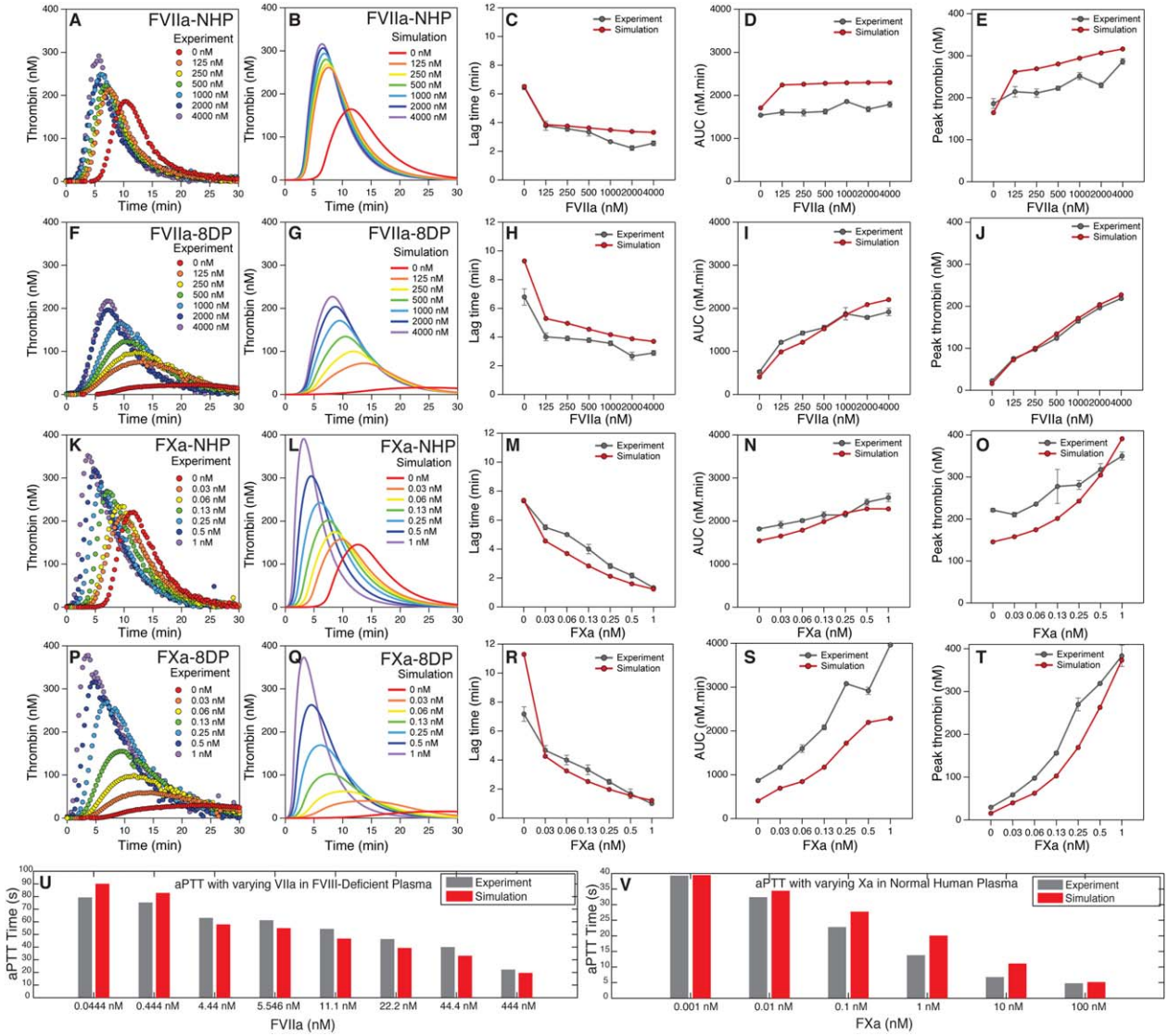

Figure 2 Model optimization against TGA and aPTT data. (**a–e**) TGA for various concentration of FVIIa added to normal human plasma (NHP). (**a**) shows the experimental data, (**b**) shows the model simulation results of the corresponding experiment. (**c–e**) Comparison between experimental (black line) vs. simulation (red line) results for lag time (**c**), AUC (**d**), and peak thrombin (**e**). (**f–j**) Comparison between experimental and simulation results when FVIIa was added to FVIII-deficient plasma (8DP) in the TGA experiment. (**k–o**) Comparison results of varying levels of FXa added to normal plasma. (**p–t**) Comparison results of varying levels of FXa added to 8DP. (**u**) Experimental (gray bar) and simulation (red bar) aPTT results when varying FVIIa added in 8DP. (**v**) Experimental (gray bar) and simulation (red bar) aPTT results when varying FXa added in NHP.

in aPTT is much more pronounced in 8DP than that in the NHP, probably due to higher nominal aPTT levels in hemophilia patients.

Among the active proteins investigated (**Figure 5**, **panel b**), FVIIIa is the most sensitive factor in 8DP. At 0.05 nM, the model predicts that it could normalize aPTT from hemophilia patients to normal subjects. FIXa is the most sensitive factor in NHP. Addition of higher levels of FIXa have a large-scale effect in aPTT, and have not been plotted so as to preserve the scale of the plot. The model predicted a relatively high concentration (120 nM) of FVIIa is needed to shorten aPTT in 8DP to NHP levels. Finally, while FV has little effect on aPTT, an increase in FVa has a moderate effect on decreasing aPTT. A similar comparison of aPTT changes in NHP and 9DP is shown as **Supplementary Figure S6**.

Effect of small variation in coagulation factor levels on the TGA response to rFVIIa treatment

There are substantial variations in the responses to FVIIa treatment.[34] We used the model to explore whether small variations in the protein levels (up to 25% in either direction) due to normal population variability could contribute to the observed variability in the response. The TGA profile resulting from treatment with 20 nM of FVIIa in 8DP was

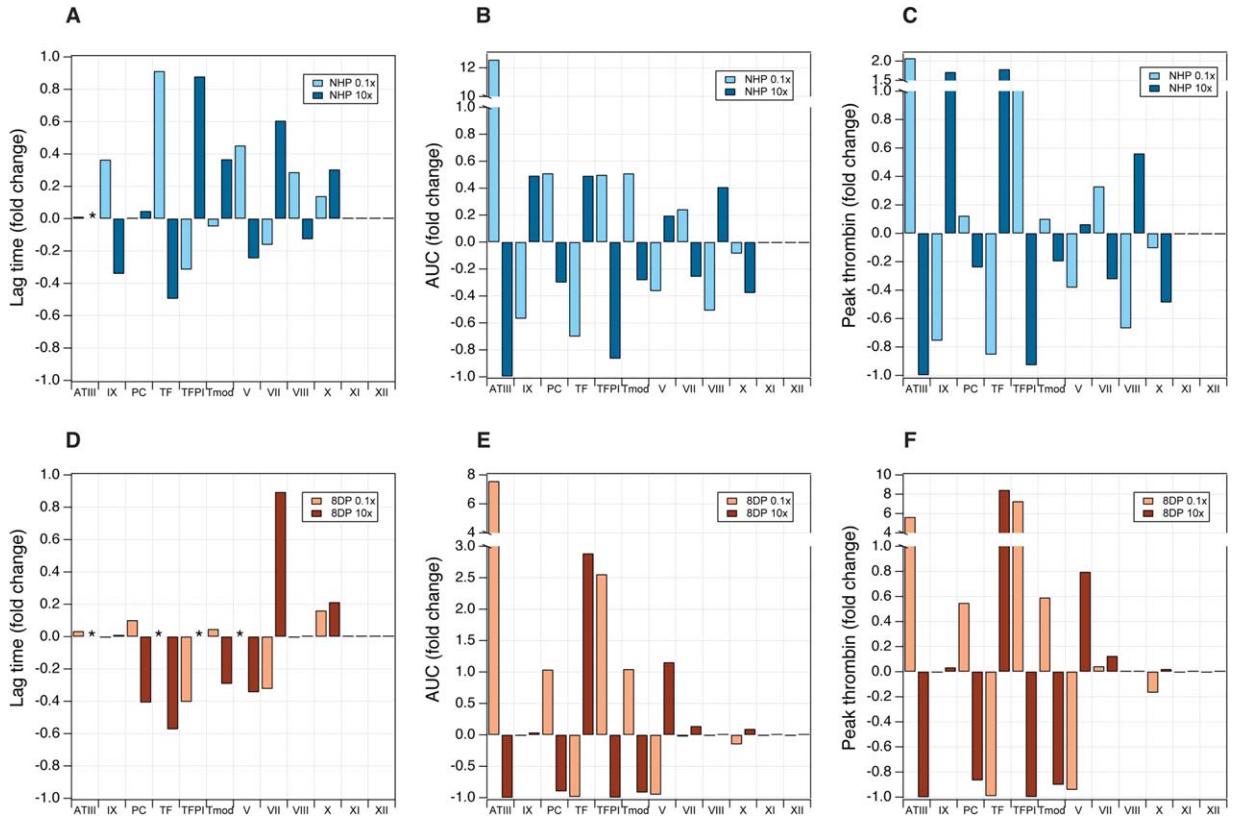

Figure 3 Effects of varying zymogen and inhibitor levels in NHP and 8DP on TGA parameters. (**a–c**) The effects on varying zymogens 10-fold lower (lighter color) or higher (darker color) than the nominal values in NHP; (**d–f**) show the same as (**a–c**), but for 8DP. Asterisk denotes that the lag time cannot be accurately determined, as the TGA profile is highly suppressed such that peak thrombin is less than 1.3 nM.

simulated for 100 subjects with small variation of coagulation protein levels (**Figure 6**, **panel a**). **Figure 6** (**panel b**) shows the variation in lag time, AUC, and peak thrombin expressed as fold change from the nominal values for 8DP. The simulation results show that substantial variability in TGA can be observed. Peak thrombin is most sensitive to changes in the initial levels of coagulation protein and lag time is least sensitive to the variation of the protein levels. To understand which protein may contribute the most to the variability in TGA response to FVIIa, we applied local sensitivity analysis to the 100 sets of initial conditions representing the 100 subjects. The mean results of the 100 sensitivity analyses are shown in **Figure 6** (**panel c**). As expected, the potent anticoagulant ATIII is very sensitive, implying that TGA levels are highly dependent on variations in ATIII. TGA is also predicted to be very sensitive to changes in the TFPI level.

DISCUSSION

We present a detailed mechanistic model of the coagulation network, constructed by combining previously published literature models and further optimized to match in-house experimental TGA and aPTT data. TGA and aPTT were chosen since they each represent the effectiveness of extrinsic and intrinsic pathway activation, respectively, and are both important assays in the clinical setting. Although PT is another commonly tested clinical assay, it is not included in the model at this time, mainly due to lack of internal data during model development. We used the optimized model to explore the effects of modulating either inactive zymogens and inhibitors or active proteins on TGA and aPTT endpoints. We also used the model to explore variability in response to FVIIa treatment.

To our knowledge, this work is a first attempt to estimate parameters in the coagulation cascade using the *in vitro* TGA and aPTT, for both normal plasma and FVIII-deficient plasma simultaneously, using an automated global optimization routine (simulated annealing). The optimized model was able to describe experimental results and to show dose-dependent changes in TGA parameters (lag time, peak thrombin, and AUC) and aPTT. Better fits can be obtained by optimizing only against a subset of experiments one at a time (**see Supplementary Figures S7–S10** for details). A potential explanation is that this large set of experiments was conducted on different days and using different batches of plasma; these circumstances contribute to data variability, which makes it difficult to define a single set of parameter values to simultaneously fit all the data. Overall, our results suggest the underlying model structure is of sufficient complexity and can be tailored to individual datasets.

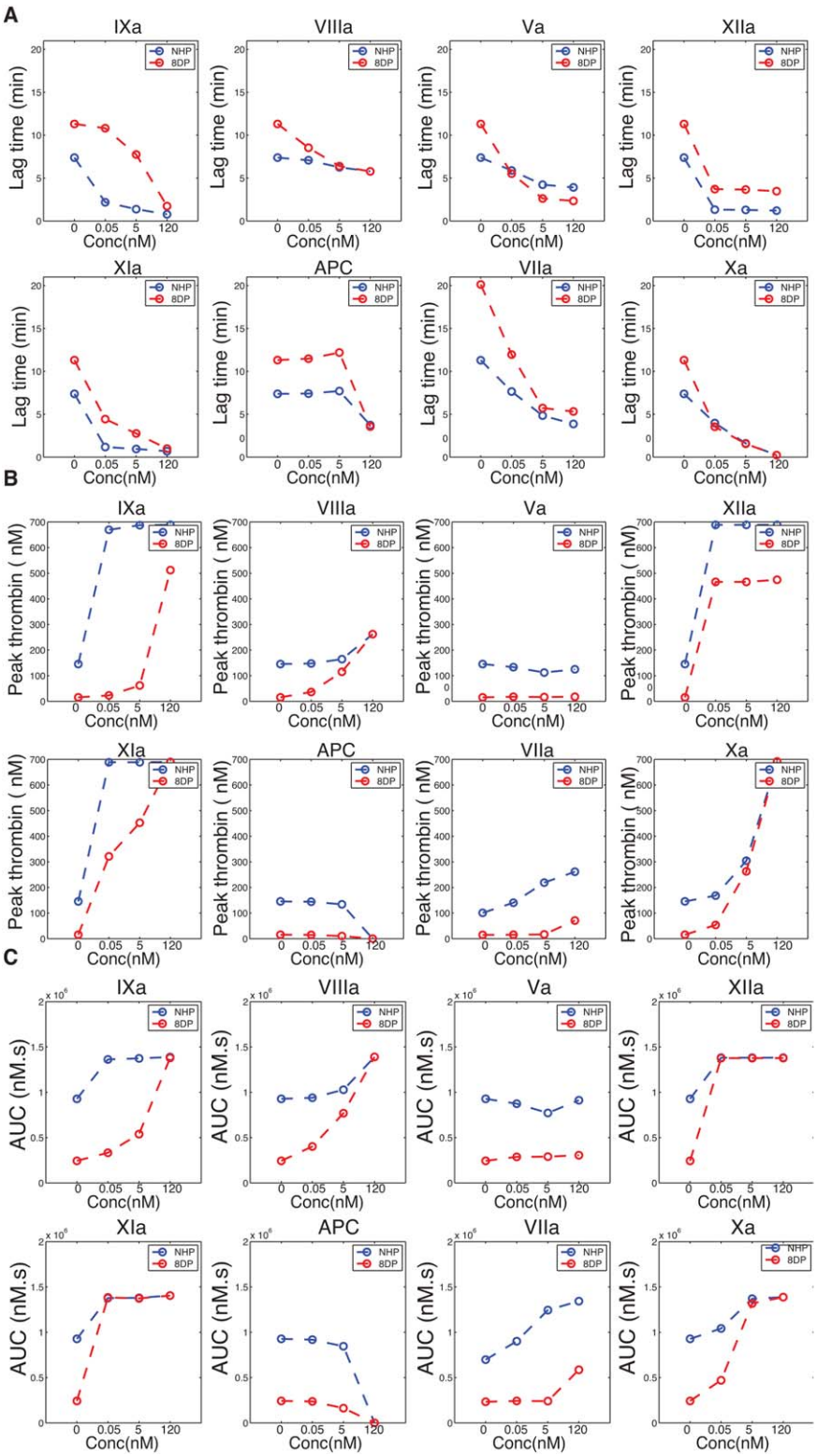

Figure 4 Effects of varying active protein levels in NHP and 8DP on TGA (lag time (**a**), peak thrombin (**b**), and AUC (**c**)). All the active protein concentrations were fixed at 0.05, 0.5, or 120 nM to simulate low, medium, and high concentrations of the active enzymes.

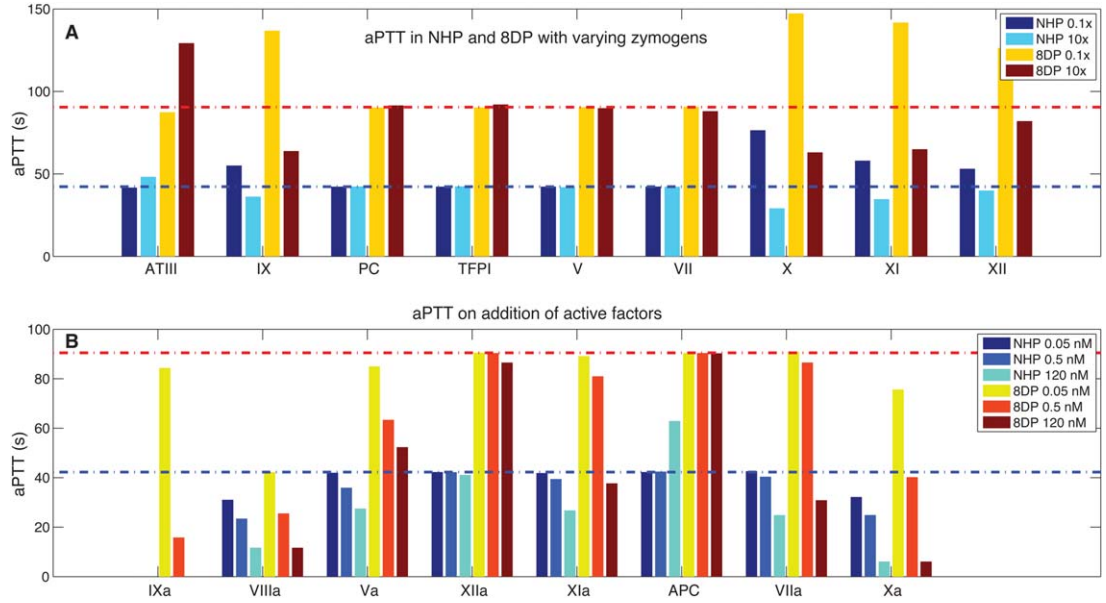

Figure 5 Effects of varying zymogen, inhibitor, and active protein levels in NHP and 8DP on aPTT. (**a**) Changes in aPTT with modulation of zymogen and inhibitor levels. (**b**) Changes in aPTT with modulation of active proteins. The dashed broken lines indicate aPTT values when all the proteins are at their nominal levels for NHP (blue line) or 8DP (red line).

The model was then used to answer pertinent questions that arise in clinical therapy for diseases such as hemophilia. In particular, the model can be used to predict the relative sensitivity of various biomarkers when a hemostasis modulating therapy is applied. To help compare the therapy effects on different endpoints, changes in initial concentrations of zymogens or inhibitors and increasing initial concentrations of active molecules on TGA parameters and aPTT are summarized in four categories in **Table 1**. Different patterns of TGA and aPTT changes were observed. Some factors, e.g., FXI and FXII, change aPTT, while having a minimal effect on the TGA parameters. FX, however, changes both the TGA profile as well as aPTT. On the other end of the spectrum, some coagulation factors, e.g.,

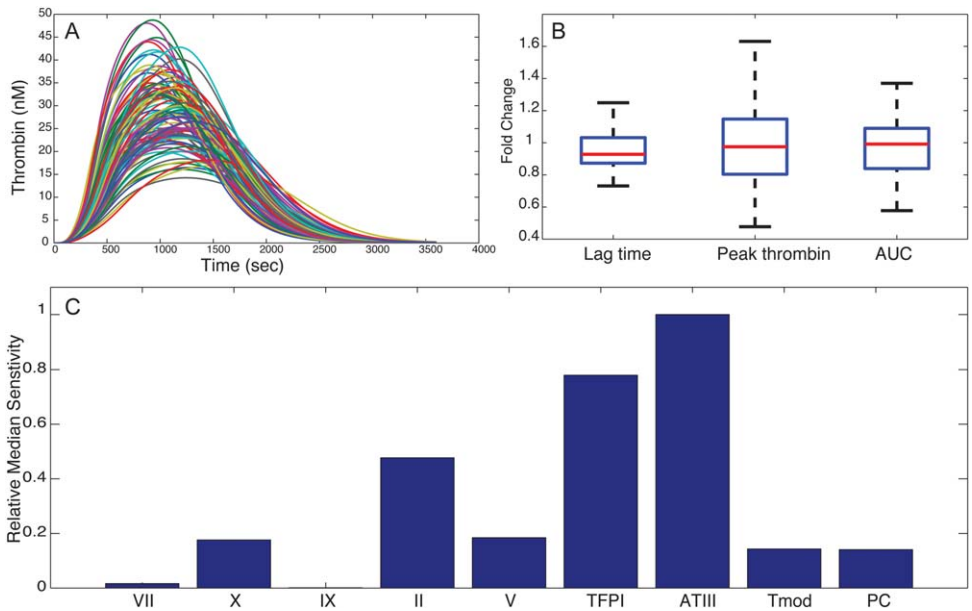

Figure 6 Effect of small variation in the levels of coagulation proteins on TGA profile in response to FVIIa treatment in 8DP by simulation. (**a**) TGA profile for 100 subjects when initial levels of zymogens or inhibitors were varied up to ±25% of their nominal values. (**b**) Boxplot of the fold change from nominal value for lag time, peak thrombin, and AUC of the TGA profile. (**c**) The relative median sensitivity values for proteins.

Table 1 Comparison between the magnitude of changes in TGA parameters and aPTT for NHP and 8DP

	Normal human plasma				FVIII-deficient plasma			
Species	Lag time	Peak thrombin	AUC	aPTT	Lag time	Peak thrombin	AUC	aPTT
ATIII	#	##	#	#	#	##	##	#
FIX	#	##	#	#	#	#	#	#
PC	#	#	#	*	#	##	##	#
TFPI	##	##	#	*	#	##	##	#
FV	#	#	#	*	#	##	##	*
FVII	##	#	#	*	##	#	#	#
FVIII	#	##	#	#	–	–	–	–
FX	#	#	#	#	#	#	#	#
FXI	*	*	*	#	*	*	*	#
FXII	*	*	*	#	*	*	*	#
FIX a	##	##	#	#	#	##	#	##
FVIIIa	#	#	#	#	#	###	#	##
FVa	#	#	#	#	##	#	#	#
FXIIa	##	##	#	*	##	###	#	*
FXI a	##	##	#	#	##	###	#	#
APC	#	#	#	*	#	###	#	*
FVIIa	##	##	##	#	##	#	#	##
FXa	##	##	#	#	##	###	#	##
TF	#	##	#	–	##	###	#	–
Tmod	#	#	#	–	#	##	##	–

The two cases being considered in this table are (i) when zymogens are varied 10x (from their nominal values) and (ii) active proteins are set at a concentration of 5 nM (see **Figures 4** and **5** for results of other cases). The difference in TGA and aPTT parameters were categorized into 4 bins according to the following scheme: relative change (Δ = TGA Parameter/TGA Parameter (nominal) – 1) < 0.01 implies – no change (shown by *), $0.01 < \Delta < 0.5$ is shown by #, $0.5 < \Delta < 5.0$ - ## and $\Delta > 5.0$ is shown by ###. The direction of change is depicted by the different colors, i.e., decrease in relative change is shown in red and an increase is shown in green.

FV and FVII, affect TGA but have no effect on aPTT. The three parameters used to characterize the TGA profile tend to either change or stay unchanged together, although the magnitude of change could be different. It is, however, important to note that in none of the simulations where initial levels of proteins were varied do we observe a maximum or minimum in the TGA parameters or aPTT. The behavior of these parameters can be characterized by an asymptotically increasing or decreasing trend. This probably suggests that there is no "sweet spot" of dose which could give maximum TGA or minimum aPTT results, although these parameters do plateau with increasing or decreasing dose. The model also predicts differences in the response of NHP and 8DP. In summary, modulating protein levels can have different effects on different biomarkers (i.e., TGA vs. aPTT), depending on their location in the intrinsic, extrinsic or common pathway, their activation status, and on the type of plasma under consideration.

As an external validation, the model prediction of varying protein levels on aPTT and TGA was compared to literature information from naturally occurring protein deficiencies. An extensive analysis of TGA profiles in congenital deficiency of coagulation factors was performed by Dieri *et al.* in which the authors measured the effect of severe, moderate, or mild deficiency of several coagulation factors, including FXII, FXI, FV, FVII, and FX on TGA.[35] Overall, our model correctly predicts the trend in TGA change due to factor depletion. On a few occasions, the model prediction does not match the experimental observation quantitatively, e.g., the impact of FV deficiency on TGA is seen only when FV levels are $\sim$1–2% of normal, but our simulations show changes in TGA parameters with 10% of FV levels (**Figure 3**, **panels a,b**). A comparison of aPTT changes observed in various coagulation factor disorders and model simulation results also showed that the model results match well qualitatively.[36,37] For example, our model correctly predicted that FVII deficiency has no impact on aPTT, FV and FX deficiencies prolong aPTT, but the clinical data are only available for severe deficiency ($<$1%).[37] Our simulations show that FV at 10% levels do not change aPTT, but at the $<$1% level, i.e., severe deficiency, aPTT was prolonged (data not shown).

We also used the model to explore whether a small variation of protein levels within the normal range can impact responses to the treatment of FVIIa. Using TGA as the endpoint, we showed significant variation in the TGA profile when the same concentration of FVIIa was added. We then used the model to further explore which of the coagulation factors may contribute most to variability in response. ATIII and TFPI were the two most sensitive parameters, suggesting that the ATIII and TFPI level could be used as a biomarker to predict how well a patient will respond to FVIIa treatment.

Based on the model simulation, modulations of different targets have clearly different effects on different endpoints. This information will be helpful when choosing endpoints to measure in clinical studies and help provide a mechanistic explanation for when changes are observed or not. The linkage of these pharmacodynamics endpoints to clinical efficacy (i.e., cessation of bleeding) has not been well established as of yet. In the future, the model could be expanded to mechanistically bridge this linkage by adding

interactions with platelets and endothelial cells at the site of clot formation, which also play a critical role in stopping blood loss at the injury site, in addition to the proteins in the circulating blood, incorporating blood flow dynamics and including dynamics of clot formation.[38,39]

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