MATHEMATICAL MODEL OF COAGULATION UNDER FLOW: UNDERSTANDING THE ROLE OF CELL SURFACES FOR INHIBITION AND A CLINICAL APPLICATION

by

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ABSTRACT

Coagulation is a self-defense mechanism to stop the bleeding at an injury site. The process involves an intricate biochemical reaction network that is balanced by pro-coagulant and anti-coagulant factors. Natural coagulation inhibitors, tissue factor pathway inhibitor (TFPI) and antithrombin (AT) effectively inhibit coagulation and thus have been identified as potential therapeutic targets for bleeding disorders and other clinical applications. However, due to the complexity of the coagulation network, biochemical experiments alone are not sufficient to provide insights into the hidden mechanisms of coagulation inhibitors in a cost-efficient and timely manner. Mathematical models are powerful tool to assess how thrombin, the main enzyme generated in coagulation, is altered under different conditions and can unveil non-intuitive mechanisms underlying the behavior. In this dissertation, we address two questions using mathematical models: 1) how do the TFPI and AT-mediated inhibitory reactions on platelet surfaces affect the coagulation system under flow? And 2) how cell surfaces can be important for the mechanism of actions of a treatment for hemophilia A, a genetic disorder characterized with inability to form a strong clot? Platelet surfaces are essential for the formation of pro-coagulant complexes that regulate the positive feedback of coagulation. However, whether platelet surfaces are also important for inhibition is not fully understood. Therefore, one motivation for this study is to mathematically investigate how platelet surface-mediated inhibitory reactions will impede thrombin production. An experimentally validated mathematical model of coagulation under flow was extended with known and newly found biochemical inhibitory reaction network mediated by TFPI and AT. Simulation results revealed that platelet surfaces play an important role for efficient inhibition by TFPI and AT, and TFPI was identified as potential therapeutic target to recover thrombin generation. This result motivated us to further apply this model in context of hemophilia A blood, and to investigate the mechanisms of action of a specific drug being developed for hemophilia A treatment. The clinical application of this model provided proof of concept of the drug’s mechanisms of actions. Simulation results demonstrated that in addition to the platelet surfaces, endothelial and subendothelial surfaces are also key regulators of coagulation.
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Figure 1.1 Diagram of coagulation reactions. Boxed proteins represent platelet surface bound proteins, and unboxed proteins are free in plasma. Blue arrows indicate chemical transport, red dashed arrows indicate activation processes, green arrows indicate binding and unbinding from platelet surface. Black solid lines are forward catalytic reactions and black dashed lines are feedback catalytic reactions. Purple dots indicate inhibitory reactions: diamond dots TFPI-mediated inhibition, triangle dots APC-mediated inhibition, and pentagon dots AT-mediated inhibition.

Figure 1.2 Structure of TFPI\(\alpha\) (A) and TFPI\(\beta\) (B).

Figure 2.1 Newly added reactions involving FV-h, FXa, and TFPI\(\alpha\). (Left) Generation of FV-h through the activation of FV by FXa and secretion from platelet stores, binding/unbinding of coagulation factors to the platelet surface, and the assembly of prothrombinase. (Right) Binding of TFPI\(\alpha\) to platelet-bound FXa, FV-h, and prothrombinase, and inhibition of prothrombinase assembly by TFPI\(\alpha\). Line styles indicate different interactions: (Dense Dashed lines) binding and unbinding of protein pairs, (Longer Dashed lines) catalytic reaction steps, (Solid Double arrow) binding and unbinding of proteins with platelet surface, and (Arrow with faded tail) secretion of protein from platelet. Light shade with black font indicate TFPI-bound and inhibited cofactors, and heavier shade with white font indicate TFPI\(\alpha\)-bound and inhibited enzymes. FV is also secreted from platelet stores and can be activated by thrombin, but this is not shown because they were included in earlier model. Circed numbers indicate reaction number listed in Table.

Figure 2.2 Effects of the TF level and shear rate on the lag time and the thrombin concentration threshold behavior. (A) Lag times and (B) thrombin concentration after 10 minutes for a range of TF densities and three different shear rates. Black curves represent the results from the new model, which includes the additional TFPI\(\alpha\)-mediated inhibition reactions, and gray curves represent the results from the old model, where TFPI\(\alpha\) could only bind to fluid phase FXa and then to TF:VIIa. All simulations were run with \([\text{TFPI}\alpha] = 0.5\) nM. Vertical dashed lines indicate TF densities of interest. The flat horizontal lines in (A,C) at lag time > 30 indicate that the thrombin concentration did not reach 1 nM within 30 minutes for the corresponding TF density.

Figure 2.3 Thrombin concentration vs. time for the indicated TFPI\(\alpha\) levels for shear rate 100/s and with (A) TF = 2.5 fmol/cm\(^2\) and (B) TF = 6 fmol/cm\(^2\). Note the different time scales.

Figure 2.4 Lag times as functions of TF and TFPI\(\alpha\) levels with the specified TFPI\(\alpha\) binding reactions. Lag times for cases in which (A) TFPI\(\alpha\) binding with both FV-h and FXa are turned off, (B) TFPI\(\alpha\) binding with FV-h is turned on and TFPI\(\alpha\) binding with FXa is turned off, (C) TFPI\(\alpha\) binding with FXa is turned on and TFPI\(\alpha\) binding with FV-h is turned off, and (D) TFPI\(\alpha\) binding to both FV-h and FXa are turned on. For parameter values in the region without color, the thrombin concentration did not reach 1 nM thrombin within 40 minutes. Lag time vs. TF density for TFPI\(\alpha\) = 0.5 nM with and without TFPI\(\alpha\) binding FV-h or/and FXa for (E) TFPI\(\alpha\) = 0.5 nM and (F) TFPI\(\alpha\) = 4.0 nM. These curves correspond to the slices in the heat map indicated by the horizontal lines in (A-D). Shear rate is fixed to 100/s.
Figure 2.5  Thrombin generation for $h_5 = 0, 0.5, 1.0$ for shear rate $100/s$. (A) The TFPIa concentration is 0.5 nM and the TF density was either 2.5 or 6 fmol/cm$^2$. (B) The TFPIa concentration is 2.5 nM and the TF density was either 4 or 10 fmol/cm$^2$.

Figure 3.1  Newly added reactions involving AT, FIXa, FXa, thrombin (IIa) and FXIa. Inactivation reactions are indicated with T-shaped arrows. FXI/FXIa exists as dimer form, and only the exposed end of FXIa can be inactivated by AT.

Figure 3.2  (A) Lag time and (B) thrombin concentration after 10 minutes of activity, with variations in TF density and shear rate. TF density was varied from 0 to 30 fmol/cm$^2$, and shear rate was either 100, 500, or 1500/s. Abbreviation "SI" in legend represents surface-dependent inactivation. Black lines represent results with AT-mediated inactivation of fluid-phase enzymes alone inactivation of surface-bound enzymes and gray lines represent the results with inactivation of both fluid-phase and surface-bound enzymes by AT. Vertical dashed lines indicate TF densities of interest. Flat curves at $\geq30$ minutes indicates that lag time larger than 30 minutes in (A).

Figure 3.3  Thrombin time courses for various AT levels, with and without surface-dependent inactivation reactions. SI denotes Surface-mediated Inactivation. Shown in top row of the figure are (A) TF=2.5 fmol/cm$^2$ and (B) TF=6 fmol/cm$^2$, each without SI. Shown in the bottom row of the figure are (C) TF=2.5 fmol/cm$^2$ and (D) TF=6 fmol/cm$^2$, each with SI. AT level was one of 0, 50, 100, or 200% of 2.4 $\mu$M. Shear rate was set to 100/s for all simulations.

Figure 3.4  Thrombin generation with TF=6 fmol/cm$^2$ and varied levels of LWMH and UFH. Thrombin generation in the presence of LMWH and UFH are shown in black and gray lines, respectively. The heparin levels were varied as 0.1, 10, 50, 100% of a standard therapeutic concentration.

Figure 3.5  Concentration time courses of (A) thrombin, (B) FXa, fluid-phase and surface-bound, (C) FIXa, fluid-phase and surface-bound, and (D) FXIa, fluid-phase and surface-bound, each with TF density of 15 fmol/cm$^2$ and shear rate of 100/s. Simulations were performed with or without surface-dependent AT inactivation reactions SI = Surface-mediated Inactivation, which are shown as gray and black curves, respectively, and for no heparin, LMWH, or UFH, shown by varying line styles.

Figure 3.6  Thrombin generation in the presence of (A) LMWH or (B) UFH. The time course is obtained from simulations in which we turn off all the AT-mediated inactivation reactions and then allow inhibition of FXa, FIXa, FXIa and thrombin, individually and one by one. Each curve thus shows thrombin generation when there is either no or only one inactivation reaction that exists in the system. TF density was set to 15 fmol/cm$^2$ and shear rate was set to 100/s. Heparin concentration is fixed to 100% of the standard therapeutic concentration.

Figure 4.1  Model schematics. The steady state model (left) predicts levels of concizumab bound to TFPIa and TFPIb. Output from the steady state model is input to the flow-mediated coagulation model (right), which simulates thrombin generation that results from reactions occurring in a small reaction zone above exposed tissue factor and subjected to flow.
Figure 4.2 Steady state model results. Concizumab exposure levels as a function of initial concizumab (A), and steady state model species with or without TFPIb (B). The red dot indicates where the initial concizumab dosage leads to a 4 nM exposure level. Steady state concentrations of all model species at the initial dosage of 21.5 nM are shown as a legend in (A). TFPIβ was set to either 0 or 18 nM in figure (B), and concentration of C:TFPIα, plasma TFPIα, and TFPIα:C:TFPIb complexes are plotted out.

Figure 4.3 Hrombin generation time courses under varied TF and concizumab levels. TF levels are set to be low, intermediate, and high (3, 6 and 20 fmol/cm²). Concizumab levels are set to 0 and 21.5 nM, which represent no treatment (-Czm) and 4 nM concizumab exposure level (+Czm) conditions. FVIII level is fixed to 1% to simulate severe hemophilic condition.

Figure 4.4 Concentration time courses of free TFPIα in plasma (A), TF:VIIa:Xa:TFPI complex (B), active FXa both in plasma and bound to a platelet surface (C), and the prothrombinase complex (D). Concizumab exposure level is set to 0 and 4 nM. TF is fixed to 7 fmol/cm² and FVIII level is fixed to 1% of its baseline value. Concizumab blocks inhibitory action of TFPI (A,B) and leads to increases in FXa and prothrombinase (C,D).

Figure 4.5 Lag time as a function of TF (A) and thrombin time courses (B) for various mechanisms of action of concizumab. FVIII is fixed to 1% and no other factors/inhibitors are varied. For no TFPI inhibition on the platelet surfaces (plt), the rate of TFPIα binding to FXa and FXa:FV-h was set to zero. For no TFPI inhibition on the subendothelium (SE), the rate of binding for TFPIα:FXa to TF:VIIa and for TFPIα to TF:VIIa:Xa was set to zero. For the reduced TFPI level condition, the plasma and upstream concentration of TFPIα was set to 0.0028 nM. In the combined case, all reactions involving TFPI, FXa and TF:VIIa were turned off and the plasma/upstream TFPI level was fixed to 0.0028 nM. For the concizumab case, 21.5 nM of concizumab is used, which leads to the 4 nM exposure level. TF level is fixed to 8 fmol/cm² for the thrombin curves in (B) to highlight case indicated by vertical dashed line in figure (A).

Figure 4.6 Hrombin generation time courses with concizumab, in the presence and absence of TFPIb (A). Different mechanisms of C:TFPIα inhibition are toggled on and off: C:TFPIα inhibits formation of prothrombinase (IF) via binding free FV-h, or it directly inhibits prothrombinase via FV-h (DI). TFPIb is set to 18 nM, IF and DI are turned on and off together. (C) TFPIb is set to zero, IF and DI are toggled on/off individually and together. TF = 7 fmol/cm².

Figure 4.7 If-flow chart of two assumptions made in this model and corresponding results in thrombin generation. There are two major assumptions made in this study: 1) TFPIβ can physically sequester TFPIα from plasma via concizumab, and 2) C:TFPIα complex can bind and inhibit FV-h. Based on true or false in different assumptions, we can predict different level of enhancement in final thrombin output.

Figure 5.1 Final thrombin concentration under range of initial concizumab dosage. Concizumab is varied between 0 to 30 nM, and TF is fixed to 6 fmol/cm².

Figure A.1 Schematic of the model reaction zone (main figure) and endothelial zone (inset).
Figure A.2  Local sensitivity analysis of clotting factor levels on thrombin metrics. The initial conditions of clotting factor and inhibitor levels were varied between 50% and 150% of their baseline values. Shown are (A,B,C) the amplitude change in lag time, maximum relative rate, and final thrombin concentration, (D,E,F) the percentage change in each of the metrics, and (G,H,I) the LS scores for each metric and for each species. Solid black triangles represent the species with LS score higher than 0.75, gray triangles for LS scores from 0.25 to 0.75, and open triangles for LS lower than 0.25. The arrow direction indicates if the variable was increased or decreased.

Figure A.3  Local sensitivity analysis of TFPI-related kinetic rates on thrombin metrics. The new kinetic parameters were varied between 90% and 110% of their baseline values. Shown are (A,B,C) the amplitude of the changes in the lag time, maximum relative rate and final thrombin concentration due to the kinetic parameter variations. The plus/minus sign indicates the association/dissociation rate, respectively. Lower case $m$ represents the components that are bound to platelet surface. The forward slash shows which two components are interacting each other, while the "ter" and "term" indicates interactions that involve a ternary complex and whether the species is in plasma or bound to the platelet surface, respectively. For example: term/FXa (+) indicates the rate of association between the platelet-bound TFPI:FV-h complex and the fluid phase FXa to form the ternary complex FXa:TFPI:FV-h.

Figure A.4  Thrombin generation time courses under different TFPI levels (0 nM and 0.5 nM) plotted in linear scale. TF level is varied by 2.5 fmol/cm$^2$ (A) and 10 fmol/cm$^2$ (B). Shear rate is fixed at 100/s.

Figure A.5  FXa concentration in the presence of LMWH (A) or UFH (B), FIXa concentration in the presence of LMWH (C) or UFH (D), FX:TF:VIIa concentration in the presence of LMWH (E) or UFH (F), and FX:tenase concentration in the presence of LMWH (G) or UFH (H). The time course is obtained from simulations in which we turn off all the AT-mediated inactivation reactions and then allow inhibition of FXa, FIXa, FXIa and thrombin, individually and one by one. Each curve thus shows thrombin/tenase generation when there is either no or only one inactivation reaction that exists in the system. TF density was set to 15 fmol/cm$^2$ and shear rate was set to 100/s. Heparin concentration is fixed to 100% of the standard therapeutic concentration.

Figure A.6  Subendothelium-attached platelet count (PLAS) and paltelet-attached-activated platelet count (PLAV) time course with varied LMWH treatment (A-C) or UFH treatment (D-F). TF level is fixed to 6 fmol/cm$^2$. We examined how heparin in the system might affect platelet deposition. We specifically looked at two types of platelets: those that are activated and bound to subendothelium (PLAS), and those that are activated and bound to deposited platelets (PLAV), and their sum. The platelets accumulate on the subendothelium (SE) and PLAV eventually plateaus due to the limited space at the SE, whereas platelets above the injury site will continue to grow. Increasing the heparin concentrations led to decreases in both platelet species through time. This is because by increasing amount of heparin, it can greatly reduce the thrombin in the reaction zone, which leads to reduced amount of platelet to be activated by thrombin. Such a reduction can cause a shift from platelet-bound platelet to subendothelium-bound platelet. The increase in subendothelium-bound platelet will physically cover up the surface, which can negatively influence the initiation phase of coagulation.
Figure A.7 Concentration time course of platelet surface bound FXa, FV-h, and their complexes with TFPI. TF level is varied by 2.5 fmol/cm% (A,C,E,G) and 10 fmol/cm$^2$ (B,D,F,H). Under each TF level, TFPI level is varied by 0.5 nM and 2.5 nM, and shear rate is varied by 100/s, 500/s and 1500/s.

Figure A.8 Instantaneous generation and removal of TAT (A,B), and accumulative concentration of TAT in plasma (C) and on the platelet membrane (D). TF level is fixed to 5 fmol/cm$^2$, and shear rate is fixed to 100/s.

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Table 2.1 List of reactions added to the model and their kinetic rate constants, with literature references. \( k^+ \) shows forward reaction rate, \( k^- \) shows backward reactions rate, and \( k^{cat} \) indicates catalytic reaction rate: a) Binding of TFPI\( \alpha \) to FV-h, \( K_D=9 \) pM from Jeremyn et al (2014). b) Activation of FV by thrombin, \( K_M=7.1(10)^{-8} \) M from Monovich and Tracy (1990) c) Binding between FV-h and FXa, \( K_D=1(10)^{-10} \) M from Mann (1987) d) FV-h binding platelet surface, \( K_D=3(10)^{-9} \) M from Krishnaswamy et al (1988) e) TFPI\( \alpha \) binding FXa, \( K_D=2(10)^{-11} \) M from Jesty et al (1994) f) Prothrombin activation by prothrombinase that has FV-h, \( K_M=0.4nM \) from Camire et al (2020) g) Inactivation of FV-h by activated protein C, \( K_M=12.5(10)^{-9} \) M from Solymoss et al (1988) h) Generation of FV-h by FXa, \( k_5^{cat}=0.046 \) s\(^{-1} \) and \( K_M=10.4(10)^{-9} \) M from Monovich and Tracy (1990).

Table 3.1 List of reactions added on top of the extension with newly added TFPI-meditated reactions, and their kinetic rate constants, with literature references. \( k^+ \) shows forward reaction rate and \( k^- \) shows backward reactions rate. Subscript LMWH and UFH indicates reaction rates when AT is bound to either LWMH or UFH respectively: a) For inhibition of FIXa by AT, \( k^+ = 4.8 \times 10^2 \). For inhibition of FXa by AT, \( k^+ = 3.5 \times 10^3 \). For inhibition of thrombin by AT, \( k^+ = 1.4 \times 10^4 \). And for inhibition of FXIa by AT, \( k^+ = 2.4 \times 10^2 \), from Olson et al. b) We assume the inhibition rate of FIXa and binding rate to platelet on specific binding site are the same as the normal binding site. c) Binding of heparin to antithrombin, \( K_D = 36nM \) for LWMH, and \( K_D = 9.7nM \) for UFH, from Olson et al. d) Accelerated inhibition of FIXa by AT:UFH complex, \( k^+ = 6.2 \times 10^8 \), and AT:LMWH complex, \( k^+ = 5 \times 10^5 \). For FXa by AT:UFH complex, \( k^+ = 6.6 \times 10^8 \), and AT:LMWH complex, \( k^+ = 1.3 \times 10^6 \). For thrombin by AT:UFH complex, \( k^+ = 4.7 \times 10^7 \), and AT:LMWH complex, \( k^+ = 5.3 \times 10^6 \). And for FXIa by AT:UFH complex, \( k^+ = 1.8 \times 10^5 \), and AT:LMWH complex, \( k^+ = 1 \times 10^4 \), from Olson et al. e) Binding of FIXa to platelet surface, \( K_D = 2.5 \times 10^{-9} \) from Ahmad et al. f) Binding of FXa to platelet surface, \( K_D = 2.5 \times 10^{-9} \) from Walsh et al. g) Binding of thrombin to platelet surface, \( K_D = 5.9 \times 10^{-7} \) from Mann et al. h) Binding of FIX to platelet surface, \( K_D = 1 \times 10^{-7} \) from Greengard et al. i) Binding of FIXa to platelet surface, \( K_D = 1.7 \times 10^{-7} \) from Miller et al.

Table 3.2 List of platelet-binding reactions added on top of the extension with newly added TFPI-meditated reactions, and their kinetic rate constants, with literature references. \( k^{on} \) shows binding rate and \( k^{off} \) shows unbinding reactions rate: e) Binding of FIXa to platelet surface, \( K_D = 2.5 \times 10^{-9} \) from Ahmad et al. f) We assume the inhibition rate of FIXa and binding rate to platelet on specific binding site are the same as the normal binding site. g) Binding of FXa to platelet surface, \( K_D = 2.5 \times 10^{-9} \) from Walsh et al. h) Binding of thrombin to platelet surface, \( K_D = 5.9 \times 10^{-7} \) from Mann et al. i) Binding of FIX to platelet surface, \( K_D = 1 \times 10^{-7} \) from Greengard et al. j) Binding of FIXa to platelet surface, \( K_D = 1.7 \times 10^{-7} \) from Miller et al.

Table 4.1 List of reactions included in the steady state model. C represents concizumab. Kinetic rates taken from a) Binding of concizumab to TFPI\( \alpha \), \( K_D = 25 \) pM. b) Binding of concizumab to TFPI\( \beta \), \( K_D = 123 \) pM.
Table 4.2  List of reactions included in the extended flow-mediated coagulation model. Superscript h indicates the partially activated form of FV (FV-h). Superscript m indicates that the model species is bound to a membrane. The units for k+ are 1/(Ms), the units for k- and kcat are 1/s. Notes: a) Activation of FX by TF:VIIa, from Lu et al. 2004 , b) TFPIα binding FXa, $K_d = 20$ pM from Jesty et al. 1994 , c) Concizumab binding TFPIα, from Hilden et al. 2012 , d) Binding of TFPIα to FV-h, $K_d = 9$ pM from Wood et al. 2013 . . . 53
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CHAPTER 1
INTRODUCTION

System biology is a topic with staggering complexity, where researchers rely on mathematical simulations to predict and reveal answers to the many mysteries of biological systems [1–3]. Hemostasis is one of such complex biological processes that occurs when a person gets injured and starts to bleed. Hemostasis involves the formation of a blood clot to physically stop the bleeding. There exists an intricate biochemical network behind coagulation and the formation of a clot [4]. Mathematical models have been developed to address the questions related to the coagulation process in various environments: clot formation in a static environment [5, 6], clot formation at a small injury site under blood flow [7–9], spatial clot formation in various sized vessels [10, 11], clot degradation [12–14] and so on. The construction of mathematical models of coagulation has not only been utilized in a basic research context but has also been applied to the pharmaceutical field to facilitate the development of drug designs. Burghaus et al developed a coagulation model to assess the portfolio of drug action mechanisms of Rivaroxaban [15], a direct activated coagulation factor X (FXa) inhibitor for the prevention of venous thromboembolism. Nagashima developed a coagulation model to study the different modes of action of anticoagulant proteases [16]. Since coagulation is balanced by pro-coagulant and anti-coagulant proteins, inhibitors of natural anti-coagulants have been identified as potential therapeutic target for bleeding disorders, such as hemophilia A [17, 18].

The focus of this thesis is the extension of a mathematical model of coagulation under flow and the application of this model for drug development. In this chapter, we first present a review of coagulation biology and two anti-coagulant proteins that we are interested in. Chapter 2 introduces an extension of the model with newly discovered tissue factor pathway inhibitor (TFPI)-mediated inhibitory reaction networks and corresponding findings. Chapter 3 introduces an extension of the model with more detailed anti-thrombin (AT)-mediated inactivation reactions to examine existing anticoagulant materials. Chapter 4 describes an application of the model for drug development related to hemophilia A treatment. Chapter 5 summarizes the major findings from the study and discusses future plans for this modeling work.

1.1 Background on Coagulation
1.1.1 Coagulation Cascade

Blood coagulation is a process involving a series of chemical reactions to form a blood clot to ameliorate blood loss. The process initiates with the exposure of tissue factor (TF) and collagen on the sub-endothelial cell surface, followed by activation and adhesion of zymogens, enzymes, and platelets [19, 20]. The end product, thrombin, cleaves fibrinogen into fibrin monomers, which polymerizes and forms fibrin meshwork.
to cover and physically stabilize the clot [21, 22]. Thrombin generation is critical to the clotting process and is accomplished by the sequential formation of three enzyme-cofactor complexes. One is formed on the injured vascular wall by TF (cofactor) and activated coagulation factor VII (FVIIa, forming TF:VIIa) [23]. The other two are formed on the surfaces of activated platelets by activated coagulation factors VIII (FVIIIa, cofactor) and IX (FIXa, the VIIIa:IXa ‘tenase’ complex) [24], and by activated coagulation factors V (FVa, cofactor) and FXa (forming Va:Xa ‘prothrombinase’ complex). The formation of these enzyme-cofactor complexes is critical to provide positive feedback of coagulation; TF:VIIa activates more FIX and FX, and the tenase complex activates more FX. Eventually, it is the prothrombinase complex that enzymatically converts the plasma protein prothrombin into thrombin [25, 26], which leads to stable clot formation. The schematic of the coagulation network can be found in Figure 1.1. Because of the essential roles of the platelet-bound enzyme complexes, the availability of binding sites on the surfaces of activated platelets for the coagulation factors that form these complexes is an important regulator of coagulation; robust thrombin generation does not occur if these complexes cannot form. Further, the requirement that important coagulation reactions occur on activated platelets helps localize coagulation to the site of injury instead of being spread throughout the vasculature by the flowing blood. The pro-coagulant activity of coagulation factors is counter-balanced by coagulation inhibitors, which are essential to control the overgrowth of the clot. A lack of sufficient inhibitors that take part in the negative feedback loops may lead to excessive clot growth and thrombosis, while excessively high concentrations of inhibitors may impede coagulation and lead to excessive bleeding [27, 28]. Hence, there are many existing regulators of clotting that are necessary to uphold this critical balance. Bleeding and clotting disorders that manifest as deficiencies or mutations in clotting proteins, hemophilia A for example, can disrupt this balance.

1.1.2 Biology of TFPI

Tissue factor pathway inhibitor (TFPI) is one of the natural anti-coagulant proteins that we are interested in. TFPI is a Kunitz type serine protease inhibitor with two different isoforms: TFPIα and TFPIβ [29, 30]. TFPIα either exists in plasma or is released from platelet upon activation [30, 31], where 20% of plasma TFPIα is in a full-length, free form and the rest is thought to be bound to lipoproteins [32]. TFPIα contains three Kunitz domains: Kunitz 1 and 2 mediate binding of TFPI to TF:VIIa and FXa respectively [33, 34], and Kunitz 3 binds to Protein S to accelerate inhibitory effect [29]. The structure and corresponding binding sites are shown in Figure 1.2A. TFPIα is well known for its inhibition of TF:VIIa during the initiation phase of coagulation by forming a quaternary complex between TF:VIIa, FXa, and TFPI [34]. Recent studies showed that TFPIα can also inhibit coagulation differently: the C-terminus end of TFPIα can recognize the acidic region of partially activated factor V (FV-h). This FV-h is shown to
Figure 1.1 Diagram of coagulation reactions. Boxed proteins represent platelet surface bound proteins, and unboxed proteins are free in plasma. Blue arrows indicate chemical transport, red dashed arrows indicate activation processes, green arrows indicate binding and unbinding from platelet surface. Black solid lines are forward catalytic reactions and black dashed lines are feedback catalytic reactions. Black line with fade indicates secretion from platelet. Purple dots indicates inhibitory reactions: diamond dots TFPI-mediated inhibition, triangle dots APC-mediated inhibition, and pentagon dots AT-mediated inhibition.

have similar co-factor activity to FVa, therefore the interaction between TFPI\(\alpha\) and FV-h can inhibit the formation of prothrombinase or directly inhibit prothrombinase [35, 36].

TFPI\(\beta\) is another form of TFPI that exists on the surface of the endothelium. TFPI\(\beta\) contains a Kunitz 1 and 2 domain, which exhibit anti-coagulant effects via FXa-dependent TF:VIIa inhibition [37]. The Kunitz 3 domain and the C terminus are replaced by GPI anchor that fixes TFPI\(\beta\) to the endothelium surface [30]. The structure of TFPI\(\beta\) is shown in Figure 1.2B.

The surface-mediated biochemical functions of both TFPI\(\alpha\) and TFPI\(\beta\) in coagulation under flow are investigated in this thesis. Chapter 2 is focused on the newly found interaction between TFPI\(\alpha\) and FV-h, which to our knowledge, has not been incorporated into any mathematical model. Therefore, one of the aims of this thesis is to explore the importance of TFPI-mediated inhibitory reactions through FV-h both in plasma and on platelet surfaces. The function of both forms of TFPIs is investigated in chapter 4 to examine their influence on the bio-availability and efficacy of a hemophilia A drug both before and after a
1.1.3 Biology of AT

Anti-thrombin (AT) is a serine protease inhibitor in plasma that negatively controls the coagulation [38–40]. AT can suppress thrombin generation via four different irreversible inactivation reactions: inactivation of activated coagulation factor IX (FIXa) [41], inactivation of FXa [42], inactivation of activated coagulation factor XI (FXIa) [43], and inactivation of thrombin [42]. AT plays a critical role in controlling coagulation where deficiency in AT can lead to excessive thrombin generation, which is related to venous thrombosis [44–46]. The anticoagulant effect of AT is greatly accelerated by heparin, one of the oldest clinical anti-coagulant drugs [47, 48]. Accelerated inhibitory effects are exerted when AT is bound to heparin [49], where the enhanced inhibitory effect depends on the specific type of heparin being used. Unfractionated heparin (UFH) is one heparin type that undergoes minimal processing and thus possesses various unwanted side effects due to its heterogeneity [50]. Low molecular weight heparin (LMWH) is another form of heparin with lower molecular weight compared to UFH due to different manufacturing processes [51, 52]. Due to a longer half-life and better bio-availability, LMWH is more favorable than UFH in the clinical field [53–57], and further synthetic heparin is expected to be introduced in the future.

Chapter 3 of this thesis focuses on the extension of the mathematical model with a more complete scheme of AT-mediated inactivation reaction both in plasma and on platelet surfaces. Heparin was also incorporated to reflect the accelerated AT-mediated inactivation effect caused by heparin treatment. Using
this extended model, we investigate the AT-mediated inactivation of coagulation factors on platelet surface and their impact on the coagulation cascade. We also aimed to determine which reaction was the major inactivation reaction mediated by AT. In addition, we explored how different types of heparin treatment would affect thrombin production behavior and highlighted a potential synthetic regimen for heparin in the future.

1.2 Review of Mathematical Model of Coagulation

In the last two decades, a number of mathematical models have been developed to inspect the complexity of the coagulation system. Hockin et al developed a mathematical model to simulate the extrinsic pathway of coagulation in static environments [5]. The accuracy of this model was further examined by Diamond et al via Monte Carlo Simulations [58]. Bungay et al developed another mathematical model of coagulation that incorporate the lipid bi-layer to allow the coagulation factors to deposit and react to each other [6]. However, none of the studies above have incorporated flow as a factor to influence the coagulation, which fails to capture the mechanistic relationship between blood flow and coagulation. In the next section, we introduce the history of the development of the mathematical model of coagulation under flow that we used in this thesis study.

1.2.1 History of Mathematical Model of Coagulation under Flow

The mathematical model of coagulation used in this thesis study was first developed by Kuharsky and Fogelson in 2001 [7]. The model simulates the biochemical reactions triggered by exposure of TF to blood plasma above a tiny injury site (10x10 um). The model includes platelet activation process, where activated platelets release agonists that activate other nearby platelets and also support coagulation factors binding to their surfaces to regulate positive feedback. The model tracks all species concentrations across time. The concentration of thrombin was the main focus for the analysis of coagulation behavior. The area above the injury site is defined as the reaction zone. Due to an assumption that all species are instantaneously well-mixed in the reaction zone, the system is mathematically translated by the law of mass action and solved by ordinary differential equations. Albeit in a simplified way, blood flow was also incorporated to predict coagulation in a more physiologically relevant environment. Simulation results demonstrated that the burst of thrombin generation is highly sensitive to the TF level in a threshold-like manner. The model shows that flow not only brings coagulation proteins to the injury site but also acts as an inhibitor by removing key coagulation proteins from the injury site and delaying thrombin generation.

The coagulation model under flow was further updated with an extra reaction zone above the endothelial region adjacent to the injury site [8]. The endothelial region enabled transportation of
thrombin and protein C (PC) via diffusion, where this PC is activated by the thrombin-thrombomodulin complex in the endothelial zone. Activated protein C (APC) then must diffuse back to the reaction zone to inhibit coagulation. Different reaction schemes for TFPI on the subendothelium were also studied to investigate the influence of TFPI-mediated inhibitory reaction on TF:VIIa. The simulation results indicate that the efficacy of APC-mediated inhibition is limited by the diffusion rate between the reaction zone and endothelial region, and further limited by the flow rate. It was also suggested that TFPI-mediated inhibitory reactions had little to no effect in thrombin generation, even though TFPI is regarded as a significant coagulation inhibitor [59].

Later, the model was updated again with a more complete picture of FXI-mediated positive feedback [9]. The model was extended to include the reactions of FXI activation by thrombin, and FIX activation by activated coagulation factor XI (FXIa). The newly added FXI-mediated positive feedback demonstrated more robust thrombin generation, which was dependent on the platelet concentration in the near wall region. The model was further used to explore the synergistic relationship between FXI and TF for a sustained thrombin generation, which was then further experimentally validated [60].

1.2.2 Application

Mathematical modeling is a strong tool for systems modeling as it allows us to thoroughly and systemically examine biological systems. The coagulation model under flow used in this thesis study has been extensively used in various applications to further examine the stability of the model as well as investigate the potential therapeutic target for bleeding disorders. Link et al performed local and global sensitivity studies on the flow model to investigate the sensitivity of the model towards the variation in blood components [61]. Plasma coagulants and inhibitors, and reaction kinetics were varied locally and globally, and clinically relevant thrombin metrics were compared. The study results were able to explore how the thrombin generation can be influenced by different parameters either via one-at-a-time variation or via variations in the combination.

The model was also used to study Hemophilia A, a genetic bleeding disorder characterized by a deficiency in coagulation factor VIII (FVIII). Patients with hemophilia A have a weaker ability to form clots due to insufficient FVIII to form tenase, and thus weaker positive feedback. A global sensitivity analysis was performed with the coagulation flow model under the hemophilia A condition [10]. The study revealed that coagulation factor V (FV) acts as a potential thrombin modifier in hemophilia. The model was used to explain this observation and revealed that this modification was due to a binding competition between FV and FVIII for FXa. By reducing the FV level, it allows more FVIII to bind to FXa and be activated, leading to an increase in tenase production, prothrombinase production, and eventually rescues
the thrombin generation. This result was experimentally validated in a microfluidic assay using whole blood from individuals with hemophilia A. This application demonstrates the strength of the model to both answer questions regarding non-intuitive relationship between coagulation factors, and highlight potential mechanisms for drug designs.

Chapter 4 of this thesis describes how this model was applied to the field of drug development. We have collaborated with Novo Nordisk for this chapter of the study, where we incorporated concizumab, a drug designed for treatment for hemophilia A, into the model to investigate the efficacy and mechanisms of actions of the drug. Concizumab is a monoclonal antibody that targets TFPI and reduces its inhibitory function [62, 63]. Specifically, concizumab binds K2 domain of TFPI, therefore reducing its inhibitory effect on FXa. We extended the coagulation model under flow with concizumab as a new species, and included its interactions with other coagulation factors and inhibitors. We developed a 'steady state' model that predicts how much drug remains in plasma under different dosages of concizumab. The predicted steady state estimates were then used for the coagulation flow model to simulate how the remaining drug concentration affects thrombin generation. The advantage of using this model to study concizumab is that the model extension with the TFPI-mediated inhibitory reactions gives us more realistic and accurate simulations to fully describe how the drug interacts with all the components that affect the drug's efficacy. Two different assumptions were made for this concizumab study, where based on the assumptions being true or false, we observed different levels of thrombin enhancement via concizumab treatment. These results suggest further experimental studies are needed for verification to fully explain the mechanism of actions of concizumab.

1.3 Motivations for this Thesis Study

The coagulation flow model has been continuously refined and applied in basic research and clinical applications. However, the model could not capture some known observations: TFPI is a significant coagulation inhibitor in experiment yet thrombin generation was not sensitive to TFPI in previous version of this model [7, 8, 61]. The variation in AT level also had little to no effect on thrombin generation in those versions of the model [61]. We hypothesized that this is because the platelet surface is very important for coagulation factors to be deposited and react to each other to stimulate the positive feedback of coagulation. TFPI and AT were only assumed to inhibit factors in plasma in previous versions of the model, and the inhibitory reactions on platelet surfaces were also not fully incorporated. It is well studied that the platelet surface is essential to promote the coagulation during hemostasis [64]. It allows the formation of tenase and prothrombinase, key co-factor-enzyme complexes that generate more FXa and thrombin, to provide positive feedback [65]. Receptors expressed on platelets can act as catalytic surfaces
to facilitate the hemostasis process [66–68]. Furthermore, platelet surfaces localize coagulation and fibrin formation to the injury site to enforce on-site sealing [64]. However, the importance of the platelet surface to the coagulation inhibitors is not yet established and often neglected in literature. If we identify that platelet surface-mediated inhibition greatly suppresses thrombin production, we shed light on a mechanism of action to exploit for anti-coagulant drug designs. One of the major aims for the studies presented in this thesis is to explore how TFPI- and AT-mediated inhibition on platelet surfaces affects thrombin production in coagulation by using the mathematical model proposed here. With our extended model, we not only demonstrated stronger sensitivity to TFPI and AT with surface-dependent inhibitions, but also able to dissect the inhibitory effect and identify which individual reactions on the platelet surface contributed the most to reduce the thrombin generation.

To our knowledge, our extended model is the first mathematical model of coagulation to incorporate the complete scheme of TFPI-mediated inhibitory reactions. This gives us an advantage for the second aim of the study: applying the model in the context of drug development. With the newly extended model, we can now further investigate how drugs targeting TFPI may interact with other coagulant proteins under flow. Concizumab is a drug that targets TFPIs to stabilize clot formation in hemophilic blood. Complete TFPI-mediated reaction scheme in the model allows us to investigate how individual protein-protein interactions may affect the thrombin generation with the concizumab treatment administered. By using this extended model, we were able to demonstrate the proof of concept of how concizumab enhances thrombin generation, and further identify the mechanisms of actions of concizumab. We discovered that concizumab greatly enhances thrombin generation by targeting TFPIα in plasma or on platelet surfaces. We also found that concizumab plays a critical role on the endothelial surface; concizumab can form ternary complex with TFPIα and TFPIβ to sequester the plasma TFPIα level, and indirectly reducing its inhibitory effect. This result indicates that not only subendothelial and platelet surfaces can be important for the coagulation, but endothelial surfaces can also be a dominant factor for the hemostasis.
CHAPTER 2
INHIBITION OF PLATELET-SURFACE-BOUND PROTEINS DURING COAGULATION UNDER FLOW I: TFPI (JOURNAL ARTICLE)

Modified from a paper published in The Biophysical Journal 1.
Kenji Miyazawa 2, Aaron Fogelson 3,4, Karin Leiderman 5,6

2.1 Abstract

Blood coagulation is a self-repair process regulated by activated platelet surfaces, clotting factors, and inhibitors. Tissue factor pathway inhibitor (TFPI) is one such inhibitor, well known for its inhibitory action on the active enzyme complex comprised of tissue factor (TF) and activated clotting factor VII. This complex forms when TF embedded in the blood vessel wall is exposed by injury, and it initiates coagulation. A different role for TFPI, independent of TF:VIIa, has recently been discovered whereby TFPI binds a partially cleaved form of clotting factor V (FV-h) and impedes thrombin generation on activated platelet surfaces. We hypothesized that this TF-independent inhibitory mechanism on platelet surfaces would be a more effective platform for TFPI than the TF-dependent one. We examined the effects of this mechanism on thrombin generation by including the relevant biochemical reactions into our previously-validated mathematical model. Additionally, we included the ability of TFPI to bind directly to and inhibit platelet-bound FXa. The new model was sensitive to TFPI levels and, under some conditions, TFPI could completely shut down thrombin generation. This sensitivity was due entirely to the surface-mediated inhibitory reactions. The addition of the new TFPI reactions increased the threshold level of TF needed to elicit a strong thrombin response under flow, but the concentration of thrombin achieved, if there was a response, was unchanged. Interestingly, we found that direct binding of TFPI to platelet-bound FXa had a greater anticoagulant effect than did TFPI binding to FV-h alone, but that the greatest effects occurred if both reactions were at play. The model includes activated-platelets' release of FV species and we explored the impact of varying the FV/FV-h composition of the releasate. We found that reducing the zymogen FV fraction of this pool, and thus increasing the fraction that is FV-h, led to acceleration of thrombin generation.

1Reprinted with permission of Biophysical Journal, 122(1), 99–113. (Figure B.1)
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2.2 Introduction

Hemostasis is a self-defense mechanism, initiated by a vascular injury, whereby a blood clot forms to stop bleeding. It consists of two intertwined processes, platelet aggregation and coagulation, that begin when the injury exposes subendothelial collagen and tissue factor (TF), respectively. Platelets adhere to the collagen and become activated, which enhances their ability to aggregate and plug the injury. They also expose phospholipid binding sites on their surfaces to which plasma proteins, called clotting factors, can bind. Activated platelets release proteins from internal stores, including additional clotting factors, inhibitors, and agonists that further platelet activation [19, 69]. Coagulation is a network of enzymatic reactions that involves clotting factors and inhibitors, and culminates in the production of the enzyme thrombin. Thrombin cleaves the soluble plasma protein fibrinogen to form insoluble fibrin, which polymerizes to form a cross-linked hydrogel that physically stabilizes the growing platelet aggregates.

Thrombin generation is necessary to the clotting process and is accomplished by the sequential formation of three enzyme-cofactor complexes. One is formed on the injured vascular wall by TF (cofactor) and activated coagulation factor VIIa (FVIIa) and the other two are formed on the surfaces of activated platelets by coagulation factors VIIIa (cofactor) and IXa (the FVIIIa:FIXa ‘tenase’ complex) and by coagulation factors Va (cofactor) and Xa (the FVa:FXa ’prothrombinase’ complex). It is the prothrombinase complex that enzymatically converts the plasma protein prothrombin into thrombin. Because of the essential roles of the platelet-bound enzyme complexes, the availability of binding sites on the surfaces of activated platelets for the coagulation factors that form these complexes is an important regulator of coagulation; robust thrombin generation does not occur if these complexes cannot form. Further, the requirement that important coagulation reactions occur on activated platelets helps localize coagulation to the site of injury instead of it being spread throughout the vasculature by the flowing blood. The coagulation process involves positive and negative feedback loops, which enable thrombin generation to exhibit threshold-like behavior; substantial thrombin generation occurs only after a sufficient level of TF is exposed. On the other hand, a lack of sufficient inhibitors, that take part in the negative feedback loops, may lead to excessive clot growth and thrombosis, while abnormally high concentrations of inhibitors may impede coagulation and lead to excessive bleeding. The many regulators of clotting normally work together to maintain a proper balance between promoting and inhibiting processes. Bleeding and clotting disorders that manifest as deficiencies or mutations in clotting proteins, such as hemophilia, can disrupt this balance with serious consequences.

Tissue factor pathway inhibitor (TFPI) has long been regarded as a key inhibitory regulator of coagulation. It is best known for its inhibition of TF:VIIa activity due to formation of a quaternary
complex that includes TF:VIIa, TFPI, and FXa, but more recently has become known to inhibit through FV, more details on this below. In humans, there are two TFPI types: TFPI\(\alpha\) and TFPI\(\beta\) [29, 30]. In this study, we focus only on TFPI\(\alpha\). TFPI\(\alpha\) is found in human plasma [30] and is also released from platelets upon the platelets’ activation [31]. TFPI\(\alpha\) contains three Kunitz domains and a C terminus tail. TFPI binds to TF:VIIa and FXa through its Kunitz 1 and 2 domain, respectively [33, 34], and to Protein S via Kunitz 3 [29]. It binds to some FV variants through the C-terminus tail, which is important for its inhibitory action.

Coagulation factor V (FV) is another essential protein for thrombin generation. In its fully cleaved/activated form (FVa), it serves as the cofactor for FXa within the prothrombinase complex that converts prothrombin to thrombin [70, 71]. In 2001, a novel bleeding disorder was discovered in a family from east Texas [72] and related to FV [73]. A genetic mutation caused increased expression of an alternative form of FV that was ‘partially’ cleaved and thus named FV-short. Later, FV-short was shown to bind tightly to TFPI\(\alpha\), which was in line with the observed, increased levels of TFPI\(\alpha\) in the East Texas family. It is now believed that the increased TFPI\(\alpha\) levels are the cause of their bleeding phenotype [74]. Since the FV-short discovery, it has been recognized that normal FV is physiologically cleaved into two different forms through distinct mechanisms: FXa cleaves what is called the basic region of the B domain, leading to what we call the partially cleaved/activated form [35, 75], and thrombin cleaves the entire B domain leading to the fully cleaved/activated form [76]. There is some evidence that FXa can fully cleave FV into FVa, but it does so rather inefficiently [77, 78].

Variants of FV are stored in a platelet’s \(\alpha\)-granules and secreted after the platelet is activated [70, 79, 80]. Platelet-secreted FV may be partially or fully cleaved, but there is little data in the literature on the detailed makeup of platelet-secreted FV molecules or on their susceptibility to be bound by TFPI\(\alpha\). In the rest of this paper, we denote the partially-cleaved or platelet stored FV as FV-h, for half-cleaved/activated, and FVa is the the fully-activated form. Of note, FV-short, FV-h, and FVa possess similar cofactor activity within the prothrombinase complex, but only FV-short or FV-h can bind to TFPI\(\alpha\) [81].

Early biochemical studies showed that TFPI\(\alpha\) has varying effects on thrombin generation depending on the forms of FV that are present [82]. Mast and Broze performed prothrombin activation assays with mixtures containing prothrombin, FXa, lipids, TFPI\(\alpha\), and varying forms of FV. With FVa and FXa, addition of TFPI\(\alpha\) produced modest inhibition of thrombin generation. With FV and FXa however, addition of TFPI\(\alpha\) substantially impeded thrombin generation. Interestingly, when FV was preincubated with FXa, subsequent addition of TFPI\(\alpha\) had a decreased inhibitory effect (i.e., more thrombin was generated) in comparison to the case where FV, FXa, and TFPI\(\alpha\) were added at the same time. These
results suggest that TFPIα inhibits thrombin generation through a variety of mechanisms that could act before and/or after the formation of prothrombinase (with either FV-h or FVa). The presence of lipids may also have enhanced or mediated the inhibitory mechanisms in that study. In later biochemical studies, TFPIα was shown to inhibit prothrombinase early in thrombin generation via FV-h [35, 36]. It was suggested that TFPIα inhibits thrombin generation through multiple mechanisms, including direct binding of TFPIα with FV-h, interactions with FV-h in ways that either prevent FXa from binding FV-h or that cause it to bind in a way that does not promote thrombin generation, and of course the well-known binding interaction between TFPIα and FXa [36]. The relative importance of these various mechanisms and interactions, along with the effects of lipid or platelet surfaces on them, remains unclear.

Mathematical models are powerful tools that can be used to gain insight into complex biological systems. Our group has developed mechanistic, experimentally validated mathematical models of flow-mediated coagulation that have led to novel insights related to phenotypic variation observed in bleeding and thrombotic disorders [7–10, 60, 61]. These models include activated platelet surfaces as the site of many coagulation reactions, thus allowing the availability of these surfaces and their properties to regulate coagulation. In simulations with our previous models, procoagulant platelet-surface reactions are critical to the production of thrombin. The availability of binding sites on the procoagulant surfaces of platelets deposited onto the injury play an important regulatory role. Other mathematical models that simulate thrombin generation in the absence of flow [5], have also shown that thrombin generation is sensitive to variation in normal levels of clotting factors in healthy individuals [83] and in hemophilia [84]; these models generally assume that the reactions occur in the presence of an excess of lipid surfaces and do not look specifically at events on limited platelet surfaces as regulators of coagulation.

Collectively, these experimental and mathematical findings raise the question: would blocking the binding of TFPIα to FXa and/or FV-h on the platelet surface rescue thrombin generation in hemophilia? Platelet surface-dependent, TFPIα-mediated inhibition has not yet been included in any mathematical models of coagulation. We extended our previous model of flow-mediated coagulation to include a detailed description of platelet surface-mediated TFPIα inhibition, via platelet-bound FXa and FV-h. In our previous model, TFPIα was assumed to bind FXa in the plasma phase and the TFPIα:Xa complex could subsequently bind to TF:VIIa. However, TFPIα had very little impact on thrombin generation through this mechanism because, the overwhelmingly dominant inhibitor was dilution of fluid-phase enzymes and active co-factors as flow carried them downstream away from the injury. This was true for all shear rates examined with the model [7]. An alternative mechanism of TFPIα inhibition of TF:VIIIa, in which TFPIα binds to TF:VIIa:Xa, was also investigated in this model and it was found for this mechanism to work, FXa would have to remain bound to TF:VIIa for a long time, in which case its own presence could
inhibit TF:VIIa without a role for TFPIα [8].

A major motivation for the current study is to explore the effectiveness of platelet-surface anticoagulant reactions in the regulation of thrombin generation under flow. To our knowledge, our studies are the first to examine the effect of TFPI- and AT-mediated inhibition occurring directly on platelet surfaces with modeling (we studied AT inhibition with and without heparin in a companion study [85]). The key new additions to the model in this paper are that (1) FV can be partially activated to FV-h by FXa, (2) FV-h can bind FXa to form active prothrombinase on the platelet surface, (3) TFPIα can directly bind to FV-h in the fluid or bound to the platelet surface, and (4) TFPIα can bind directly to FXa that is bound to the platelet surface. The aforementioned experiments suggested that early in the coagulation reactions, before much thrombin has been generated, active prothrombinase forms on activated platelet surfaces by binding of FXa and FV-h, and that the activity of FXa, FV-h, and/or the prothrombinase formed from them is significantly impacted by TFPIα.

The assumptions regarding TFPIα resulted in many binding combinations that either interfered with the formation of prothrombinase or directly inhibited the already formed complex. We studied how the additional TFPIα inhibition affected the TF threshold for various shear rates and whether inhibition via FXa or FV-h had a larger inhibitory effect. Our results demonstrated new and significant sensitivity of simulated thrombin generation to TFPIα levels that was entirely due to inhibition reactions occurring on platelet surfaces. Direct binding of TFPIα to FXa on the platelet surface had a somewhat stronger inhibitory effect than binding to FV-h when each was considered in isolation, but the greatest inhibitory effect occurred when both mechanisms were at play.

2.3 Materials and Methods

2.3.1 Previous Mathematical Model Review

Here we give a brief review of our previously developed mathematical model of flow-mediated coagulation [7–9]. More details about this model and its sensitivity to parameters can be found elsewhere [61]. The model simulates the coagulation reactions occurring in a small reaction zone (RZ) above an injury where TF in the subendothelium (SE) is exposed, see Figure S1 in the supplemental information. Clotting factors and platelets are transported into and out of the RZ by a combination of flow and diffusion, using a mass transfer coefficient whose value is a function of vessel and injury size, the flow’s shear rate, and the species’ diffusivity. Clotting factor concentrations in the RZ change due to their involvement in the coagulation reactions, their binding with activated platelets, and by transport in and out of the zone. Similarly, platelet concentrations change as platelets adhere to the injured wall, become activated, and as other platelets are transported in and out of the zone. As platelets build up in the
reaction zone, the height and volume of the reaction zone increase with the volume of plasma and concentration of platelets in it changing accordingly. Deposition of platelets also blocks the activity of TF:VIIa on the subendothelium in proportion to the fraction of the subendothelium which the platelets cover. The concentration of each species in the reaction zone plasma is tracked with an ordinary differential equation; this choice relies on the assumption that each species is uniformly distributed (well-mixed) within the reaction zone. An additional well-mixed endothelial zone (EZ) is located adjacent to the RZ, in the direction perpendicular to the flow with height equal to that of the RZ and width dependent on the flow shear rate and protein diffusion coefficients. The EZ is where active protein C (APC) is produced by a complex formed by thrombomodulin in that zone and thrombin which has diffused to the EZ from the RZ. This APC either diffuses into the RZ or is carried away by the flow.

There are three forms of platelets in the model: unactivated platelets that exist in the plasma phase, activated platelets that are directly attached to SE, and activated platelets in the thrombus that are not directly attached to SE. Activation of platelets is achieved by contact with the SE, interaction with thrombin, or by exposure to already activated platelets (this is an indirect way to model release of agonists from platelet stores). Activated platelets provide the membrane surface necessary for coagulation factors to bind and react. Each activated platelet expresses specified types and numbers of binding sites to which coagulation proteins can selectively bind.

### 2.3.2 New Model Extensions

The key new extensions to the model are described in the next few sections. They involve partially-activated FV (FV-h) and its interaction with FXa and TFPIα. The additional reactions describe the generation of FV-h, TFPIα binding to FV-h and FXa, direct inhibition of FV-h or FXa within prothrombinase by TFPIα, inhibition of FV-h:FXa prothrombinase assembly, and full activation of FV-h by thrombin. All of these additional reactions involving FV-h, FXa, and TFPI are sketched in Fig 2.1, and listed in Table 2.1 with any available kinetic rate constants from the literature. In Eq. 2.1, we show the evolution equation for the concentration of one of the new species as an example of the nature of the model’s equations. A full listing of the model equations and parameter values is given in the section S2 of Supplemental Information. Note that for this paper, the ODEs labeled 1-104 comprise the model.

The remaining equations are added for the companion paper.

The model uses the following notation: $Z_i$ and $E_i$ to refer to a specific zymogen or pro-cofactor species and the corresponding enzyme or co-factor species when they are in the plasma and $Z^m_i$ and $E^m_i$ refer to the surface-bound versions of these proteins (e.g., $E^m_7$ refers to the TF:VIIa complex on the subendothelium, and $E_5$ and $E^m_5$ refer to factor Va in the plasma and bound to a platelet surface,
respectively). The new species, partially cleaved factor V is denoted $E^5_v$. The concentrations of the proteins are denoted similarly, but with lower case $e$. So, $e^{5}_v$ is the concentration of platelet-bound factor Va. The symbols $TF, P_2, P_5, P_8, P_9, P_{10}$, and $P_{11}$ are used to denote tissue factor and the platelet binding sites for prothrombin, FV/FVα, FVIII/FVIIIa, FIX/FIXα, FX/FXα, and FXI, respectively. For the platelet binding sites specific to thrombin, factor IXα, and factor XIα, we use the symbols $P^*_2, P^*_9$, and $P^*_11$. The concentrations of binding sites are indicated similarly but with lower case $p$. We denote the complex of $Z_i$ and $E_j$ by $Z_i : E_j$ and its concentration by $[Z_i : E_j]$, so, for example, $TFPIα : E^{10}_v$ denotes TFPIα bound to platelet-bound Factor Xα, and $[TFPIα : E^{10}_v]$ refers to its concentration.

Figure 2.1 Newly added reactions involving FV-h, FXα, and TFPIα. (Left) Generation of FV-h through the activation of FV by FXα and secretion from platelet stores, binding/unbinding of coagulation factors to the platelet surface, and the assembly of prothrombinase. (Right) Binding of TFPIα to platelet-bound FXα, FV-h, and prothrombinase, and inhibition of prothrombinase assembly by TFPIα. Line styles indicate different interactions: (Dense Dashed lines) binding and unbinding of protein pairs, (Longer Dashed lines) catalytic reaction steps, (Solid Double arrow) binding and unbinding of proteins with platelet surface, and (Arrow with faded tail) secretion of protein from platelet. Light shade with black font indicate TFPIα-bound and inhibited cofactors, and heavier shade with white font indicate TFPIα-bound and inhibited enzymes. FV is also secreted from platelet stores and can be activated by thrombin, but this is not shown because they were included in earlier model. Circled numbers indicate reaction number listed in Table.

2.3.3 Generation and Activation of FV-h

We assumed there are two major sources of FV-h: activation of FV by FXα (Reaction 25 from Table 2.1), and secretion by platelets upon their activation. We assume here that FXα can only partially activate FV to FV-h. We also assume that a total of 3000 FV and FV-h molecules are released per activated platelet [86] and denote by $h_5$ the fraction of these which are FV-h molecules. For most of the simulations in this study we fixed $h_5 = 0.5$, but we also reported on simulations examining the model result’s sensitivity to variations in $h_5$. We assumed that FV and FV-h bind to the same platelet binding sites ($P_5$) and that platelet-bound FV-h and FXα can bind to one another to produce an early form of prothrombinase FV-h:FXα. We assumed that FV-h can be fully activated by thrombin, in the plasma, on
the platelet surface, and within the FV-h:FXa complex (Reactions 2,6,9 in Table 2.1).

2.3.4 TFPIα Binding to FV-h and FXa

In our previous model [7–9], TFPIα could bind only to fluid-phase FXa. Here, we also allowed TFPIα to bind directly from the plasma to platelet-bound Xa (Reaction 22 in Table 2.1) and to FV-h in the plasma or bound to a platelet (Reactions 1 and 5 in Table 2.1). We assumed that TFPIα cannot bind to FVa because the FV’s B domain is completely removed upon full activation. This assumption is supported by the fact that TFPI binds FV in a form where only part of the B domain is cleaved upon activation by FXa, leaving the acidic region to bind the C terminus of TFPI [35]. We assumed that the entire 20% of plasma TFPI is the full length form, which can interact with both FV-h and FXa. Differentiation between the truncated form and full length forms of TFPI in the plasma is the subject for future research. Furthermore, we assumed that when FXa or FV-h is bound to TFPIα, it cannot subsequently form a FV-h:FXa prothrombinase complex. We based this assumption on the proximity of the binding sites on FV-h, FXa, and TFPIα for each other, the large size and flexibility of a TFPIα molecule, and the previous suggestion that this reaction is blocked [35, 36, 87]. We do not consider the secretion of TFPIα from platelets in this model extension.

2.3.5 Direct Inhibition of Prothrombinase

We assume that prothrombinase is formed by the binding of platelet-bound FXa to platelet-bound FVa (FVa:FXa), as in our previous model, or to platelet-bound FV-h (FV-h:FXa, Reaction 3 in Table 2.1). Since FXa and FV-h can both be bound to TFPIα, via its Kunitz 2 domain and C-terminus region, respectively, we assume that FV-h:FXa can be directly inhibited by TFPIα binding either to FXa or to FV-h in the complex (Reactions 7 and 8 in Table 2.1, respectively). This is illustrated by the reactions shown on the top surface of the activated platelet in Fig. Figure 2.1(right). The binding kinetics for TFPIα binding to FXa or FV-h in the FV-h:FXa complex are considered to be the same as those for TFPIα’s binding to FXa or FV-h outside of the complex. Both forms of prothrombinase (FVa:FXa and FV-h:FXa) are assumed to be active and able to cleave prothrombin [81], but the enzymatic function of FV-h:FXa is assumed to stop when TFPIα is bound to either the FV-h or FXa part of the complex. TFPIα does not inhibit the enzymatic function of standard prothrombinase FVa:FXa.

2.3.6 Inhibition of Prothrombinase Assembly

As mentioned above, we assume that TFPIα can be bound to both FXa and FV-h at the same time via its Kunitz 2 domain and C-terminus, respectively, but binding interactions between FXa and FV-h in that case are blocked. In this situation, TFPIα is inhibiting the assembly of FXa and FV-h into
prothrombinase. The inhibition of assembly can occur through two different, two-step reactions: TFPIα binds to FV-h via its C terminus first and then binds to FXa via its K2 domain (Reaction 11 in Table 2.1), or TFPIα binds to FXa via its K2 domain first and then binds to FV-h via its C terminus (Reaction 12 in Table 2.1). This is illustrated by the reactions shown on the bottom surface of the activated platelet in Fig. Figure 2.1(right). Each coagulation factor to which TFPIα is bound can either be platelet-bound or in the fluid. Thus, there are multiple situations to consider, including ones in which one of the coagulation factors in the FV-h:TFPIα:FXa complex is bound to the platelet membrane and the other factor is not bound to the membrane, but is held close to the platelet through its interaction with TFPIα (Reactions 16-21 in Table 2.1). In such cases, the free end of the ternary complex can become attached to the membrane, or the membrane-bound end can detach from the surface and release the entire ternary complex into the fluid. The fluid-phase ternary complex can rebind to a platelet surface by either its FV-h or FXa, or it may be washed away by the flow.

2.3.7 Model Equations

The reactions in Table 2.1 are translated into mathematical equations using the law of mass action. Equation (2.1) is an example of the type of equations that comprise the model. It describes the rate of change of the concentration of FV-h in the fluid, by the processes of the binding and unbinding of FV-h from the platelet surface, its delivery or removal due to flow, its secretion from the platelet, its full activation by thrombin, and its binding to and unbinding from TFPIα:

\[
\frac{de_5^h}{dt} = -k_{on}^h e_5^h e_5^m \cdot p_{avail}^h + k_{off}^h e_5^m + k_{flow} (e_5^{h,up} - e_5^h) + h_5 \cdot n_5 \cdot \frac{d}{dt} ([PL_a^s] + [PL_a^u]) - k_{e_5^h:e_2}^+ e_2 e_5^h + k_{e_5^h:TFPI}^- [E_5^h : TFPI] (2.1)
\]

In this equation, \( p_{avail}^h \) denotes the concentration of platelet binding sites for FV, FV-h, and FVa that are not already bound to one of these species, \( n_5 \) denotes the total number of FV and FV-h molecules released by a platelet when it is activated, \( e_5^{h,up} \) is the concentration of FV-h in the bulk plasma (generally set to 0), and \( \frac{d}{dt} ([PL_a^s] + [PL_a^u]) \) is the rate at which platelets are activated. The other quantities are defined in Table 2.1.
2.4 Results

2.4.1 Tissue Factor and Shear Rate Dependency

Here we examined how the variation in TF density and shear rate affected thrombin production with
the additional TFPIα inhibitory mechanisms and compared the outcomes to those from our previous
model. We focused on how TF and shear rate dependency differ for low (0.5 nM) and high (2.5 nM)
TFPIα plasma concentrations. For various TF densities in the range 0 to 20 fmol/cm² and for shear rates
100/s, 500/s and 1500/s we performed simulations with the old and new models. We looked at two output
metrics, the lag time, which we define as the time-point at which the thrombin concentration first reaches 1
nM, and the thrombin concentration at 10 minutes. Fig. (Figure 2.2A,C) show the lag times and Fig.
(Figure 2.2B,D) show the thrombin concentrations at 10 minutes. For both old and new models and for
both TFPIα levels, the lag time decreased as the TF density increased and/or the shear rate decreased.
The reasons for these behaviors is that a higher TF density provides a larger initial stimulus and
decreasing the shear rate slows the loss of essential enzymes from the RZ. Also, the thrombin
concentrations at 10 minutes increased with the TF density, sharply at low TF densities and more
gradually at high ones. In fact, the results indicated a threshold dependence on TF density in all cases
examined. (We refer to curves of thrombin at 10 minutes vs. TF density as threshold curves.) The
thrombin concentration at 10 minutes was also affected by the shear rate; in particular, the level of TF
necessary to achieve a high thrombin concentration depended on the shear rate and, for high TF, the
thrombin concentration at 10 minutes was somewhat higher for lower shear rate.

In Fig. (Figure 2.2) results for the old and new models are indicated by gray and black lines,
respectively. In Fig. (Figure 2.2A,C), we see that, for a given TF density, the newly added
TFPIα inhibition increases the lag time, and that to achieve a particular lag time, a larger TF density was
needed with the new model. This is because the additional inhibition reactions made it harder for the
system to accumulate FXa on platelet surfaces and this slowed prothrombinase formation and thrombin
generation. We see for both TFPIα levels that the change in lag time between the two models was greater
at higher shear rate. For the low TFPIα level (Fig. (Figure 2.2A)), we see that for each shear rate, the
curves for the two models converged as the TF density was increased, indicating that the effect of the
additional inhibition is more pronounced at low TF densities. Compared to these results, we see that for
the higher TFPIα level (Fig. (Figure 2.2C)), the differences between the two models were greater for each
TF density and that a substantial effect of the additional inhibition persists to much higher TF densities.

Turning to Fig. (Figure 2.2B,D), we look at how the thrombin concentration at 10 minutes differs
between the two models for different TF densities and shear rates. For each shear rate, the threshold TF
density was increased by the additional TFPIα inhibition reactions, that is, the threshold curves were shifted to the right. For low TFPIα, the effect was small for shear rate 100/s and progressively increased as the shear rate was increased first to 500/s and then to 1500/s. For the range of TF densities that were above threshold for the old model and below threshold for the new one, there was a notable difference in the thrombin concentrations at 10 minutes. For TF densities that were below threshold for both models or above threshold for both models, there was little effect of the additional inhibition reactions. In particular, at high TF densities, the thrombin concentrations at 10 minutes are very similar. For high TFPIα, the effects of the model changes are more dramatic. There is a much broader range of TF densities which for the old model are above threshold and for the new model are below threshold, and for TF in this range there was a substantial difference in the thrombin concentrations achieved with the two models.

In summary, a major effect of the additional platelet-surface-mediated TFPIα inhibition reactions was to increase the TF threshold for substantial thrombin production to a degree that increased with increasing plasma TFPIα levels. For TF densities that were above threshold for both models, the lag time was larger with the additional reactions. The magnitude of the extra delay in thrombin production engendered by the additional inhibition varied with the TF density and shear rate and was substantial for a range of TF densities and shear rate 1500/s for low TFPIα and for a much broader range of TF densities and all shear rates considered for high TFPIα. The thrombin after 10 minutes for a sufficiently high TF density was affected little by the additional reactions for both TFPIα levels and all shear rates considered.

2.4.2 Impact of TFPIα Concentration on Thrombin Generation

The previous results were based on variations in TF and shear rate for two fixed plasma concentrations of TFPIα. Next, we fixed the TF density at 2.5 fmol/cm² or 10 fmol/cm² and the shear rate at 100/s and studied how varying the TFPIα levels from 0 to 2.5 nM in increments of 0.5 nM affected thrombin generation. We did this only with the new model as the old model’s results varied negligibly with the TFPIα level. The variation of thrombin with time for these experiments is shown in Fig. (Figure 2.3). In Fig. (Figure 2.3A) two qualitatively different outcomes are seen for a TF density of 2.5 fmol/cm² depending on the TFPIα level. As the TFPIα concentration was increased from 0 nM to 0.5 nM and then to 1.0 nM, there was a substantial prolongation of the lag time from 923s to about 1400s to about 1980s. The thrombin concentrations at 40 minutes in these simulations were 333, 303, and 261 nM for TFPIα concentrations of 0, 0.5, and 1.0 nM, so the new inhibition reactions also affected, albeit modestly, the thrombin concentrations at this time. For higher TFPIα concentrations (1.5, 2.0, and 2.5 nM) the thrombin curves peaked at levels below 1 nM and were in decline during the latter part of the simulations, indicating that robust thrombin production would never occur. These results are reflected in and explained
Figure 2.2 Effects of the TF level and shear rate on the lag time and the thrombin concentration threshold behavior. (A) Lag times and (B) thrombin concentration after 10 minutes for a range of TF densities and three different shear rates. Black curves represent the results from the new model, which includes the additional TFPI\(\alpha\)-mediated inhibition reactions, and gray curves represent the results from the old model, where TFPI\(\alpha\) could only bind to fluid phase FXa and then to TF:VIIa. All simulations were run with \([\text{TFPI}\alpha]\) = 0.5 nM. Vertical dashed lines indicate TF densities of interest. The flat horizontal lines in (A,C) at lag time > 30 indicate that the thrombin concentration did not reach 1 nM within 30 minutes for the corresponding TF density.

by the concentration time course of FXa, FV-h, and their complexes with TFPI\(\alpha\) under different TF, shear rate and TFPI levels, as shown in Figure S7. Similar to what we observed for thrombin, higher TF levels lead to higher concentrations of platelet-bound FXa and FV-h, whereas responses to increases in shear rate are more sensitive at low TF levels, see Figure S7.

The thrombin curves for a TF density of 10 fmol/cm\(^2\) are shown in Fig. (Figure 2.3B). At this TF density, the variations in TFPI\(\alpha\) levels prolonged the lag time modestly, and robust thrombin production
Figure 2.3 Thrombin concentration vs. time for the indicated TFPIα levels for shear rate 100/s and with (A) TF = 2.5 fmol/cm² and (B) TF = 6 fmol/cm². Note the different time scales.

occurred for all the TFPIα concentrations examined. These results reinforced our earlier observation that the additional TFPIα-mediated inhibition reactions can strongly influence thrombin dynamics with the greatest impact happening for low TF densities. These results are in line with experimental ones showing the major inhibitory effects of TFPI under flow occur at low TF [93]. In that study, TFPI antagonism was shown to decrease the lag time of fibrin deposition at low TF and to a lesser extent at higher TF levels. Our results for thrombin generation with TFPI = 0 and 0.5 nM, plotted on a linear scale (Figure S4), compare well with their time courses of fibrin deposition and differences they see in lag times with and without TFPI antagonism and under different TF levels.

2.4.3 Examination of the Major Inhibitory Reaction Step(s)

Our model included two new ways that TFPI could impede thrombin generation, through direct binding with platelet-bound FXa and through interactions with FV-h. To investigate whether one binding reaction more greatly affects the lag time, and to determine for which TF levels that occurred, we computed the lag times as we varied the TF density and the TFPIα concentration with and without the binding of TFPIα to FXa and FV-h. The results are shown in the four heat maps in Fig. (Figure 2.4). In the heat maps, the color indicates the lag time in minutes (white cells indicate that the thrombin concentration did not achieve 1 nM within 40 min), a ”-” indicates that the binding reaction is ”turned off” and a ”+” indicates that the binding reaction is ”turned on”. To ”turn off” the binding of TFPIα to FXa, we set all association rates between TFPIα and FXa to zero (Reactions 7,11,17,22 and 24 in Table 2.1). Similarly, to ”turn off” TFPIα binding with FV-h, we set all association rates between TFPI
and FV-h to zero (Reactions 1, 5, 8, 12, 16 and 23 in Table 2.1).

As expected, when we turned off TFPIα binding to both FXa and FV-h, shown in Fig. (Figure 2.4A), we observed that the lag time was dependent only on TF (displaying threshold dependence) and was independent of the TFPIα concentration. For simulations in which we turned on only the binding between TFPIα and FV-h, the lag time exhibits dependence on TFPIα, but this effect saturated at a TFPIα concentration greater than about 3 nM, see Fig. (Figure 2.4B). Also, the influence of the TFPIα concentration on the lag time was much diminished for TF densities above about 6 fmol/cm².

If instead, we turned on only the binding of TFPIα to FXa, there was a slightly greater dependence of lag time on both TFPIα and TF, and this effect did not saturate with TFPIα level, at least up to 5 nM, see Fig. (Figure 2.4C). Turning on both binding reactions, as seen in Fig. (Figure 2.4D), the lag time threshold was shifted to a higher TF level than in either of the previous cases, consistent with the shift in the TF threshold in the new model revealed in Fig. Figure 2.2. Also notice that the red vertical line at TF 2.5 fmol/cm² represents the same information as that at the intersections of the thrombin curves and the 1 nM level line (red horizontal line) in Fig. Figure 2.3, where the TF density was fixed to 2.5 fmol/cm².

Comparing the heat maps in Fig. (Figure 2.4B-C), we conclude that binding of TFPI to one of either FV-h or FXa significantly altered the lag time, but that the TFPIα-FXa binding had a stronger influence.

For a more detailed comparison of the lag times for the 4 cases at specific TFPI levels, Fig. (Figure 2.4E,F) shows plots of the lag times as a function of TF density for two specific TFPIα concentrations, 0.5 nM and 4 nM, indicated with horizontal black lines in the heatmaps. These plots allow a clearer comparison of the effects of each of the binding reactions on lag times. In particular Fig. (Figure 2.4E) shows that at the lower TFPIα, lag times are increased in the order of adding TFPIα/FV-h reactions alone, adding TFPIα/FXa reactions alone, and finally both reactions together (the curves shift slightly to the right and never cross). However, for the higher TFPIα shown in Fig. (Figure 2.4F), the larger lag times are clearer but additionally, a larger effect of one of the individual reactions alone depends on the TF density (gray dashed line and black solid line cross at TF 4 fmol/cm²). The greatest increase in lag time occurred when both reactions were at play (Fig. Figure 2.4D).

2.4.4 Significance of Platelet Derived FV

To further investigate the importance of FV-h in the coagulation system, we explored the effect of the FV-h derived from platelets that is secreted upon activation. We did this by varying the parameter $h_5$ that denotes the fraction of all of the FV-type molecules released by the platelet that are half-activated FV-h. This parameter is used in Eq. (2.1) above. Fig. (Figure 2.5) shows the thrombin generation over time for various $h_5$ and TF values for low (0.5 nM) and high (2.5 nM) TFPIα plasma concentrations. We vary $h_5$
Figure 2.4 Lag times as functions of TF and TFPIα levels with the specified TFPIα binding reactions. Lag times for cases in which (A) TFPIα binding with both FV-h and FXa are turned off, (B) TFPIα binding with FV-h is turned on and TFPIα binding with FXa is turned off, (C) TFPIα binding with FXa is turned on and TFPIα binding with FV-h is turned off, and (D) TFPIα binding to both FV-h and FXa are turned on. For parameter values in the region without color, the thrombin concentration did not reach 1 nM thrombin within 40 minutes. Lag time vs. TF density for TFPIα = 0.5 nM with and without TFPIα binding FV-h or/and FXa for (E) TFPIα = 0.5 nM and (F) TFPIα = 4.0 nM. These curves correspond to the slices in the heat map indicated by the horizontal lines in (A-D). Shear rate is fixed to 100/s.

among 0, 0.5 and 1, which correspond, respectively, to only zymogen FV, a mixture of half FV and half partially activated FV-h, and only FV-h being released from platelets upon activation. At the low TFPIα concentration, we considered TF densities 2.5 and 6.0 fmol/cm^2 and for the high TFPIα concentration, we ran simulations at TF densities 4.0 and 10.0 fmol/cm^2. These values, indicated by vertical lines in Fig. (Figure 2.3B,D), bracket the range of TF values over which the thrombin
concentration at 10 minutes changed rapidly as the TF density was varied for shear rate 100/s and the low and high TFPIα concentrations respectively.

For both TFPIα concentrations, the resulting behaviors were qualitatively similar but differed in magnitude. In Fig. (Figure 2.5A), we see that for TFPIα = 0.5 nM and for the higher TF density (6.0 fmol/cm²), there was little difference in the thrombin curves for the different $h_5$ values. In contrast, for the lower TF density (2.5 fmol/cm²), the effect of increasing $h_5$ was to shorten the lag time, from 1419s when only zymogen is released by the platelets, to 1080s for the 50/50 mixture, to 914s when only partially-activated FV-h was released. For both TF densities, the thrombin concentration at 40 minutes changed little with changes in $h_5$. Fig. (Figure 2.5B) shows that for TFPIα = 2.5 nM, there was almost negligible difference in the thrombin curves for the higher TF density 10.0 fmol/cm²), and that, while the variation in lag time with $h_5$ was greater for TF density 4.0 fmol/cm²), it was much less than for the combination of low TFPIα concentration and low TF density shown by the black curves in Fig. (Figure 2.5A). To summarize, an increase of FV-h content within the platelets shortened the lag times. When TFPIα was low, the effect was the greatest for a low TF density.

2.4.5 Local Sensitivity Analysis

We performed a local, one-at-a-time, sensitivity analysis (SA) of the new model to the new parameters relating to TFPI binding with FV-h. All of the details and the results are shown in the Supplemental Information (section S4, Figure S2 and S3) and we will give only a brief summary of them here. The SA
methods were based on those developed by Saltelli [94, 95], and those we used to analyze our previous model [61]. We chose three thrombin metrics: lag time, maximum rate of thrombin generation, and final thrombin concentration, as the quantities of interest and then ranked the sensitivities of these quantities to levels of all clotting factors and inhibitors except for TFPIα, as in our previous work [61]. The lag times in the new model showed a higher sensitivity to TFPIα levels than in our old model, as expected from the previous results shown above; the effects of TFPIα on the maximum rates and final thrombin concentration were negligible, as they were with the previous model. However, in comparison to our previous SA results, FX became increasingly important for the lag times and maximal rates with the new model, which makes sense due to the direct inhibition of FXa on platelet surfaces by TFPIα. Next, a local sensitivity analysis was performed on the kinetic rates. Forward and reverse kinetic rates for each of the TFPIα/FV-h reactions were varied by 10% and the changes in the three thrombin metrics were measured. These small perturbations in reaction rates had minimal effects on all thrombin metrics; none of the perturbations led to more than a 1% change from baseline metric values.

2.5 Discussion

Our previous mathematical model, in which TFPIα acts only through FXa and TF:VIIa, showed minor inhibitory effects of TFPIα on thrombin generation. In that model, most FXa that was produced on the subendothelium and subsequently bound to TFPIα in the fluid was quickly washed away by the flow before it could rebind to the TF:VIIa in the small injury zone. It is possible that for a longer injury, the fluid-phase FXa:TFPIα complexes might bind to TF:VIIa downstream and inhibit its action. Other reaction schemes for this pathway have been considered [8, 91, 96–98], which has led to some disagreement regarding the effects of TFPIα under flow. Further mathematical investigation of different schemes and injury sizes is warranted to better understand TFPIα inhibition via TF:VIIa under flow.

To the best of our knowledge, the current study is the first mathematical modeling approach to investigate TFPIα inhibition on the surface of activated platelets. We extended our previous model to include generation and activation of FV-h, as well as its inhibition by TFPIα on activated platelet surfaces. Additionally, we included TFPIα binding to FXa bound to an activated platelet surface that leads to its inhibition. These extensions relate solely to platelet surface reactions, and essentially all of the inhibitory effects of TFPIα we observe in the extended model are due to these reactions, since TFPIα had very little influence in our earlier model. We studied how the newly added TFPIα inhibition pathways affected thrombin generation related to the TF threshold behavior, thrombin lag times, the system’s sensitivity to shear rate, and how thrombin generation was affected by varying TFPIα levels at low and high TF densities. In general, we found that the newly added TFPIα reactions created a new sensitivity of the
model system to TFPIα levels, especially at low TF levels. At high TF densities, the same TFPIα concentration increased the lag time but still led to a robust thrombin response. In fact, when a strong thrombin response was generated, under variations of TFPIα from 0 to 5 nM, the system produced approximately the same final thrombin concentrations.

Our general results about TFPIα inhibition under flow are in line with previous experimental studies performed with microfluidics [93]. In those studies, it was observed that TFPIα antagonism enhanced fibrin formation (thrombin generation in our model can be thought of as a surrogate for fibrin formation) when normal blood or plasma was flowed over TF/collagen microspots, but this effect was seen only at low TF densities. The experiments did not run long enough to see if the final fibrin fluorescence achieved was similar with and without TFPIα antagonism, however there was a significant fibrin response that was delayed with TFPIα. Taken together with our results, this suggests that platelet-surface dependent TFPIα inhibition affects the timing but not the magnitude of a thrombin response and that these effects are most pronounced at low TF densities. TFPI leads to delayed lag times under static conditions as well. van’t Veer and Mann performed thrombin generation assays with and without TFPI and with varying levels of TF [99]. In comparing the lag times with TFPI to those without, they found that it was always increased and that the increases were more prominent for lower TF levels. Although these assays were performed with lipid vesicles and not platelets, the surface-dependent mechanism could still be at play.

Our mathematical model gives as output the concentration of every model species at every point in time, and also gives us the freedom to turn on and turn off specific pathways. These capabilities allowed us to investigate TFPIα binding with FXa and FV-h to characterize their individual and combined inhibitory behavior. In turning off these pathways separately and together, we found that TFPIα binding with both FXa and FV-h has a strong inhibitory role. When TFPIα could bind with FV-h, but not with FXa in either plasma or on platelet surfaces, the inhibitory effect of TFPIα saturated at about a 3 nM TFPIα concentration. With the FXa inhibition pathway on, but without TFPIα/FV-h binding, the inhibitory effects of TFPIα increased with increasing TFPIα concentration, up to the maximum that we simulated (5 nM). These results can be explained by considering what constitutes prothrombinase in the system. In this new model, there are two forms of prothrombinase, FXa:FV-h and FXa:FVa, both of which can actively convert prothrombin to thrombin. In our model, to form either type of prothrombinase complex, FV must first be converted to FV-h by FXa or to FVa by thrombin. Only FXa is present in the initial stage of coagulation, so FV-h is available first. This allows formation of FV-h:FXa which generates the first thrombin. Once a sufficient level of thrombin is reached, thrombin becomes the primary activator of FV, fully activating it to FVa, leading to FXa:FVa formation, which is protected from TFPIa inhibition and further supports propagation of thrombin. This is in line with the proposed scheme of Schuijt et
al., suggesting that FXa-dependent FV activation is pivotal to the initiation phase of coagulation. Essentially, FV-h is needed early to generate the first thrombin and start the positive feedback, then FVa is the primary cofactor for FXa. In other words, the prothrombinase concentration is limited by the amount of FXa and not by the amount of FV-h and this explains the observed differences between the two inhibitory pathways.

The composition of platelet-released FV forms was important in the new model. Increasing the amount of FV-h relative to FV within the platelets resulted in acceleration of thrombin generation with the most significant effects at low TF and effects that are mostly washed out at higher TF. At low TF, if all platelet-released FV was FV-h, then there would be no need for FXa to activate it first, thus removing a reaction step and accelerating coagulation. Additionally, the released FV-h would be able to immediately bind to FXa on activated platelet surfaces to form active prothrombinase (FV-h:FXa) during the early stages of coagulation. Our simulations in this study were performed under the assumption of normal healthy blood and it is possible these effects are magnified in the case of hemophilia A. In our previous study [10], in which the model did not account for FV-h, we found that lowering the FV levels in plasma and in platelets could enhance thrombin generation in hemophilia A, where there is a deficiency in FVIII. In that study, the model suggested that the reason for this is that FV and FVIII compete for FXa in the early stage of coagulation and that lowering FV weakens this competition so that more of the albeit small quantity of FVIII can be activated; this leads to more tenase, more prothrombinase, and ultimately to more thrombin. In our new model we have introduced a new species that also competes for FXa, namely FV-h. Thus there is now competition between three species for FXa: FV, FVIII, and FV-h. It will be interesting to see how this changes our results for hemophilia A blood and this is a topic of immediate future research.

It seems plausible then that a reduction or modulation of inhibitors could offset complications associated with bleeding disorders. For example, TFPIα levels have been shown to affect clotting in individuals with hemophilia A and B, the bleeding disorders characterized by deficiencies in clotting factors VIII (FVIII) and IX (FIX), respectively. Several biochemical experiments have revealed relationships between TFPIα and the early stages of coagulation in hemophilia blood [17]. One study showed that TFPIα modulates the generation of FXa by inhibiting TF:VIIa in the presence of FVIII and FIX [100], suggesting that TFPIα itself could be inhibited to enhance coagulation. This hypothesis was examined in various animal models as well: a recent mouse study demonstrated that an anti-TFPIα antibody used in mice under hemophilic conditions decreased the hemoglobin lost over 20 min following tail transection. This indicated that direct inhibition of TFPIα effectively suppressed tail bleeding in mice with hemophilia [101]. Another study showed that rabbits made temporarily hemophilic with anti-FVIII antibodies had reduced mean bleeding times after injection with anti-TFPIα antibodies [102]. This study demonstrated
that partial inhibition of TFPIα mitigates bleeding under hemophilia conditions. Together with our previous findings regarding FV and hemophilia, these studies suggest the potential for TFPIα and FV manipulation in the treatment of hemophilia. Further investigation of interactions between TFPIα and FV and their effects on coagulation in hemophilia is thus warranted. As a first step, we have developed a mathematical model of coagulation under flow that includes the relevant TFPIα and FV interactions with the goal to make possible future studies focused on hemophilia.

The reason we studied TFPIα at 0.5 and 2.5 nM is because it is still not clear how much free TFPIα is in the blood. Although TFPI is often reported to be 2.5 nM in plasma, TFPIα has been suggested to account for only 10-30% of the total TFPI pool in the blood [76] with platelet TFPI accounting for about 7-8% of this total [31]. Approximately 80% of plasma TFPI is a C-terminus truncated form (cannot bind to FV-h) and is thought to be bound to lipoprotein [32]. Although one biochemical study showed that the lipoprotein-bound form of TFPI can actively inhibit thrombin generation [103], it is not entirely clear how inhibitory this form is in vivo. The remaining 20% is free in the plasma and consists of a mixture of a full length TFPIα form and the truncated form. Thus, the 0.5 nM levels studied here represent that 20%.

One limitation of our model is the absence of explicit inclusion of protein S in the coagulation reactions. Protein S is known to be a critical regulator of coagulation, playing the role of cofactor for both activated protein C and TFPIα in the inactivation of FVa and inhibition of FXa, respectively [104]. Protein S can bind directly to lipid surfaces, and thus could localize TFPIα bound to the Protein S near the lipid surface. This is likely part of a mechanism that enhances the rate of encounter between platelet-bound FXa and TFPIα. FV-short and protein S have recently been shown to be synergistic cofactors for TFPIα, leading to much stronger inhibition of FXa together than when TFPIα is bound to only one of FV-h or Protein S [105]. This synergy seems to occur via formation of a trimolecular complex of TFPIα, FV-short, and protein S [106, 107] and it was hypothesized that these complexes are present in circulating plasma at subnanomolar concentrations [107]. Considering the high concentrations of protein S in plasma, it was further suggested that nearly all of the TFPIα in plasma may be bound within such trimolecular complexes, and that no or very little free FV-short or free TFPIα exist in plasma [106], however data supporting that hypothesis are sparse, and as mentioned above, it is still unclear how much free TFPIα exists in circulating plasma. While we do not include Protein S explicitly in our model, some of our simulation results may still shed light on its potential effects. For example, our simulations with high levels of plasma TFPIα can be thought of as corresponding to lower levels of the Protein S-FV short-TFPIα complex which has 10-fold higher power to inhibit FXa than does TFPIα alone [104]. Similarly, if almost all plasma TFPIα is in complex with Protein S and FV-short, then little TFPIα would be available to bind with FV-h produced by FXa or released from platelets, while FXa-inhibiting capability
would still be present. Our simulations in which we “turned off” TFPI\(\alpha\) binding to FV-h would correspond, at least approximately, to that situation. Thus, even in scenarios in which TFPI\(\alpha\) circulates in complex with Protein S and FV-short, our model provides strong evidence that direct inhibition of platelet-bound FXa is an important mechanism for regulating thrombin generation under flow. In future research, we plan to explicitly include in the model protein S and its cofactor activity for TFPI\(\alpha\) and activated protein C.

Finally, it is also known that platelets secrete TFPI\(\alpha\) upon activation with thrombin [31, 108]. The exact location of these TFPI\(\alpha\) molecules within the platelet is unknown but it is known that it is not the \(\alpha\)-granules [108] where FV is stored. It is possible that this stored and released TFPI\(\alpha\) pool could play an important role in regulating thrombin generation, especially if protein S can quickly localize it to the platelet surface. This is another topic of immediate future research.

2.6 Conclusion

In this study, we extended a mathematical model of flow-mediated coagulation to explore the effects of TFPI\(\alpha\)-mediated inhibitory reactions that take place on activated platelet surfaces. Results from our new model show that the surface-dependent TFPI inhibition is much stronger than the TFPI inhibition on the subendothelium and as such, the TF threshold density is shifted to higher values in our new model compared with our old one. Further, these reactions could lead to significant delays in thrombin generation at low TF density if TFPI\(\alpha\) levels were increased. Ultimately, the new model was sensitive to changes in TFPI\(\alpha\) levels, especially at low TF density, and gave TFPI\(\alpha\) potential to be a significant modifier of thrombin generation. There are still some additional limitations in this model that we need to address in future work: (1) we do not include TFPI\(\alpha\) secretion from platelets, (2) we do not consider inhibition by TFPI\(\beta\) in the endothelial region, and (3) some reaction kinetics are unknown and so we made assumptions about their values and more experimental data would be useful for defining more precise values. Nevertheless, we developed an updated version of an experimentally validated mathematical model of flow-mediated coagulation that is sensitive to TFPI\(\alpha\) and successfully exhibits the experimentally observed inhibitory effects of TFPI\(\alpha\) under flow. More importantly, this model revealed the importance of the platelet surface as a platform for efficient inhibition. It is known that the platelet surface dependence of procoagulant enzyme reactions is key to localizing coagulation to the site of injury. Thus, it makes sense that the inhibition of these key enzymes should also be most effective where they are needed most. Finally, TFPI\(\alpha\) may be a potential therapeutic target to rescue thrombin generation in various bleeding disorders, and the current model can serve as a foundational tool to assess the effectiveness of TFPI\(\alpha\) as a modifier of thrombin generation in these cases.
Table 2.1 List of reactions added to the model and their kinetic rate constants, with literature references.

k⁺ shows forward reaction rate, k⁻ shows backward reactions rate, and kcat indicates catalytic reaction rate:

a) Binding of TFPIα to FV-h, Kₐ = 9 pM from Jeremy et al (2014) [76].
b) Activation of FV by thrombin, Kₐ = 7.4(10⁻⁵) M from Monicovic and Tracy (1990) [88].
c) Binding between FV-h and FXa, Kₐ = 1(10⁻¹⁰) M from Mann (1987) [89].
d) FV-h binding platelet surface, Kₐ = 3(10⁻⁹) M from Krishnaswamy et al (1988) [90].
e) TFPIα binding FXa, Kₐ = 2(10⁻¹¹) M from Jesty et al (1994) [91].
f) Prothrombin activation by prothrombinase that has FV-h, Kₐ = 0.4uM from Camire et al (2020) [81].
g) Inactivation of FV-h by activated protein C, Kₐ = 12.5(10⁻⁵) M from Solynose and et al (1988) [92].
h) Generation of FV-h by FXa, kcat = 0.046 s⁻¹ and Kₐ = 10.4(10⁻⁹) M from Monicovic and Tracy (1990) [88].

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CHAPTER 3
INHIBITION OF PLATELET-SURFACE-BOUND PROTEINS DURING COAGULATION UNDER FLOW II: THE ROLE OF ANTITHROMBIN AND HEPARIN (JOURNAL ARTICLE)

Modified from a paper published in The Biophysical Journal. Kenji Miyazawa, Aaron Fogelson, Karin Leiderman

3.1 abstract

Blood coagulation is a self-repair process regulated by activated platelet surfaces, clotting factors, and inhibitors. Antithrombin (AT) is one such inhibitor that impedes coagulation by targeting and inactivating several key coagulation enzymes. The effect of AT is greatly enhanced in the presence of heparin, a common anticoagulant drug. When heparin binds to AT and either bridges with the target enzyme or induces allosteric changes in AT leading to more favorable binding with the target enzyme. Antithrombin inhibition of fluid-phase enzymes caused little suppression of thrombin generation in our previous mathematical models of blood coagulation under flow. This is because in that model, flow itself was a greater inhibitor of the fluid-phase enzymes than AT. From clinical observations, it is clear that AT and heparin should have strong inhibitory effects on thrombin generation, and thus we hypothesized that AT could be inhibiting enzymes bound to activated platelet surfaces that are not subject to being washed away by flow. We extended our mathematical model to include the relevant reactions of AT inhibition at the activated platelet surfaces as well as those for unfractionated heparin and a low molecular weight heparin. Our results show that antithrombin alone is only an effective inhibitor at low tissue factor densities, but in the presence of heparin, it can greatly alter and in some cases shut down thrombin generation. Additionally, we studied each target enzyme separately and found that inactivation of no single enzyme could substantially suppress thrombin generation.

3.2 Introduction

Antithrombin (AT) is an inhibitor of blood coagulation that belongs to the serine protease inhibitor (serpin) superfamily and is found in plasma. It is continually generated in the liver to maintain a normal level in plasma of approximately 2.4 µM. The anticoagulant activity of AT involves the irreversible inactivation of four key serine proteases generated during coagulation: FIXa, FXa, FXIa, and thrombin. Inactivation of FIXa reduces tenase formation; inactivation of FXa reduces prothrombinase formation; inactivation of FXIa weakens its positive feedback; and inactivation of
thrombin reduces positive feedback, platelet activation, and overall clot formation [42]. A deficiency in AT can lead to excessive thrombin generation [44], which is associated with venous thromboembolism [45].

Although AT is thought to be an important inhibitor by itself, the range of its inactivation rates for coagulation enzymes is quite large, from 10-14,000/(M · s), which covers measurements at both 25 and 37 degrees Celsius [111, 112]. Its anticoagulant activity is significantly enhanced in the presence of heparin, which is the oldest anticoagulant drug used in clinical medicine, discovered first by Mclean in 1916 [47, 48]. Heparin is a naturally occurring glycosaminoglycan that exerts its anticoagulant properties by forming a complex with AT and facilitating an enhanced inhibitory effect of AT on activated coagulation factors [49]. Unfractionated heparin (UFH) is a minimally processed form of the natural heparin and thus was the first to be used in medicine. The structure and length of UFH were found to be quite heterogeneous, and this led to unwanted and unpredictable side effects and the need for continuous monitoring during its use as an anticoagulant drug. Further processing to shorten and standardize lengths was desirable to achieve more predictable outcomes [47]. These low molecular weight heparins (LMWHs) are derived from UFH using different but controlled manufacturing processes that lead to mean molecular weights that are less than half that of UFH [51, 52]. Compared to UFH, LMWHs have longer circulating half-lives and higher bio-availability, more predictable outcomes and less monitoring, and are thus largely favored for clinical use [54–57].

The anticoagulant activity of heparin is its ability to accelerate the inactivation of activated coagulation factors via AT. This activity and the molecular weight of heparin have an interesting and complex relationship. It is now understood that there are two main mechanisms for heparin’s anticoagulant activity. There is allosteric activation of AT by heparin, which alters the structure of AT and enhances its recognition by the various coagulation factors [113, 114], and there is also the fact that heparin provides a template to which both AT and coagulation factors can bind and form a ternary bridging complex [115, 116]. It is thought that the longer the heparin, the more significant the bridging effect can be [117]. The allosteric activation mechanism works to inactivate FIXa and FXa but not thrombin or FXIa [113, 114]. The bridging mechanism can potentially affect all four species, to varying degrees, but thrombin inhibition is solely dependent on this mechanism; thrombin inhibition is significantly enhanced by UFH when there is sufficient room on it for thrombin to bind but much less so by LMWHs [115, 116]. Enhanced inhibition of FXIa occurs mainly by the bridging mechanism but with a slight variation: FXIa has two binding sites for heparin, one noncatalytic site through which the bridging with AT can occur, and a catalytic site through which heparin can trigger allosteric modulation of FXIa functional activity (in contrast to the allosteric effects on AT described above) [110, 118]. To summarize, both LMWH and UFH can affect all four enzymes, but they do so to varying degrees, depending on their lengths. In this study, we
used a mathematical model of flow-mediated coagulation to explore the effects of LMWH and UFH on inhibition of each individual enzyme and all of them together. We chose to use Nadroparin as the LMWH since there are literature values for its kinetic rate constants along with rates for UFH within the same experimental study [112].

In our companion study focused on tissue factor pathway inhibitor (TFPI), we showed that platelet surfaces played an important yet indirect role in the inhibitory mechanisms of TFPI [85]. Platelet-surface bound enzymes are necessary in coagulation since they help localize coagulation to the site of injury and are many orders of magnitude more efficient than their fluid-phase analogues. However, they are limited by the number of activated platelets and the corresponding binding sites on those platelets’ surfaces. We hypothesized that direct binding of these platelet-bound enzymes by fluid-phase inhibitors would have a stronger overall inhibitory effect on coagulation compared to their binding of fluid-phase enzymes since the fluid-phase complexes are subject to being washed away by flow. Our simulation results confirmed our hypothesis in the case of TFPI and this led us to hypothesize that AT (with and without heparin) may be working in a similar manner.

To investigate this, we extended the model presented in our companion paper [85] to include surface-dependent inactivation by AT. In our previous model, AT could directly bind and inactivate fluid-phase enzymes only: FIXa, FXa and thrombin, but these inhibitory reactions showed little to no affect on thrombin generation [61] except under the near-stasis conditions of venous thrombosis [119]. Here we have included the inactivation of FXa, FIXa and thrombin bound to the platelet surface, and additionally the inactivation of FXIa in both fluid phase and bound to the platelet surface. Heparin (UFH and LMWH) was explicitly introduced into the model to examine its effects on anticoagulant activity via AT. Our results demonstrated that the inclusion of surface-dependent inactivation magnified the effects of AT, and did so to a greater extent when heparin was present. AT in the new version of the model altered thrombin generation at low but not high tissue factor, and almost entirely through its effect on surface-bound enzymes. In the presence of heparin, thrombin generation could be significantly delayed and reduced and these behaviors, too, were completely dependent on direct inhibition of the surface-bound enzymes. In summary, we tested two targets for inhibition of thrombin generation under flow: fluid phase and platelet-bound enzymes. We identified direct binding to platelet surface bound enzymes by fluid-phase inhibitors to be the primary mechanism for effective overall inhibition of thrombin generation under flow.
3.3 Materials and Method

3.3.1 Mathematical model review

Here we give a brief review of our previously developed mathematical model of flow-mediated coagulation [7–9] and the details of the extensions we have made to it. More details about this model and its sensitivity to parameters can be found elsewhere [61]. The model simulates the coagulation reactions occurring in a small reaction zone (RZ) above an injury where TF in the subendothelium (SE) is exposed, see model schematic in Figure S1. Clotting factors and platelets are transported into and out of the RZ by a combination of flow and diffusion, using a mass transfer coefficient whose value is a function of vessel and injury size, the flow’s shear rate, and the species’ diffusivity. Clotting factor concentrations in the RZ change due to their involvement in the coagulation reactions and by transport in and out of the zone. Similarly, platelet concentrations change as platelets adhere to the injured wall, become activated, and as other platelets are transported in and out of the zone. As platelets build up in the reaction zone the height and volume of the reaction zone increase with the volume of plasma and concentration of platelets in it changing accordingly. The concentration of each species in the reaction zone is tracked with an ordinary differential equation; this choice relies on the assumption that each species is uniformly distributed (well-mixed) within the reaction zone. An additional well-mixed endothelial zone (EZ) is located adjacent to the RZ, in the direction perpendicular to the flow with height equal to that of the RZ and width dependent on the flow shear rate and protein diffusion coefficients. The EZ is where active protein C (APC) is produced by a complex formed by thrombomodulin in that zone and thrombin which has diffused to the EZ from the RZ. This APC either diffuses into the RZ or is carried away by the flow.

There are three forms of platelets in the model: unactivated platelets that exist in the plasma phase, activated platelets that are directly attached to SE, and activated platelets in the thrombus that are not directly attached to SE. Activation of platelets is achieved by contact with the SE, interaction with thrombin, or by exposure to already activated platelets (this is an indirect way to model release of agonists from platelet stores). Activated platelets provide the membrane surface necessary for coagulation factors to bind and react. Each activated platelet expresses specified types and numbers of binding sites to which coagulation proteins can selectively bind.

In our companion paper [85], we extended the model to include novel TFPI-mediated inhibition reactions that allow TFPI to act directly on activated platelet surface bound species. These reactions enabled enhanced inhibitory effects compared to our previous version(s) of the model. Here, we included an additional extension to incorporate the inactivation of coagulation enzymes by AT and the AT-heparin complex, specifically on the platelet surface.
Table 3.1 List of reactions added on top of the extension with newly added TFPI-mediated reactions, and their kinetic rate constants, with literature references. $k^+$ shows forward reaction rate and $k^-$ shows backward reaction rate. Subscript LWMH and UFH indicates reaction rates when AT is bound to either LWMH or UFH respectively:

a) For inhibition of FIXa by AT, $k^+ = 4.8 \times 10^2$. For inhibition of FXa by AT, $k^+ = 3.5 \times 10^3$. For inhibition of thrombin by AT, $k^+ = 1.4 \times 10^4$. And for inhibition of FXIa by AT, $k^+ = 2.4 \times 10^2$, from Olson et al. [111]

b) We assume the inhibition rate of FIXa and binding rate to platelet on specific binding site are the same as the normal binding site.

c) Binding of heparin to antithrombin, $K_D = 36nM$ for LWMH, and $K_D = 9.7nM$ for UFH, from Olson et al. [113]

d) Accelerated inhibition of FIXa by AT:UFH complex, $k^+ = 6.2 \times 10^6$, and AT:LMWH complex, $k^+ = 5 \times 10^5$. For FXa by AT:UFH complex, $k^+ = 6.6 \times 10^6$, and AT:LMWH complex, $k^+ = 1.3 \times 10^6$. For thrombin by AT:UFH complex, $k^+ = 4.7 \times 10^7$, and AT:LMWH complex, $k^+ = 5.3 \times 10^6$. And for FXIa by AT:UFH complex, $k^+ = 1.8 \times 10^5$, and AT:LMWH complex, $k^+ = 1 \times 10^4$, from Olson et al. [112]

e) Binding of FXIa to platelet surface, $K_D = 2.5 \times 10^{-9}$ from Ahmad et al. [120]

f) Binding of FXa to platelet surface, $K_D = 2.5 \times 10^{-9}$ from Walsh et al. [121]

g) Binding of thrombin to platelet surface, $K_D = 5.9 \times 10^{-7}$ from Mann et al. [122]

h) Binding of FIX to platelet surface, $K_D = 1 \times 10^{-7}$ from Greengard et al. [123]

i) Binding of FIXa to platelet surface, $K_D = 1.7 \times 10^{-7}$ from Miller et al. [124]

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Table 3.2 List of platelet-binding reactions added on top of the extension with newly added TFPI-meditated reactions, and their kinetic rate constants, with literature references. $k^{on}$ shows binding rate and $k^{off}$ shows unbinding reactions rate:

e) Binding of FIXa to platelet surface, $K_D = 2.5 \times 10^{-9}$ from Ahmad et al. [120]

f) We assume the inhibition rate of FIXa and binding rate to platelet on specific binding site are the same as the normal binding site.

g) Binding of FXa to platelet surface, $K_D = 2.5 \times 10^{-9}$ from Walsh et al. [121]

h) Binding of thrombin to platelet surface, $K_D = 5.9 \times 10^{-7}$ from Mann et al. [122]

i) Binding of FIX to platelet surface, $K_D = 1 \times 10^{-7}$ from Greengard et al. [123]

j) Binding of FIXa to platelet surface, $K_D = 1.7 \times 10^{-7}$ from Miller et al. [124]

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### 3.3.2 Extension in the new model

The new AT/heparin-mediated inactivation reactions include the inactivation of FXa, FIXa and thrombin bound to the platelet surface, and inactivation of FXIa both in the fluid phase and bound to the platelet surface. We previously assumed that the AT-mediated inactivation occurred with a pseudo first-order reaction rate due to the high concentration of AT in the plasma. To track intermediate species more carefully in this study we have explicitly introduced both AT and heparin as new species. We allow the interaction between AT and heparin to include the formation of complexes, which then have an accelerated inactivation rate that depends on the heparin type (UFH or LMWH). The standard concentration of AT is set to 2.4 µM, and the concentration of heparin depends on different type of heparin (refer to section S3 in the Supplemental Material). The kinetic rates we used are based on the experimentally observed data [117]. Additionally, we allow AT to diffuse into the endothelial region and further inhibit FXa, FIXa and thrombin that resides there.

All of the new reactions involving AT and heparin are sketched in Fig. 3.1 and the corresponding reactions are listed in Table 3.1 and Table 3.2. In Eq. 3.1, we show the evolution equation for the concentration of one of the new species as an example of the nature of the
model’s equations. A full listing of the model equations and parameter values is given in section S2 of the Supplemental Material. Note that the ODEs labeled 1-104 comprise the model from our companion paper [85] and the remaining equations represent the new species in this paper.

The model uses the following notation: $Z_i$ and $E_i$ refer to a specific zymogen or pro-cofactor species and the corresponding enzyme or co-factor species when they are in the plasma and $Z_i^m$ and $E_i^m$ refer to the surface-bound versions of these proteins (e.g., $E_7^m$ refers to the TF:VIIa complex on the subendothelium, and $E_8$ and $E_8^m$ refer to factor VIIIa in the plasma and bound to a platelet surface, respectively. The concentrations of the proteins are denoted similarly, but with lower case $z$ and $e$. So, $e_8^m$ is the concentration of platelet-bound factor VIIIa. The symbols $TF$, $P_2$, $P_5$, $P_8$, $P_9$, $P_{10}$, and $P_{11}$ are used to denote tissue factor and the platelet binding sites for prothrombin, FV/FVa, FVIII/FVIIia, FIX/FIXa, FX/FXa, and FXI, respectively. For the platelet binding sites specific to thrombin, factor IXa, and factor XIa, we use the symbols $P^*_2$, $P^*_9$, and $P^*_11$. The concentrations of binding sites are indicated similarly but with lower case $p$. We denote the complex of $Z_i$ and $E_j$ by $Z_i : E_j$ and its concentration by $[Z_i : E_j]$, so, for example, $AT : E_9^m$ denotes AT bound to platelet-bound Factor IXa, and $[AT : E_9^m]$ refers to its concentration.

Figure 3.1 Newly added reactions involving AT, FIXa, FXa, thrombin (IIa) and FXIa. Inactivation reactions are indicated with T-shaped arrows. FXI/FXIIa exists as dimer form, and only the exposed end of FXIIa can be inactivated by AT.
3.3.3 Inhibition of FXa/FIXa/thrombin by AT

In our previous models [7–9], AT inactivated FXa, FIXa and thrombin in the fluid-phase only. Now AT can additionally inactivate all of these enzymes when they are bound to the platelet surface (Reactions. 1-4 in Table 3.1). We assume the inactivation rates to be the same for both fluid-phase and platelet-bound enzymes, and allow the inactivated AT-enzyme complex to bind/unbind from the platelet surface with kinetic rates that match those of the enzyme itself. We also assume that platelet-bound enzymes, which are also bound to AT, occupy their corresponding binding site. For example, one AT:FXa complex on the membrane, \( AT : E_{10}^{m} \), takes up one FX/FXa binding site.

3.3.4 Inactivation of FXIa by AT

In our new model, we introduce the inactivation of FXIa by AT, in both fluid- and platelet-bound phase (Reactions 5-9 in Table 1). Since FXI and FXIa are dimers, we have included inactivation of only the FXIa part of the several dimeric forms that involve FXI and FXIa: FXI:FXI, FXI:FXIa, and FXIa:FXIa in the fluid. Each of these can also be bound to the platelet surface. We assume that when FXIa is bound directly to a platelet binding site, it does not participate in any reactions, and that AT can only inhibit the free FXIa end of the dimer that is not directly bound. For example, AT can bind and inhibit FXIa in FXIa:FXIa complex in the fluid phase on either or both ends (Reaction 6 in Table 1), but can only inhibit the FXIa that is exposed to the fluid when the other end is bound to the platelet (Reactions 8 and 9 in Table 1, respectively). The detailed FXI/FXIa complexes and available inhibition sites are also shown in the reaction schematic in Fig. Figure 3.1.

3.3.5 Introduction of heparin

Heparin is introduced as a new species with a fixed upstream concentration set to 253 and 759 nM for UFH and LMWH respectively based on the recommended therapeutic range 0.3-0.7 U/ml [125]. The conversion of heparin potency to molar concentration can be found in section S3 in the Supplemental Material. We also varied this concentration to understand how this affects heparin’s impact.

3.3.6 Inactivation by AT:Heparin complex

We assume that AT can bind to heparin and form the complex we call \( ATH \) (Reaction 10 in Table 1). The AT:Heparin complex inactivates FXa, FIXa, FXIa and thrombin in either the fluid- or platelet-bound phase by direct binding (Reactions. 11-19 in Table 1). When AT is bound to LMWH, we simply change the kinetic rate constants to represent Nadroparin, which greatly accelerates the inactivation of FIXa (by 3 orders of magnitude), accelerates inactivation of FXa and thrombin to a lesser extent (by a factor of 370),
and accelerates FXIa inactivation by a yet smaller amount (by a factor of 40). When AT is bound to UFH, we use the reaction kinetic reported from the same experiment, which indicate that it accelerates inactivation of all of the targeted enzymes (750-13000 times increase) more potently than does LMWH. Platelet-bound enzymes that are inactivated by AT:Heparin complexes can also bind/unbind from the platelet surface, and, while bound, occupy the corresponding enzyme binding site on the platelet (Reactions 20-31 in Table 2).

3.3.7 Example Equation

Newly added reactions listed in Table 1 were expressed mathematically using the law of mass action. For example, the following equation describes the rate of change of the platelet-bound concentration of FXa bound to AT. It accounts for the binding and unbinding of the complex FXa:AT with the platelet surface, and the direct binding of AT from the fluid to platelet-bound FXa:

\[
\frac{d[E_{m10} : AT]}{dt} = k_{on10}[E_{m10} : AT]p_{av10} - k_{off10}[E_{m10} : AT] + k_{in10}^{+}e_{m10}^{-}AT \cdot \text{Inactivation of platelet-bound FXa by AT} \tag{3.1}
\]

Here, \( p_{av10} \) is the concentration of FX/FXa binding sites on activated platelets which are not occupied by a FX- or FXa-containing species.

3.4 Results

3.4.1 Tissue Factor and Shear Rate Dependence

Here we examined how the variation in TF density and shear rate affected thrombin production with the new AT inhibitory mechanisms at the platelet surface and compared the outcomes to those without that surface inhibition. For various TF densities in the range 0 to 30 fmol/cm\(^2\) and for shear rates 100/s, 500/s and 1500/s we performed simulations with and without surface inhibition (denoted SI in the figure). We looked at two output metrics, the lag time, which we define as the time-point at the thrombin concentration first reaches 1 nM, and the thrombin concentration at 10 minutes. Fig. (Figure 3.2A) shows the lag times and Fig. (Figure 3.2B) shows the thrombin concentrations at 10 minutes.

With and without the surface inhibition, the lag time decreased as the TF density increased and/or the shear rate decreased. These behaviors occur because a higher TF density provides a larger initial stimulus and decreasing the shear rate slows the loss of essential enzymes from the RZ. Also, the thrombin concentrations at 10 minutes increased with the TF density, sharply at low TF densities and more gradually at high ones. In fact, the results indicated a threshold dependence on TF density in all cases.
Figure 3.2 (A) Lag time and (B) thrombin concentration after 10 minutes of activity, with variations in TF density and shear rate. TF density was varied from 0 to 30 fmol/cm$^2$, and shear rate was either 100, 500, or 1500/s. Abbreviation "SI" in legend represents surface-dependent inactivation. Black lines represent results with AT-mediated inactivation of fluid-phase enzymes alone inactivation of surface-bound enzymes and gray lines represent the results with inactivation of both fluid-phase and surface-bound enzymes by AT. Vertical dashed lines indicate TF densities of interest. Flat curves at >30 minutes indicates that lag time larger than 30 minutes in (A).

Influence of AT Level on Thrombin Generation

Next, we compared how thrombin generation was affected by various AT levels at low versus high TF density. We varied the AT level from 0-200% of its physiological concentration (2.4 µM), and used 4 fmol/cm$^2$ and 15 fmol/cm$^2$ for the low and high TF densities, respectively. Fig. Figure 3.3 shows plots of thrombin generation with (C,D) and without (A,B) surface inhibition by AT, for low TF (A,C) and high TF (B,D). In the absence of the surface AT reactions, there is no to little change in the thrombin generation for all levels of AT (Fig. Figure 3.3A vs. B). When the AT surface reactions are present, thrombin generation is significantly delayed at the low but not the high TF density (Fig. Figure 3.3C,D). Another observation is that the concentration of thrombin after 20 minutes of activity remains almost the same in all cases; similar to our TFPI study, the AT affects mainly the timing of the thrombin burst. In summary, surface-dependent inactivation by AT affects thrombin generation, but this is more prominent at a low TF density. Since measurements of plasma levels of thrombin-antithrombin (TAT) are used clinically, we calculated the instantaneous rates of generation of...
Figure 3.3 Thrombin time courses for various AT levels, with and without surface-dependent inactivation reactions. SI denotes Surface-mediated Inactivation. Shown in top row of the figure are (A) TF=2.5 fmol/cm² and (B) TF=6 fmol/cm², each without SI. Shown in the bottom row of the figure are (C) TF=2.5 fmol/cm² and (D) TF=6 fmol/cm², each with SI. AT level was one of 0, 50, 100, or 200% of 2.4 µM. Shear rate was set to 100/s for all simulations.

TAT complexes (in the plasma and on the platelet surfaces) and their removal by flow, for various levels of AT. We found that more TAT was generated than carried away by flow, with the majority staying bound to platelet surfaces, as shown in Figure S8 the Supplemental Material.

3.4.2 Influence of Heparin Level on Thrombin Generation

The effect of different heparin concentrations was also examined. The thrombin time courses shown in Fig. Figure 3.4 were generated for different heparin concentrations (0.1%, 10%, 50% and 100%) for both LWMH (where 100% = 253 nM) and UFH (for which 100% = 759 nM). The values for 100% were based on recommended dosages and the conversions and references are in the Supplemental Material. As the level of heparin was increased, the delay in thrombin generation was increased, and at the therapeutic concentration, both types of heparin prevented the thrombin concentration from reaching 1 nM. As expected, UFH had a higher overall inhibitory effect. Our new model exhibits the distinct and clinically-observed effects on anticoagulant activity of LMWH and UFH.
Figure 3.4 Thrombin generation with TF=6 fmol/cm$^2$ and varied levels of LWMH and UFH. Thrombin generation in the presence of LMWH and UFH are shown in black and gray lines, respectively. The heparin levels were varied as 0.1, 10, 50, 100% of a standard therapeutic concentration.

3.4.3 Effects of Surface-Dependent AT Inactivation on thrombin, FIXa, FXa, and FXIa

To study the individual enzymes and the effect of inactivation on their concentrations, we tracked the time courses of thrombin, FXa, FIXa and FXIa. Fig. Figure 3.5 shows plots of the sum of both the fluid-phase and platelet-bound species. We examined these concentrations in the absence and presence of the surface-dependent inactivation by AT, and under the condition of no heparin, with LWMH or with UFH at 25% of their therapeutic concentrations. For all species, the cases without surface-dependent inactivation by AT or ATH showed almost indistinguishable curves, meaning that AT and ATH were not effective at reducing enzyme concentrations when inactivating fluid phase enzymes only. In the presence of surface-dependent inactivation by AT but in the absence of heparin, the concentrations of all species look similar to the case with no surface-dependent inactivation. With surface-dependent inactivation, both the LMWH and UFH substantially reduced the concentrations of all enzymes, with the strongest effects from UFH. These results collectively show that the surface-dependent inhibition reactions are critical and necessary to induce observable effects of heparin on its target enzymes.

3.4.4 Examination of the Major Inactivation Reactions

To understand the effects of inactivating each enzyme alone, we separately kept individual inactivation reactions “turned on”, while “turning off” the remaining reactions; turning off a reaction here means that we set the corresponding association rates to zero. For example, to focus on effects of inactivating FIXa alone, we keep AT inactivation of FIXa turned on while setting AT association rates for FXa, FXIa and
Figure 3.5 Concentration time courses of (A) thrombin, (B) FXa, fluid-phase and surface-bound, (C) FIXa, fluid-phase and surface-bound, and (D) FXIa, fluid-phase and surface-bound, each with TF density of 15 fmol/cm² and shear rate of 100/s. Simulations were performed with or without surface-dependent AT inactivation reactions SI = Surface-mediated Inactivation, which are shown as gray and black curves, respectively, and for no heparin, LMWH, or UFH, shown by varying line styles.

thrombin to zero. For these studies, we used a TF density of 15 fmol/cm² and a shear rate of 100/s. Fig.

Figure 3.6 shows the resulting thrombin time courses under the influence of either LMWH (A) or UFH (B). Simulations with LMWH and UFH showed similar trends. In both cases, inactivating FXIa had a negligible effect. Inactivating either FIXa, FXa, or thrombin increased the lag time; with LMWH the lag times were increased by about 200s, with UFH they were increased by about 400s for FIXa and thrombin inactivation and by about 800s for FXa inactivation. The thrombin concentrations after 20 minutes were about the same for individual inactivation of FIXa, FXa, and FXIa, around 150 nM. For inactivation of thrombin, the thrombin concentration after 20 minutes is reduced to about 100 nM with LMWH and about 20 nM with UFH; this is due to a reduction in both positive feedback and the direct inactivation itself. When LMWH and UFH are working to their full potential, i.e., they are inactivating all four enzymes to to their respective degrees, the thrombin generation is essentially shut off, with concentrations that never even reach 1 pM. In summary, our model shows that inactivation of any of the single target
enzymes alone is not enough to prevent robust thrombin generation; inhibiting multiple targets together enables accumulative inhibition to completely extinguish thrombin generation.

### 3.5 Discussion

In our previous mathematical models of flow-mediated coagulation [7–9], AT inactivated FIXa, FXa and thrombin in the fluid-phase only, and this had little effect on thrombin production since the fluid-phase species, whether inactivated or not, were subject to flow and could easily be washed away. Our extended model, which includes direct binding of fluid-phase AT to platelet-bound species shows a new sensitivity of the model coagulation system to AT. In particular, we find that AT can dramatically increase the lag time of thrombin generation at low TF density through this surface-dependent inhibition mechanism. However, at high TF, even when AT was increased to 200% of its normal concentration, the changes in thrombin generation are slight. Addition of these new reactions does not significantly alter the TF density threshold or shear rate dependence. We found only small increases in the lag time and slight changes in thrombin concentration after 10 minutes of clotting activity, when compared to simulations run in the absence of the AT-mediated inactivation reactions, over a wide range of TF densities. As observed in our previous studies [7–9], increasing the flow shear rate increases the lag time and reduces the thrombin concentration after 10 minutes of clotting activity.
The presence of heparin greatly magnifies the sensitivity of the system to AT, and this sensitivity is due entirely to the direct binding of platelet-bound species by fluid-phase ATH. When the AT-mediated inactivation of platelet-surface-bound enzymes is turned off, neither type of heparin has any noticeable effect on the system. This highlights the critical role that the platelet surface plays in this process. We find that UFH at 10% of a therapeutic level or LMWH at 100% of a therapeutic level can completely shut down thrombin generation, even at high TF densities. These results are generally in line with observed responses to heparin therapies in the sense that UFH is a stronger inhibitor than LMWH; this is because UFH affects the inactivation of FIXa, FXa, FXIa, and thrombin, whereas LMWH affects mainly the inactivation of FIXa and FXa, and has less influence on the inactivation of thrombin and FXIa.

In our model we do not include direct effects of heparin on the rate of platelet deposition in the reactions. The only way that platelet deposition can be affected by heparin in this model is indirectly through its effect on thrombin generation and thrombin's activation of platelets. In particular, less thrombin from the heparin will reduce positive feedback and will also reduce the number of platelets activated by thrombin. Some plots of the platelet dynamics with varying types and concentrations of heparin are shown in the Figure S6 in the Supplemental Material; heparin indeed reduces the platelet deposition.

It has been shown that the addition of heparin can also accelerate the inhibitory effect of TFPI, as shown in prothrombinase activity assays initiated with FXa, FV, prothrombin, and lipids, but such an effect was greatly diminished when FXa was pre-incubated with partially activated FV [82]. This data is in line with several observations showing procoagulant effects of heparin when AT is not present in the system [82, 126]. Wood et al. further explored these ideas and showed that the negatively charged heparin molecule can block the interaction between TFPIα and partially activated FV (FV-h) [35]. Therefore, addition of heparin can reduce the inhibitory effect of TFPIα towards prothrombinase that is made with FV-h, but has no effect on prothrombinase made with fully activated FVa. Our model does not reflect the reduced binding interaction between TFPIα and FV-h by heparin treatment, but our model and results can still give some insight about what might happen under these conditions. The results in our companion TFPI study [85] showed that the coagulation response in the absence of binding between TFPIα and FV-h was enhanced. That scenario resembles the situation where heparin would block the binding of TFPIα to FV-h. We note that AT-mediated inactivation in that version of the model had no effect because it only acted on fluid-phase enzymes, thus we can consider it to be a case in which there is essentially no AT, isolating the procoagulant effects of heparin to be through TFPIα/FV-h interactions. Nevertheless, further exploration of procoagulant effects of heparin and the extension of heparin-TFPIα would be an interesting topic for future work.
The model allowed us to do simulations in which we could isolate the effect of inhibiting one coagulation enzyme at a time by setting the rate constants for other enzymes to zero. In doing this systematically, we found that no one single enzyme inactivation was enough to prevent substantial thrombin generation under flow for the TF densities examined. Inactivating FXIa alone had almost no effect. Inactivating each of FIXa, FXa, or thrombin individually by either LMWH or UFH led to increased lag time. The lag times were increased more for UFH than LMWH. Substantial thrombin was still produced by 20 minutes in these cases. The strength of heparin inhibition seems to be the simultaneous enzyme targets at multiple steps in the coagulation system.

There are other types of LMWH heparins and derivatives of UFH that have been developed for use as anticoagulant drugs [47, 51, 112] that we did not study in this work. There is a large body of clinical research to understand which heparins and their derivatives work best for various indications, for example treatment of deep vein thrombosis versus thromboprophylaxis after surgery. Complete details are beyond the scope of this paper but we point interested readers to a few published reviews [127, 128]. Researchers are still trying to identify individual risk factors associated with bleeding when using heparin as an anticoagulant treatment [129, 130]. Even though newer types of anticoagulants are being developed, advanced, and frequently used in place of heparins [131], both LMWH and UFH, were effective in managing clotting complications of COVID-19 [132, 133].

3.6 Conclusion

In this study, we explored the effects of surface-dependent inactivation by AT in a model coagulation system under flow. As we concluded in our companion [85] TFPI study, targeting the enzymes bound to activated platelet surfaces was required for efficient inhibition of thrombin generation. We showed that AT alone can delay thrombin generation when TF density is low but not when TF density is high. This inhibition was completely dependent on the platelet surface reactions. Our results show that AT in the presence of heparin can drastically inhibit thrombin generation. We developed a new version of our mathematical model that is sensitive to AT and heparin in ways that have been observed clinically, i.e., the magnitude of the effects of LMWH versus UFH. We found that inactivating single enzymes only was ineffective at suppressing thrombin generation. Combinations of two or three targets could be examined to aid in design of new anticoagulant drugs. The model we developed in this study and in our companion TFPI study [85] will serve as a powerful tool for such use in future studies.
CHAPTER 4
STUDYING THE MECHANISM(S) OF ACTION OF CONCIZUMAB USING A MATHEMATICAL
MODEL OF FLOW-MEDIATED COAGULATION

NOTE: This paper draft is under discussion and edit with Novo Nordisk for the future submission.

4.1 Abstract

Background: Concizumab is a monoclonal antibody that targets tissue factor pathway inhibitor
(TFPI) to reduce bleeding in hemophilia A.

Objectives: To determine the relative influence of multiple possible mechanisms through which
conzizumab enhances thrombin generation in hemophilia A.

Methods: We developed a mathematical model to estimate the plasma concentrations of concizumab
and TFPI for specified doses of concizumab and given values of TFPI\textsubscript{\(\alpha\)} and TFPI\textsubscript{\(\beta\)}. These estimates were
used as input to another mathematical model that simulates thrombin generation under flow. Thrombin
time courses were simulated for variations in tissue factor (TF), TFPI, and concizumab to determine the
effects of different possible mechanisms of action of concizumab. The mechanisms explored included
formation of ternary complexes of concizumab, TFPI\textsubscript{\(\alpha\)}, and TFPI\textsubscript{\(\beta\)}, reducing inhibition at the
subendothelium and on activated platelets, and the possibility that TFPI\textsubscript{\(\alpha\)} bound to concizumab could
bind to partially activated factor V.

Results: Concizumab improved thrombin generation in hemophilia A for all TF levels. The effects of
conzizumab were an accumulation of reducing inhibition at three cellular surfaces: the subendothelium, the
endothelium, and activated platelet surfaces. Endothelial TFPI\textsubscript{\(\beta\)} trapped TFPI\textsubscript{\(\alpha\)} through a ternary
complex with concizumab and significantly reduced the plasma concentration of TFPI\textsubscript{\(\alpha\)} and
conzizumab-bound TFPI\textsubscript{\(\alpha\)}. Without TFPI\textsubscript{\(\beta\)}, there was more plasma concizumab-bound TFPI\textsubscript{\(\alpha\)}, which if
allowed to bind partially activated FV, decreased the efficacy of concizumab. Conclusions: Concizumab
enhances thrombin generation in hemophilia A in a mathematical model of flow-mediated coagulation. Its
effectiveness is dependent on biochemical mechanisms not yet verified. Future experimental work to test
these mechanisms is proposed.

4.2 Introduction

Hemophilia A is a genetic bleeding disorder characterized by a deficiency in coagulation factor VIII
(FVIII) due to the defect in F8 gene. The severity of hemophilia is classified by the plasma level of FVIII:
severe if < 1%, moderate if between 1 to 5 %, and mild between 5 and 40% [134]. Patients with severe
hemophilia may receive FVIII concentrates intravenously several times per week, but this intensive prophylactic regimen cannot always stop bleeding incidents and may lead to arthropathy over time [135–137]. Gene therapy has been proposed as an alternative treatment method, with higher achieved levels of FVIII and significant improvements in the frequency of bleeds, factor activity level and duration of expression, and chronic pain [138]. However, limitations still exist with gene therapy, including only temporary expression of FVIII and serious side effects such as liver function abnormalities and toxicity [139, 140].

Other methods of treatment include designing antibodies against endogenous inhibitors of coagulation. Concizumab is a humanized monoclonal antibody against tissue factor (TF) pathway inhibitor (TFPI) that is under development as a subcutaneous treatment for patients with hemophilia A [62, 141]. Concizumab targets the Kunitz 2 (K2) domain of TFPI, an active domain through which TFPI binds to activated coagulation factor X (FXa) [63, 142, 143]. There are two isoforms of TFPI: TFPIα that is in plasma and TFPIβ that is bound to endothelial cell surfaces. TFPIα inhibits the initiation phase of coagulation by either forming a complex with FXa and then binding TF:VIIa or by binding TF:VIIa:Xa directly; either mechanism leads to the inhibitory quaternary complex TF:VIIa:Xa:TFPIα, which reduces the activity of TF:VIIa. TFPIα can also bind from the plasma to activated platelet surface-bound FXa and partially activated FV (FV-h). Both of these mechanisms are inhibitory as they can either prevent the formation of prothrombinase or directly inhibit activity of prothrombinase complexes already formed. Concizumab reduces inhibition by blocking any of the interactions between TFPI and FXa. The efficacy of concizumab has been verified through in vivo and ex vivo experiments during Phase 1 studies, where thrombin generation was greatly accelerated in thrombin generation assays in a dose-dependent manner in hemophilic patients’ blood [144]. Phase 2 trials of concizumab assessment have also shown its safety and potential to protect from bleeding incidents in hemophilia [62, 145].

Overall, clinical trials seem to show positive results of concizumab as a treatment for hemophilia. However, there are other potential mechanisms through which concizumab may be working, i.e., binding TFPIα versus TFPIβ, or modulating binding between TFPI and FV-h [81]. The importance of each individual mechanism has not yet been examined in a rigorous way, which could give a more holistic view of the effects of concizumab. In this study, the goal was to systematically investigate multiple potential mechanisms of action of concizumab and determine their influence on thrombin generation.

Mathematical models are safe and powerful tools to probe complex biological systems like blood clotting and coagulation. Our group has developed experimentally validated models of flow-mediated blood coagulation that have led to novel insights related to phenotypic variation observed in bleeding and thrombotic disorders [7–10, 60, 61]. The models include platelet adhesion, aggregation, and thrombin
generation initiated by TF exposure at an injury site. The models can simulate and track the dynamics of multiple proteins, complexes, as well as mobile/bound and unactivated/activated platelets. Our recent work showed that direct binding of TFPI\(\alpha\) to platelet-bound FXa and FV-h gave stronger inhibitory effects compared to these reactions occurring in plasma only. Through these reactions, TFPI\(\alpha\) in the model could be targeted to enhance thrombin generation [85, 146]. Our previous models did not include the TFPI\(\beta\) isoform. Here, we have developed a new ‘steady state’ model to study the interactions with TFPI\(\beta\) and estimate plasma levels of concizumab, TFPI, and their complexes after giving specific doses of concizumab. Those estimated plasma levels were then used as input into our flow-mediated coagulation model, which was updated to include all interactions with concizumab. The combined models were used to simulate thrombin generation curves that could be analyzed to investigate various mechanisms of action of concizumab. We made two major assumptions in our modeling: (1) one molecule of concizumab can form a ternary complex with two TFPI\(\alpha\) molecules or with one TFPI\(\alpha\) and one TFPI\(\beta\) molecule, and (2) TFPI\(\alpha\) bound to concizumab can inhibit FV-h. The result of the first assumption is that TFPI\(\beta\) can sequester TFPI\(\alpha\) to the endothelium, which is a procoagulant effect. The result of the second assumption is an anticoagulant effect. We analyzed thrombin generation in our model with combinations of these assumptions being true or false. Our results show that concizumab is effective at enhancing thrombin generation in hemophilia A for all cases, but has the strongest enhancement when concizumab can bind both TFPI\(\alpha\) and TFPI\(\beta\), and concizumab bound to TFPI\(\alpha\) cannot inhibit FV-h.

4.3 Methods

Two mathematical models were used for study: a steady-state model and a flow-mediated coagulation model, see Figure 4.1 for a schematic. The steady-state model simulates reactions occurring within a single blood ‘compartment’ for a long enough time so that the concentrations stop changing in time, which is when they are in a ‘steady state’. The reactions include the binding of concizumab to TFPI\(\alpha\) in the plasma and to TFPI\(\beta\) on endothelial cells and ternary complex formation. The model outputs are plasma concentrations of all included proteins and their complexes, given a specified concentration of concizumab, volume of plasma, and TFPI levels. These outputs are then used as inputs to the flow-mediated coagulation mathematical model to simulate how the remaining levels of concizumab, plasma TFPI\(\alpha\) and concizumab-bound TFPI\(\alpha\) (C:TFPI\(\alpha\)) influenced thrombin generation upon exposure to TF.

4.3.1 Steady State Mathematical Model

The five reactions listed in Table 4.1 were assumed to occur in a single blood compartment. Metabolism, degradation, and clearance of drug particles were neglected. The reactions were translated
Figure 4.1 Model schematics. The steady state model (left) predicts levels of concizumab bound to TFPIα and TFPIβ. Output from the steady state model is input to the flow-mediated coagulation model (right), which simulates thrombin generation that results from reactions occurring in a small reaction zone above exposed tissue factor and subjected to flow.

into mathematical equations (ordinary differential equations) using the law of mass action, and then simulated to track each model species (e.g., concizumab, TFPIα, etc.) dynamically through time. Since concizumab is categorized as IgG4 isotype monoclonal antibody, there are two epitopes for the K2 domain of TFPI on each concizumab molecule [63, 147]. Therefore, the major reactions are concizumab binding to TFPIα (to one or two molecules), to TFPIβ (3), and the formation of a ternary complex TFPIα:C:TFPIβ.

The blood compartment requires a specified blood volume, plasma levels of concizumab and TFPIα, and an estimated concentration of TFPIβ (see supplemental material for this calculation). Here we used 2.5 nM TFPIα and 18 nM TFPIβ. We define the concizumab exposure level as the summation of free plasma concizumab, concizumab bound to one or two TFPIα, at steady state. Previous studies report a geometric mean of concizumab exposure level in patients’ plasma to be 4 nM, so this was set to be the primary concentration used in this study. For various initial concizumab concentrations, the reactions were simulated until a steady state was reached (numerical solutions had negligible changes for at least 5 minutes) and the resulting exposure level was calculated. Other steady-state concentrations for each plasma species were recorded and used as input to the coagulation model.
Table 4.1 List of reactions included in the steady state model. C represents concizumab. Kinetic rates taken from a) Binding of concizumab to TFPIα, KD = 25 pM [63]. b) Binding of concizumab to TFPIβ, KD = 123 pM [63].

<table>
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<th>No.</th>
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<th>Products</th>
<th>$k^+$ (M$^{-1}$s$^{-1}$)</th>
<th>$k^-$ (s$^{-1}$)</th>
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<td>1e-4</td>
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<td>1e-4</td>
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<td>8.1e5</td>
<td>1e-4</td>
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<td>4e6</td>
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<td>a</td>
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4.3.2 Mathematical Model of Coagulation under Flow

Here we give a brief overview of our mathematical model of flow-mediated coagulation that has been described in detail elsewhere [85, 146]. The model simulates tissue factor (TF) initiated coagulation reactions occurring in a reaction zone (RZ) situated just above a small injury (10x10 um) at the subendothelial surface. All reactants and intermediates are assumed to be instantaneously well-mixed, therefore changes in space are neglected and only concentrations in time are considered. The influence of flow and diffusion to and from the RZ is considered with a simplified, single rate of mass transfer. The model includes three different types of platelets: unactivated platelets in plasma, activated and attached to the subendothelium, and activated and attached to other platelets. Activated platelets can activate other platelets via release of agonists. Coagulation reactions occur on the subendothelium, in plasma, and on activated platelet surfaces, where thrombin generation occurs. Initial concentrations of all model species are specified, the corresponding ordinary differential equations are solved, and the evolution of each species is tracked in time. The initial concentration for TFPIα and all its complexes are set to be 20% of the final concentrations estimated from the steady-state model. This assumption is due to the reports that only 20% of plasma TFPIα is free and full length [32], and the rest may be bound to lipoproteins. Even though there is one biochemical experiment reported that lipoprotein-bound TFPI can still actively inhibit thrombin generation, it is unclear how such a form can act in vivo [103]. We extended our previous model [85] to include the new reactions involving concizumab and TFPIα, as described in the next few sections, and also listed in Table 4.2.

4.3.3 Concizumab binding Kunitz 2 domain of TFPIα

Concizumab binds the Kunitz 2 (K2) domain of TFPIα and blocks its ability to bind to FXa [63]. We assumed that TFPIα is a flexible molecule so that concizumab can bind to exposed K2 domains in TFPIα in any form, which includes free plasma TFPIα, TFPIα:FV-h complexes in plasma or on platelet surface,
and TFPI\(\alpha\) bound to prothrombinase via FV-h (reaction 1, 3, 5 and 7 in Table 4.2). There are two binding sites on concizumab for TFPI\(\alpha\) [63, 147], therefore we include the reaction where C:TFPI\(\alpha\) complex can bind an additional free TFPI\(\alpha\) to form the ternary complex TFPI\(\alpha\):C:TFPI\(\alpha\) (reaction 2 in Table 4.2).

### 4.3.4 C:TFPI\(\alpha\) complex binding FV-h

Another assumption based on the flexibility of TFPI\(\alpha\) is that its C terminus is available to bind FV-h, even if the K2 domain is bound to concizumab. Previous studies have suggested that TFPI\(\alpha\) without a K2 domain can mildly increase the lag time in prothrombinase activity assays [36]. Thus, we have included possible reactions where the C:TFPI\(\alpha\) complex binds FV-h both in plasma and bound to the platelet surface (reaction 4 and 6 in Table 4.2), and the C:TFPI\(\alpha\) complex binds FV-h within a prothrombinase complex (reaction 8 in Table 4.2).

### 4.3.5 Example Equations

Biochemical reactions were translated into ordinary differential equations based on the law of mass action. For example, the following equation describes the rate of change of concizumab (C) in the steady state model, which depends on concizumab binding to TFPI\(\alpha\) and TFPI\(\beta\). The rate of change is balanced with a negative rate (loss of C due to binding TFPI) and positive rate (C becoming free after dissociation from TFPI). Binding and unbinding rates are indicated by superscript on and off respectively. Species connected with a colon in a bracket represents a complex, e.g., [C:TFPI\(\alpha\)] represents the complex of concizumab bound to TFPI\(\alpha\).

\[
\frac{dC}{dt} = -k_{on}^{C:TFPI\alpha}[C:TFPI\alpha] + k_{off}^{C:TFPI\alpha}[C:TFPI\alpha] - k_{on}^{C:TFPI\beta}[C:TFPI\beta] + k_{off}^{C:TFPI\beta}[C:TFPI\beta]
\] (4.1)

### 4.4 Results

#### 4.4.1 Steady state model and plasma level estimations

Each run of the steady state model with different initial concizumab dose leads to a different estimated concizumab exposure level. The exposure level is defined as the detectable concizumab concentration in plasma and is calculated as the sum of free plasma concizumab, C:TFPI\(\alpha\), and TFPI\(\alpha\):C:TFPI\(\alpha\) complex. Using the reactions and rates listed in Table 4.1, we varied the initial concizumab dose from 0 to 30 nM to determine which dose led to an exposure level near 4 nM. Figure 4.2A shows the results of these simulations where an initial dosage of 21.5 nM leads to a 4 nM of exposure level; therefore, and initial
Table 4.2 List of reactions included in the extended flow-mediated coagulation model. Superscript h indicates the partially activated form of FV (FV-h). Superscript m indicates that the model species is bound to a membrane. The units for k+ are 1/(Ms), the units for k- and kcat are 1/s. Notes: a) Activation of FX by TF:VIIa, from Lu et al. 2004 [148], b) TFPIα binding FXa, Kd = 20 pM from Jesty et al. 1994 [149], c) Concizumab binding TFPIα, from Hilden et al. 2012 [63], d) Binding of TFPIα to FV-h, Kd = 9 pM from Wood et al. 2013 [35]

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<td>C:TFPIα + FV₇₅₅₇₄</td>
<td>C:TFPIα:FV₇₅₅₇₄</td>
<td>C:TFPIα:FV₇₅₅₇₄</td>
<td>5e7</td>
<td>0.0045</td>
<td>1.92e6</td>
<td>d</td>
</tr>
<tr>
<td>10</td>
<td>C+TFPIα:PRO₇₅₅₇₄</td>
<td>C:TFPIα:PRO₇₅₅₇₄</td>
<td>C:TFPIα:PRO₇₅₅₇₄</td>
<td>4e6</td>
<td>1e-4</td>
<td>1.92e6</td>
<td>c</td>
</tr>
<tr>
<td>11</td>
<td>C:TFPIα:PRO₇₅₅₇₄</td>
<td>C:TFPIα:PRO₇₅₅₇₄</td>
<td>C:TFPIα:PRO₇₅₅₇₄</td>
<td>5e7</td>
<td>0.0045</td>
<td>1.92e6</td>
<td>d</td>
</tr>
</tbody>
</table>

Dosage of 21.5 nM of concizumab dosage was chosen for the rest of the simulations in this study. A strength of using a mathematical model is that we can track the concentration of all species in the model. So, in addition to finding the exposure level/initial dose pair, we can also report the amount of each TFPIβ species at steady state. These values are reported in the table inset of Figure 4.2A and show that at the 4 nM exposure level, most of the TFPIα is trapped at the endothelial surface in ternary complexes with concizumab and TFPIβ (1.85 nM of the 2.5 nM TFPI). To further understand the impact of TFPIβ on these steady state estimates, we performed the same variation in initial concizumab doses but without the TFPIβ reactions. The results of these simulations are shown in Figure 4.2B, where C:TFPIα, TFPIα, and the TFPIα:C:TFPIβ complex are plotted for the cases with TFPIβ (solid curves) and without TFPIβ (dotted curves). The most notable differences are seen in the plasma C:TFPIα levels, where they are much lower (to 0.5 nM compared to 2.1 nM) when TFPIβ is able to trap them at the endothelium.

4.4.2 Thrombin generation with tissue factor and concizumab variation

Next, we explored the effects of concizumab on thrombin generation using the flow-mediated coagulation model. We varied the tissue factor (TF) levels in the presence and absence of concizumab and generated the thrombin time courses, as shown in Figure 4.3. In all these simulations, the FVIII level was fixed to 1% to create severe hemophilia A conditions, and the initial concizumab dose was either 0 and 21.5 nM to simulate scenarios with no concizumab (-Czm) or with a 4 nM concizumab exposure level (+Czm). We used TF levels assumed to be low, intermediate, and high (3, 6, and 20 fmol/cm²), as based on model behaviors in our previous studies [85, 146], and these variations are...
Figure 4.2 Steady state model results. Concizumab exposure levels as a function of initial concizumab (A), and steady state model species with or without TFPI\(\beta\) (B). The red dot indicates where the initial concizumab dosage leads to a 4 nM exposure level. Steady state concentrations of all model species at the initial dosage of 21.5 nM are shown as a legend in (A). TFPI\(\beta\) was set to either 0 or 18 nM in figure (B), and concentration of C:TFPI\(\alpha\), plasma TFPI\(\alpha\), and TFPI\(\alpha\):C:TFPI\(\beta\) complexes are plotted out.

represented in the figure with different line styles. Increasing the TF level increases the thrombin generation with and without concizumab treatment, as expected. By comparing the solid and dotted curves, concizumab is shown to enhance thrombin generation for all TF levels. However, the enhancement is most apparent at the intermediate TF level where concizumab completely rescues thrombin generation from a subnanomolar concentration to one that is over 100 nM. Concizumab alone was not sufficient to fully recover the thrombin generation when TF is low but leads to a moderate decrease in lag time and similar maximum thrombin concentrations at high TF.

4.4.3 Time courses of additional model species for proof of concept

To demonstrate proof of concept of the effects of concizumab in our model, we generated the time courses of additional relevant model species, as shown in Figure 4.4. The model species are free TFPI\(\alpha\) in plasma (A), the TF:VIIa:Xa:TFPI\(\alpha\) complex (B), the sum of free FXa in plasma and bound to platelet surfaces (C), and prothrombinase (D), and each species is shown for cases with and without concizumab (0 or 4 nM exposure level). In all cases, FVIII level is fixed to 1% of baseline value and TF = 7 fmol/cm\(^2\). Concizumab was effective at blocking inhibitory actions of TFPI\(\alpha\); it greatly reduced the free TFPI\(\alpha\) level in the plasma (A), which led to a reduced level of TF:VIIa:Xa:TFPI (B). The reduced TFPI\(\alpha\) led to more FXa (C), and thus more prothrombinase (FXa:FVa and FXa:FV-h). These changes are what gave rise to the higher thrombin concentrations shown previously in Figure 4.3. In summary, our mathematical model
Figure 4.3 shows thrombin generation time courses under varied TF and concizumab levels. TF levels are set to be low, intermediate, and high (3, 6 and 20 fmol/cm$^2$). Concizumab levels are set to 0 and 21.5 nM, which represent no treatment (-Czm) and 4 nM concizumab exposure level (+Czm) conditions. FVIII level is fixed to 1% to simulate severe hemophilic condition.

is sensitive to concizumab and shows the effectiveness of concizumab in recovering thrombin generation under hemophilic conditions.

4.4.4 Concizumab mechanism(s) of action

TFPI$\alpha$ inhibits FXa in plasma, on platelet surfaces, and at the subendothelium as well as FV-h in plasma and on platelet surfaces. Thus, concizumab is enhancing enzyme generation and activity through multiple, indirect, mechanisms of action. The effects of these mechanisms on thrombin generation could be measured and compared using our mathematical model by setting some reaction rates to zero to turn on or off individual mechanisms. We recorded the lag times (time required to generate 1 nM thrombin) as a function of TF, and the thrombin time courses for six different scenarios: (1) No concizumab, all other reactions intact, (2) Turning off TFPI$\alpha$ binding to FXa and FXa within FXa:FV-h on platelet surfaces (3) Turning off TFPI$\alpha$ binding TF:VIIa:FXa and TFPI:FXa binding to TF:VIIa at the subendothelium, (4) Reducing the TFPI$\alpha$ level in the plasma and the upstream concentration to be 0.0028 nM (this was the value from the steady state with 21.5 nM concizumab), (5) Combining scenarios 2-4, and (6) with concizumab, and all other reactions intact. The lag times are shown in the Figure 4.5A. Comparing the cases without and with concizumab (black solid to dotted), there is a clear shortening of the lag time for all TF levels from 0 to 20 fmol/cm$^2$. In fact, every condition compared to the case without concizumab reduced the lag time. Directly reducing the TFPI$\alpha$ level had a stronger effect compared to blocking reactions at the platelet surface or on the subendothelium. The greatest reduction in lag time was observed...
Figure 4.4 Concentration time courses of free TFPIα in plasma (A), TF:VIIa:xa:TFPI complex (B), active FXa both in plasma and bound to a platelet surface (C), and the prothrombinase complex (D). Concizumab exposure level is set to 0 and 4 nM. TF is fixed to 7 fmol/cm² and FVIII level is fixed to 1% of its baseline value. Concizumab blocks inhibitory action of TFPI (A,B) and leads to increases in FXa and prothrombinase (C,D).

when all three of those mechanisms were combined, which interestingly was the same reduction due to treatment with concizumab (dotted black line compared to lightest green line). The reduction in lag times was most significant for intermediate TF levels of about 6-10 fmol/cm². For a closer look at the thrombin dynamics in that range, we plotted the thrombin time courses in Figure 4.5B with a fixed value of TF = 8 fmol/cm² and under the same 6 conditions as in Figure 4.5A. Here we can clearly see the lag time reduction from about 40 min (no concizumab) to 20 min (with concizumab), with the various conditions in between. At this TF level, all the scenarios led to similar final thrombin concentrations, which means the effect of inhibiting TFPIα here was to make thrombin more quickly but not necessarily to make more thrombin. At higher TF levels, the corresponding curves in Figure 4.5B would become much closer together since the effects of increasing TF outweigh the effects of TFPIα inhibition in this model. These results suggest the mechanisms of action of concizumab are really a combined attack of TFPIα in the plasma, on platelet surfaces, and at the subendothelium.
4.4.5 TFPI\(\beta\) and FV-h

The assumption that concizumab could bind two molecules of TFPI at the same time enabled TFPI\(\beta\) to sequester TFPI\(\alpha\) and concizumab bound to TFPI\(\alpha\) (C: TFPI\(\alpha\)) at the endothelium. Recall that the major difference in the steady state model results with and without TFPI\(\beta\) was the amount of C:TFPI\(\alpha\) in plasma, where without TFPI\(\beta\) there was a much higher concentration of C:TFPI\(\alpha\) in plasma than with TFPI\(\beta\), see Figure 4.2. The second assumption was that C:TFPI\(\alpha\) can bind to and inhibit cofactor activity of FV-h. Here we examined thrombin generation for different combinations of these assumptions being true or false, with the baseline being that both were true. First, to measure the effects of TFPI\(\beta\) sequestration, we simply set the TFPI\(\beta\) concentration to either 0 or 18 nM, ran the steady state model with 21.5 nM concizumab, and input these to the coagulation model to compare thrombin generation. Figure 4.6A shows the two resulting thrombin time courses, where we observe that removing TFPI\(\beta\) actually increases the lag time by nearly 10 minutes. Since the main difference in these scenarios is the plasma concentration of C:TFPI\(\alpha\), we hypothesized that the assumed inhibitory effects C:TFPI\(\alpha\) on FV-h were partly responsible for this. We note that this inhibition can work through two mechanisms: C:TFPI\(\alpha\) binding to FV-h and inhibiting the formation (IF) of prothrombinase, or C:TFPI\(\alpha\) binding to FV-h already in complex with
FXa so that it is directly inhibiting (DI) prothrombinase. Figure 4.6B compares the thrombin time courses with and without both of the IF and DI mechanisms, in the presence of TFPIβ. Without the IF and DI mechanisms but with TFPIβ, we observe a slight reduction in the lag time. We wondered which of the IF and DI mechanisms were more effective, together or separate, in the absence of TFPIβ. In Figure 4.6C, we toggled each of the IF and DI mechanisms on and off, in the absence of TFPIβ. Comparing to the baseline case where both are present (the lightest green curve), the greatest reduction in lag time occurs when both IF and DI mechanisms are absent (the darkest green curve). When we turn off individual mechanisms, turning off DI alone is more effective than turning off IF alone. This means that if the assumption that C:TFPIα is inhibitory holds, it is most inhibitory action occurs by direct inhibition of prothrombinase.

![Figure 4.6A](image1.png)  
**Figure 4.6A** Thrombin generation time courses with concizumab, in the presence and absence of TFPIb (A). Different mechanisms of C:TFPIα inhibition are toggled on and off: C:TFPIα inhibits formation of prothrombinase (IF) via binding free FV-h, or it directly inhibits prothrombinase via FV-h (DI). (B) TFPIb is set to 18 nM, IF and DI are turned on and off together. (C) TFPIb is set to zero, IF and DI are toggled on/off individually and together. TF = 7 fmol/cm².

4.4.6 Uncertainty in dissociation rates for concizumab

There is a range of dissociation rates for concizumab binding TFPIα or TFPIβ stemming from the different types of assays used for measurement. Thus, we performed an uncertainty study to examine how this range affected the model output. Both dissociation rates were varied in a range that encompasses reported literature values. Specifically, binding rate of concizumab to TFPIα is varied between 15-45 pM to include the uncertainty in 25±8 pM, and binding rate to TFPIβ is varied between 100-500 pM to include the variation of kinetic data acquired from ELISA (123 pM) or SPR experiments (73 pM). We recorded how the concizumab exposure level, TFPIα, TFPIα:C:TFPIβ, and the corresponding lag times changed, which are all shown in supplementary materials. Variation in binding kinetics between concizumab and TFPIα had little to no change in any of the outputs. Variation in the binding rate between concizumab and TFPIβ had a mild effect: maximum increase of 25% in exposure level, and less
than 10% change in the TFPIα, TFPIα:C:TFPIβ, and lag time. These results show that the model results and conclusions are not sensitive to the binding kinetics of concizumab to TFPI.

4.5 DISCUSSION

Concizumab is a novel monoclonal antibody designed to target the K2 domain of TFPI to enhance thrombin generation and reduce bleeding incidents in patients with hemophilia A. This study explored the underlying mechanism(s) of action of concizumab using two different mathematical models, one that estimated the steady state plasma concentrations of concizumab and TFPIα after specific doses of concizumab were applied, and one where TF was exposed to plasma with those steady state concentrations and dynamic thrombin generation could be simulated.

Thrombin generation was simulated for various TF levels with and without concizumab. Overall, the effects of concizumab in the model were to enhance thrombin generation. The efficacy of concizumab with FVIII deficiency was dependent on TF level: At low TF levels (\(<6 \text{ fmol/cm}^2\)), the amount of thrombin was increased, and the lag time was reduced, but not enough to generate more than 1 nM after 40 minutes. At intermediate TF levels (6-10 fmol/cm\(^2\)), concizumab caused more substantial increases in thrombin (\(>1\) nM). At higher TF, there were still small reductions in lag time, but the final thrombin concentrations were similar. This is because the TF level itself could overcome the lack of FVIII, providing enough stimulus even without concizumab, which indicates that TF may be an endogenous safety net for concizumab treatment. The main concept of concizumab is to block the K2 domain of TFPIα to reduce the inhibition of FXa by TFPIα. Incorporating this mechanism into our model shows that it works as a mechanism for thrombin enhancement. As shown in Figure 4.4, the addition of concizumab greatly reduced active TFPIα in plasma that inhibits FXa. However, this mechanism alone did not fully explain the overall effectiveness of concizumab. It has been previously thought that inhibition of TF:VIIa during the initiation phase of coagulation is the major inhibitory mechanism by TFPIα. However, our previous studies have shown that direct inhibition of FXa on the platelet surface by TFPIα also plays an important role. Here, we hypothesized there were three likely mechanisms of how concizumab rescues thrombin generation: reducing inhibition at the subendothelium (through TF), reducing inhibition at the platelet surface (through FXa and prothrombinase), and reducing the plasma TFPIα by trapping it at the endothelium through TFPIβ. We found that each of these individual mechanisms reduced thrombin lag times, but the direct reduction in plasma TFPIα had the greatest effect. This can be explained by the fact that directly reducing TFPIα level also reduced the inhibitory reactions. However, the reduction in plasma TFPIα did not decrease the lag time as much as concizumab. Only when we combined reducing plasma TFPIα and the inhibitory mechanisms did the reduction in lag time match that of concizumab treatment. This
demonstrates that the effects of concizumab are an accumulation of events at three cellular surfaces: the subendothelium, on activated platelet surfaces, and at the endothelium.

Besides blocking the K2 domain of TFPIα, concizumab may also affect the way that TFPIα binds to FV-h. Previous work suggests that TFPIα lacking the K2 domain can slightly inhibit the formation of prothrombinase [36]. The result in that study is in line with ours showing that the inhibition of formation of prothrombinase by C:TFPIα has a relatively minor effect on thrombin generation. However, the direct inhibition of prothrombinase, where C:TFPIα binds to FV-h in prothrombinase that is then deemed inactive in the model, has a much larger effect. It is currently unknown if this latter reaction occurs and if it does, what its effects are on prothrombinase activity. In the study of Wood et al., FV-h was preincubated with TFPIα and thus they could not assess the independent effects of direct inhibition of prothrombinase [36]. Our results suggest that direct inhibition of prothrombinase by C:TFPIα may decrease the overall efficacy of concizumab.

The TFPIβ in the steady state model trapped large amounts of TFPIα through ternary complexes with concizumab, which ultimately led to less TFPIα and C:TFPIα in the plasma. It was surprising to us that in the absence of TFPIβ, thrombin generation was significantly delayed (by about 10 min for TF = 8 fmol/cm²) with concizumab. We expected that saturating TFPIα with concizumab would have the same effect as trapping it at the endothelium, that is, making it effectively inactive or removed from the system. Without the TFPIβ, however, almost all the TFPIα is saturated with concizumab, but as mentioned above, it may still affect thrombin generation if it can bind FV-h will in the C:TFPIα complex. We observed variation in concizumab efficacy that was due to two main assumptions: 1) that TFPIβ can sequester TFPIα through concizumab, and 2) that the C:TFPIα complex can bind and inhibit FV-h. Based on whether each of the assumptions is true or false, we observed stronger or weaker thrombin enhancement by concizumab. We predict that the strongest enhancement occurs when TFPIβ sequesters TFPIα and C:TFPIα does not bind FV-h, and the weakest enhancement if TFPIβ does not sequester TFPIα and C:TFPIα does bind FV-h. The variation in efficacy under different assumptions is demonstrated in the flow chart shown in Figure 4.7. Both assumptions are based on the fact that concizumab possesses dual binding sites for K2 domain of TFPI [63, 147], and that TFPIα is a large but very flexible molecule [76]. However, to our knowledge, there is no existing experimental data to indicate whether either assumption is true or false. The advantage of using a mathematical modeling approach is that we can simulate any reaction and reaction rate we are interested in, and the model can give some insight about the possible consequences of these choices that may not be immediately intuitive. For example, our model suggests that TFPIβ, which is thought to be inhibitory, enhances thrombin generation when in combination with concizumab.
Figure 4.7 If-flow chart of two assumptions made in this model and corresponding results in thrombin generation. There are two major assumptions made in this study: 1) TFPIβ can physically sequester TFPIα from plasma via concizumab, and 2) C:TFPIα complex can bind and inhibit FV-h. Based on true or false in different assumptions, we can predict different level of enhancement in final thrombin output.

4.6 CONCLUSION

To our knowledge, this is the first mathematical model to study the effects of concizumab on thrombin generation. The advantage of this study is that we constructed a mathematical modeling framework that consists of two sub-models: a simple steady state model to estimate the plasma levels of a drug, and a coagulation model to simulate how the remaining drug in the plasma can affect thrombin generation. By using these models together, we have a wider view of how concizumab works in circulation and in coagulation. Our model gave proof of concept for the efficacy of concizumab in reducing the inhibitory effects of TFPIα, but also revealed that the bivalent nature of concizumab can further enhance thrombin generation by helping TFPIβ trap TFPIα at the endothelium. We also showed that the interaction between FV-h and the C:TFPIα complex can decrease the overall efficacy of concizumab, and that this effect can be reduced by TFPIβ. We plan to further investigate the interaction between C:TFPI and FV-h experimentally to better estimate its effects on concizumab. Other future work includes a virtual population study to identify coagulation factors or combinations of factors that may modulate the efficacy of concizumab.
5.1 Conclusion

The mathematical models of coagulation under flow described in this thesis focused on two major inhibitory reaction networks: TFPI-mediated inhibitory reactions and AT-mediated inactivation reactions. Both reaction networks were modeled in the plasma phase and on platelet surfaces under flow. We demonstrated that the platelet surface is critical for TFPI and AT to be efficient inhibitors under flow; thrombin generation is minimally affected by TFPI and AT without platelet surface-dependent inhibition, and the addition of this surface inhibition greatly enhanced the model sensitivity to the inhibitors. We also observed an accelerated inhibitory effect with heparin through surface inhibition, which gives us insight into a potential synthesis regimen for heparin.

The strength of our modeling approach is that we can use it to study bleeding disorders more broadly. We used our approach to investigate the mechanisms of actions of concizumab, a drug for hemophilia A that is currently under development. We demonstrated the proof of concept of concizumab in the model, showing it can rescue thrombin generation. The true mechanism of concizumab was to reduce the inhibition on subendothelium, endothelium, and on platelet surfaces at the same time. This application also showed how TFPI$^\beta$ is working in a non-intuitive way: TFPI$^\beta$ can sequester TFPI$^\alpha$ from plasma via a concizumab particle, which reduces TFPI$^\alpha$ mediated inhibitory effects during thrombin generation. TFPI$^\beta$ has long been regarded as a strong coagulation inhibitor, where our simulation results indicate a potential pro-coagulant effect of TFPI$^\beta$ under concizumab treatment.

One of the major motivations for this thesis study was to explore the importance of platelet surfaces for inhibiting coagulation. We demonstrated that the platelet surfaces not only enhance positive feedback, but also the inhibitory effects of TFPI and AT. Our results in the concizumab study also showed that endothelial surface is importance for the drug’s efficacy. Thus, cell surfaces, including the subendothelium, endothelium, and platelet surfaces, strongly affect and regulate thrombin generation under flow.

There are several limitations of our model. First, this model simulate thrombin generation at a small injury site, thus it is not able to predict the spatial growth of a clot that fully obstructs blood flow in a vessel. Second, our model does not include fibrin polymerization and degradation, so we miss the breakdown of the clot through fibrinolysis. Nevertheless, this experimentally validated model is extremely useful to explore the underlying mechanisms of different coagulation players.
5.2 Future Study

5.2.1 Additional Study of Concizumab

The model can be used to study the dosage-dependent behavior of thrombin generation in the concizumab study. Our preliminary results indicate that there might exist an optimal concentration of concizumab for the best therapeutic effect in the recovery of thrombin generation. We have plotted the final thrombin concentration across the range of concizumab (0-30 nM) in Figure 5.1. Increasing concizumab dosage up to 7 nM continuously enhances the final thrombin concentration, but further increasing concizumab levels may lead to less enhancement in thrombin. We suspect this happens because as concizumab dosage increases, the amount of concizumab bound TFPIα in plasma increases as well, shown in Figure 4.2B. By increasing the amount of plasma concizumab-TFPIα complex, this eventually leads to a stronger inhibitory effect and leads to less thrombin production.

The coagulation model we have used for the concizumab study can also be connected with the pharmacokinetic data of concizumab to construct a more detailed quantitative system pharmacological model (QSP model) of concizumab. By incorporating the administrated dosage, circulation rate, and drug elimination, degradation, and metabolism, we can more accurately predict the drug remaining in circulation for a longer time scale. By connecting this drug concentration profile with the coagulation flow model, we can predict the efficacy profile of concizumab upon injury at any time after the drug administration. For example, if the dosage-dependent behavior described above is true, we may observe an increase in concizumab efficacy as concizumab is eliminated from the body, reaching the optimal concentration, and a decrease in the efficacy if an additional dosage of concizumab is reapplied.

Local and global sensitivity analysis of the model can be conducted to investigate how phenotypic variation in hemophilic blood may affect efficacy of concizumab. Coagulation factors and inhibitors can be varied one at a time or in a combination. Sobol analysis can be performed to analyze the variation in the thrombin output due to the changes in the initial plasma concentrations of different species. Based on the result, we may identify specific coagulation factors or inhibitors that may impede or enhance the rescue of thrombin generation via concizumab.

5.2.2 Inclusion of Protein S in the Mathematical Model of Coagulation under Flow

The mathematical model of coagulation under flow can be further extended with protein S-mediated accelerated inhibitory reactions. Protein S acts as a cofactor of coagulation inhibitors (TFPI and APC) to down-regulate coagulation [150]. A deficiency in protein S will break the balance between pro-coagulant and anti-coagulant activities in hemostasis and increase the chance of thrombus formation. The potential extension for protein S can include the inclusion of protein S facilitated TFPI mediated inhibition, as well
as the inclusion of protein S facilitated APC mediated inhibition. The significance of this study is that this potentially extended model would then incorporate four major inhibitory systems in the coagulation: TFPI, antithrombin, APC-mediated inhibitory systems, as well as the enhancement by protein S. This would be very helpful to provide a mechanistic explanation of how protein S affects coagulation, and furnishes a bigger picture in how there can be un-intuitive relationships between coagulation factors and inhibitors.

5.2.3 Application in Other Bleeding Disorders

This thesis has been focused on studying hemophilia A by using the coagulation flow model. This was done by simply adjusting the initial conditions of the plasma coagulation factors to recreate the hemophilia A blood condition. The strength of this model is that we can easily turn on and off specific reaction kinetics we are interested in or adjust the initial conditions of any species for different plasma environments. Therefore, this model can be applied to other bleeding disorders as well. Hemophilia B, another form of hemophilia that is characterized by a lower level of FIX [151], can also be investigated using this model by reducing FIX levels in plasma. FXI deficiency is an autosomal recessive bleeding disorder characterized by a lower level of FXI that causes increased bleeding tendency [152]. With a better scheme to represent AT-mediated inactivation reactions towards FXIa, we may also use the model to study potential thrombin modifiers in FXI deficiency conditions via local and global sensitivity analysis. With a throughout TFPI-mediated inhibitory reaction scheme, we can also utilize the model to study TFPIα related bleeding disorders. East Texas bleeding disorder, for example, is a rare genetic disorder with a mutation in F5 gene that leads to the generation of alternatively spliced forms of FV, which is susceptible to TFPIα [73]. This most-updated coagulation flow model would be a strong and helpful tool to examine the inhibition of coagulation due to this genetic defect.
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A.1 Model Schematic

![Figure A.1 Schematic of the model reaction zone (main figure) and endothelial zone (inset).](image)

A.2 Model Equations

Below we have listed the full model equations for all species. The model detailed here includes extensions of our previous work [7–9]. In total, the model consists of 130 species (and their corresponding ordinary differential equations) and 239 parameters including kinetic rates and initial/upstream concentrations. The solution of the model equations was carried out with our in-house fortran code that uses DLSODE for the numerical solution of the differential equations; each run of the model that simulates 40 minutes of clotting activity takes less than 10 seconds on a linux-based laptop. Simulations of this model (in the absence of heparin) can be performed with our online coagulation simulator: ClotSims.
\[
\begin{align*}
\frac{d}{dt} z_{10} &= -k_{on}^{z_{10}} [\text{TF}]^\text{avail} + k_{off}^{z_{10}} \\
&\quad -k_{z_{10}:e_{10}}^{+} z_{10} e_{2} + k_{z_{10}:e_{10}}^{-} [Z_{10} : E_{2}] \\
&\quad -k_{z_{7}:e_{10}}^{+} z_{7} e_{10} + k_{z_{7}:e_{10}}^{-} [Z_{7} : E_{10}] \\
&\quad + k_{\text{flow}}(z_{10}^{\text{up}} - z_{10}) \\
&\quad -k_{z_{7}:e_{9}}^{+} z_{7} e_{9} + k_{z_{7}:e_{9}}^{-} [Z_{7} : E_{9}] \\
\frac{d}{dt} e_{7} &= -k_{on}^{e_{7}} [\text{TF}]^\text{avail} + k_{off}^{e_{7}} \\
&\quad + k_{z_{7}:e_{2}}^{cat} [Z_{7} : E_{2}] + k_{z_{7}:e_{10}}^{cat} [Z_{7} : E_{10}] \\
&\quad + k_{\text{flow}}(e_{7}^{\text{up}} - e_{7}) + k_{z_{7}:e_{9}}^{cat} [Z_{7} : E_{9}] \\
\frac{d}{dt} z_{10} &= -k_{on}^{z_{10}} [\text{P}]^\text{avail} + k_{off}^{z_{10}} z_{10} \\
&\quad -k_{z_{10}:e_{10}}^{+} e_{10} + k_{z_{10}:e_{10}}^{-} [Z_{10} : E_{10}] \\
&\quad + k_{\text{flow}}(z_{10}^{\text{up}} - z_{10}) \\
\frac{d}{dt} e_{10} &= -k_{on}^{e_{10}} [\text{P}]^\text{avail} + k_{off}^{e_{10}} e_{10} \\
&\quad + k_{z_{10}:e_{10}}^{cat} [Z_{10} : E_{7}] \\
&\quad + k_{z_{10}:e_{10}}^{-} [Z_{10} : E_{10}] - k_{z_{7}:e_{10}}^{+} e_{10} z_{7} \\
&\quad - k_{TFPI:e_{10}}^{+} [TFPI] + k_{TFPI:e_{10}}^{-} [TFPI : E_{10}] \\
&\quad + k_{\text{flow}}(e_{10}^{\text{up}} - e_{10}) \\
&\quad - k_{AT:e_{10}}^{in} e_{10} \\
&\quad - k_{AT:e_{10}}^{diff} (e_{10} - e_{10}^{cc}) \\
&\quad - k_{TFPI:e_{5}:e_{10}}^{+} [TFPI : E_{5}] \\
&\quad - k_{TFPI:e_{5}:e_{10}}^{-} [E_{10} : TFPI : E_{5}] \\
&\quad - k_{TFPI:e_{5}:e_{10}}^{+} [E_{10} : TFPI : E_{5}] \\
&\quad - k_{TFPI:e_{5}:e_{10}}^{-} [E_{10} : TFPI : E_{5}] \\
&\quad - k_{AT:e_{10}}^{in} e_{10} [AT] \\
&\quad + k_{AT:e_{10}}^{ATH} e_{10} [AT : Hep] \\
\frac{d}{dt} z_{10}^{m} &= k_{on}^{z_{10}^{m}} [\text{P}]^\text{avail} - k_{off}^{z_{10}^{m}} z_{10}^{m} \\
&\quad + k_{z_{10}^{m}:TEN}^{+} z_{10}^{m} [TEN] + k_{z_{10}^{m}:TEN}^{-} [Z_{10}^{m} : TEN] \\
&\quad - k_{z_{10}^{m}:TEN}^{+} [TEN^{*}] + k_{z_{10}^{m}:TEN}^{-} [Z_{10}^{m} : TEN^{*}] \\
\end{align*}
\]
\[
\frac{d}{dt} e_{10}^m = k_{10}^m e_{10}^{\text{avail}} - k_{10}^{\text{off}} e_{10}^m \\
+ k_{\text{cat}}^{\text{TFPI}} e_{10}^m [\text{TEN}] + (k_e^{\text{cat}} + k_e^{\text{m}}) e_{10}^m Z_{10}^m : E_{10}^m \\
- k_{\text{z}_5^{m}, e_{10}^{m}} e_{10}^m + (k_{\text{z}_5^{m}, e_{10}^{m}} + k_{\text{z}_5^{m}, e_{10}^{m}}) Z_{10}^m : E_{10}^m \\
- k_{\text{z}_5^{m}, e_{10}^{m}} e_{10}^m \\
+ k_{\text{cat}}^{\text{TEN}} e_{10}^m [\text{TEN}] + k_{\text{cat}}^{\text{TEN}} e_{10}^m [\text{TEN}^*] \\
- k_{\text{z}_5^{m}, e_{10}^{m}} e_{10}^m + k_{\text{z}_5^{m}, e_{10}^{m}} PRO^h \\
- k_{\text{TFPI}, e_{10}^m} e_{10}^m [\text{TFPI}] + k_{\text{TFPI}, e_{10}^m} [\text{TFPI} : E_{10}^m] \\
- k_{\text{TFPI}, e_{10}^m} e_{10}^m [\text{TFPI}] + k_{\text{TFPI}, e_{10}^m} [\text{TFPI} : E_{10}^m] \\
+ k_{\text{TFPI}, e_{10}^m} e_{10}^m [\text{TFPI} : PRO^h] \\
- k_{\text{AT}, e_{10}^m} e_{10}^m \text{[AT]} \\
- k_{\text{AT}, e_{10}^m} e_{10}^m \text{[AT : Hep]} \\
\]

\[
\frac{d}{dt} z_5 = -k_5^{\text{on}} z_5^{\text{avail}} + k_5^{\text{off}} z_5 \\
- k_{\text{z}_5^{m}, e_{10}^{m}} Z_5 : E_2 \\
k_{\text{flow}} (e_5^{\text{up}} - z_5) \\
+ k_{\text{adhl}} e_{10}^{\text{avail}} + k_{\text{act}} ([PL^v] + [PL^e]) + k_{\text{act}} e_2 \frac{e_2}{e_2 + 0.001} [PL] \\
\]

\[
\frac{d}{dt} e_5 = -k_5^{\text{on}} e_5^{\text{avail}} + k_5^{\text{off}} e_5 \\
+ k_{\text{z}_5^{m}, e_{10}^{m}} Z_5 : E_2 \\
k_{\text{flow}} (e_5^{\text{up}} - e_5) \\
k_{\text{APC}}, [APC : E_5] - k_{\text{APC}, e_5^{m}} [APC] \\
k_{\text{cat}}^{\text{APC}} [E_5] : E_2 \\
\]

\[
\frac{d}{dt} z_{10}^m = k_5^{\text{on}} z_{10}^{\text{avail}} - k_5^{\text{off}} z_{10}^m \\
- k_{\text{z}_5^{m}, e_{10}^{m}} Z_{10}^m : E_{10}^m \\
k_{\text{z}_5^{m}, e_{10}^{m}} z_{10}^m + k_{\text{z}_5^{m}, e_{10}^{m}} Z_{10}^m : E_{10}^m \\
- k_{\text{z}_5^{m}, e_{10}^{m}} z_{10}^m + k_{\text{z}_5^{m}, e_{10}^{m}} Z_{10}^m : E_{10}^m \\
+ k_{\text{cat}}^{\text{APC}} [APC : E_{10}^m] \\
k_{\text{cat}}^{\text{APC}} [APC] \\
k_{\text{cat}}^{\text{APC}} [E_{10}^m] : E_{10}^m \\
+ k_{\text{cat}}^{\text{APC}} [APC] \\
\]

\[
\frac{d}{dt} e_{10}^m = k_5^{\text{on}} e_{10}^{\text{avail}} - k_5^{\text{off}} e_{10}^m \\
+ k_{\text{cat}}^{\text{APC}} [APC : E_{10}^m] \\
k_{\text{cat}}^{\text{APC}} [APC] \\
k_{\text{cat}}^{\text{APC}} [E_{10}^m] : E_{10}^m \\
+ k_{\text{cat}}^{\text{APC}} [APC] \\
\]

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\[
\frac{d}{dt} z_8 = -k_{on} z_8 p_{avail} + k_{off} z_8^m \\
+ k_{flow} (z_{ap} - z_8) \\
- k_{z_8:e_2}^+ z_8 e_2 + k_{z_8:e_2}^- [Z_8 : E_2] \\
\]

\[
\frac{d}{dt} e_8 = -k_{on} e_8 p_{avail} + k_{off} e_8^m \\
+ k_{z_8:e_2}^- - 0.005 e_8 \\
+ k_{e_8:APC} [APC : E_8] - k_{e_8:APC e_8} [APC] \\
\]

\[
\frac{d}{dt} z_8^m = k_{on} z_8 p_{avail} - k_{off} z_8^m \\
- k_{z_8:e_10}^+ z_8^m e_{10} + k_{z_8:e_10}^- [Z_8^m : E_{10}^m] \\
- k_{z_8:e_2}^+ z_8^m e_2 \\
+ k_{z_8:e_2}^- [Z_8^m : E_2^m] \\
\]

\[
\frac{d}{dt} e_8^m = k_{on} e_8 p_{avail} - k_{off} e_8^m \\
k_{e_8:APC e_8^m} [APC] + k_{e_8:APC e_8^m} [APC : E_8^m] \\
- k_{e_8:e_9}^+ e_9^m e_9 + k_{e_8:e_9}^- [TEN] - 0.005 e_8^m \\
- k_{e_8:e_9}^+ e_9^m e_9 + k_{e_8:e_9}^- [TEN] \\
\]

\[
\frac{d}{dt} z_9 = k_{flow} (z_{ap} - z_9) - k_{on} p_9 z_9 + k_{off} z_9^m \\
- k_{z_9:e_7}^+ z_9 e_7 + k_{z_9:e_7}^- [Z_9 : E_7^m] \\
- k_{z_9:e_11}^+ e_{11} + k_{z_9:e_11}^- [Z_9 : E_{11}^m] \\
- k_{z_9:e_11}^+ z_9 e_{11} + k_{z_9:e_11}^- [Z_9 : E_{11}^m] \\
\]

\[
\frac{d}{dt} e_9 = f_{flow} (e_{ap} - e_9) \\
- k_{on} p_9 e_9 + k_{off} e_9^m \\
k_{e_9:e_9}^+ [Z_9 : E_9^m] \\
- k_{AT e_9}^+ e_9 - k_{z_9:e_9}^+ z_9 e_9 \\
+ (k_{z_9:e_9}^- + k_{z_9:e_9}^+ [Z_9 : E_9] \\
+ (k_{z_9:e_9}^+ + k_{z_9:e_9}^-) [Z_9 : E_9] - k_{z_9:e_9}^+ z_9 e_9 \\
- k_{on} p_9 e_9 + k_{off} e_9^m \\
- k_{diff} (e_9 - e_9^c) \\
+ k_{e_9:AT} [Z_9 : E_{11}^m] + k_{AT} [Z_9 : E_{11}^m] \\
- k_{e_9 e_9} [AT] \\
- k_{ATH e_9} [AT : Hep] \\
\]

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\[
\frac{d}{dt} z_2 = -k_2 n_{\text{p}_2}^\text{av} z_2 + k_2 f_{z_2} + k_{\text{flow}} (z_2^\text{up} - z_2)
\] (A.19)

\[
\frac{d}{dt} e_2 = k_{\text{flow}} (e_2^\text{up} - e_2)
\] (A.20)

\[
\frac{d}{dt} e_9 = k_9 n_{\text{p}_9}^\text{av} e_9 - k_9 f_{e_9} e_9
\] (A.17)

\[
\frac{d}{dt} e_9 = k_9 n_{\text{p}_9}^\text{av} e_9 - k_9 f_{e_9} e_9
\] (A.18)

\[
\frac{d}{dt} z_2 = -k_2 n_{\text{p}_2}^\text{av} z_2 + k_2 f_{z_2} + k_{\text{flow}} (z_2^\text{up} - z_2)
\] (A.19)

\[
\frac{d}{dt} e_2 = k_{\text{flow}} (e_2^\text{up} - e_2)
\] (A.20)
\[
\frac{d}{dt} e_2^m = k_{2s}^m p_{2}^{avail} e_2 - k_{2s}^{off} e_2^m + (k_{cat}^{e_2^m} + k_{z_2^m}^{e_2^m}) [Z_5^m : E_2^m] - k_{z_2^m}^{e_2^m} e_2^m + (k_{cat}^{e_2^m} + k_{z_2^m}^{e_2^m}) [Z_8^m : E_2^m] - k_{z_2^m}^{e_2^m} e_2^m + (k_{cat}^{e_2^m} + k_{z_2^m}^{e_2^m}) [Z_{11}^m : E_2^m] - k_{z_2^m}^{e_2^m} e_2^m + (k_{cat}^{e_2^m} + k_{z_2^m}^{e_2^m}) [E_{11}^{hms} : E_2^m] - k_{cat}^{e_2^m} [E_{5}^m : E_2^m]
\]

\[
\frac{d}{dt} [TEN] = -k_{e_6^m}^{cat} [TEN] + (k_{cat}^{e_6^m} + k_{z_{10}^m}^{e_6^m}) [Z_{10}^m : TEN] - k_{z_{10}^m}^{e_6^m} [TEN] + (k_{cat}^{e_6^m} + k_{z_{10}^m}^{e_6^m}) [Z_{10}^m : TEN] - k_{z_{10}^m}^{e_6^m} [TEN]
\]

\[
\frac{d}{dt} [PRO] = -k_{e_1^m}^{cat} [PRO] + (k_{cat}^{e_1^m} + k_{z_{27}^m}^{e_1^m}) [Z_{27}^m : PRO] - k_{z_{27}^m}^{e_1^m} [PRO] + (k_{cat}^{e_1^m} + k_{z_{27}^m}^{e_1^m}) [Z_{27}^m : PRO] - k_{z_{27}^m}^{e_1^m} [PRO]
\]

\[
\frac{d}{dt} [PL_a^s] = k_{cat}^{adp} [PL_a^s] + k_{cat}^{adh} [PL_a^s] + k_{cat}^{adh} [PL_a^s] * P_{PLAS}^a
\]

\[
\frac{d}{dt} [PL] = k_p^{flow} ([PL]_{up} - [PL]) - k_{cat}^{adh} [PL] + (k_{cat}^{adp} ([PL_a^s] + [PL_a^s]) + k_{cat}^{adh} e_2^m / e_2 + 0.001) \]

\[
\frac{d}{dt} [PL_a^v] = k_{cat}^{adh} [PL_a^v] - k_{cat}^{adh} [PL_a^v] P_{PLAS}^a + (k_{cat}^{adp} ([PL_a^v] + [PL_a^v]) + k_{cat}^{adh} e_2^m \]

\[
\frac{d}{dt} z_7^m = k_{cat}^{z_7} [TF]^{avail} - k_{off}^{z_7} z_7^m - k_{z_7}^{e_10} z_7^m e_10 - k_{z_7}^{e_2} z_7^m e_2 + k_{z_7}^{e_10} [Z_5^m : E_10] + k_{z_7}^{e_2} [Z_5^m : E_2] - k_{z_7}^{e_2} [Z_7^m : E_2] + z_7^m / 0.001 \]

\[
-\frac{1}{p_{PLAS}^a}
\]

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\[
\frac{d}{dt} e_7^m = k_5^m e_7^m [TF]_{\text{avail}} - k_{\text{off}}^m e_7^m \tag{A.29}
\]
\[
k_5^{\text{TFPI.c10.e7}_m} e_7^m [TFPI : E_{10}] + k_5^{-\text{TFPI.c10.e7}_m} [TFPI : E_{7}^m]
\]
\[
+k_7_{\text{cat}}^m e_7^m [Z_{7}^m : E_{10}] + k_{\text{cat}}^m e_7^m [Z_{7}^m : E_{2}]
\]
\[
+(k_5^m e_7^m + k_{\text{z10.e7}_m}) [Z_{10} : E_{7}^m]
\]
\[
-k_7_{\text{z10.e7}_m} e_7^m z_{10} - k_5^{-z_{10.e7}_m} e_7^m z_{9}
\]
\[
+(k_5^m e_7^m + k_{\text{z9.e7}_m}) [Z_{9} : E_{7}^m]
\]
\[
+k_7_{\text{z9.e7}_m} [Z_{7}^m : E_{9}] - e_7^m \frac{d}{dt}[PL_s] \frac{1}{p_{\text{PLAS}}}
\]

\[
\frac{d}{dt} [TFPI] = -k_5^{\text{TFPI.c10.e7}_m} e_7^m [TFPI] + k_5^{-\text{TFPI.c10.e7}_m} [TFPI : E_{10}] \tag{A.30}
\]
\[
k_{\text{flow}}([TFPI]_{\text{up}} - [TFPI])
\]
\[
-k_5^{\text{TFPI.c5.e7}_m} e_7^m [TFPI] + k_5^{-\text{TFPI.c5.e7}_m} [TFPI : E_{7}^m]
\]
\[
-k_5^{\text{TFPI.c5.e7}_m} e_7^m [TFPI] + k_5^{-\text{TFPI.c5.e7}_m} [TFPI : E_{5}]
\]
\[
-k_5^{\text{TFPI.c10.e7}_m} e_7^m [TFPI] + k_5^{-\text{TFPI.c10.e7}_m} [TFPI : E_{10}]
\]
\[
-k_5^{\text{TFPI.PRO.c10.e7}} PRO^h [TFPI]
\]
\[
+k_5^{\text{TFPI.PRO.c10.e7}} [TFPI : PRO^h] e_{10}
\]
\[
-k_5^{\text{TFPI.PRO.c5.e7}} PRO^h [TFPI]
\]
\[
+k_5^{\text{TFPI.PRO.c5.e7}} [TFPI : PRO^h] e_{5}
\]
\[
-k_5^{\text{C.TFPPI.C[TFPPI] + k_{\text{off}}^{C.TFPPI}[C : TFPPI]}}
\]
\[
-k_5^{\text{C.TFPPI}[TFPI][C : TFPPI] + k_{\text{off}}^{C.TFPPI}[TFPI : C : TFPPI]}
\]

\[
\frac{d}{dt} [TFPI : E_{10}] = k_5^{\text{TFPI.c10.e7}_m} e_7^m [TFPI] - k_5^{-\text{TFPI.c10.e7}_m} [TFPI : E_{10}] \tag{A.31}
\]
\[
+k_5^{\text{TFPI.c10.e7}_m} [TFPI : E_{10} : E_{7}^m]
\]
\[
-k_5^{\text{TFPI.c10.e7}_m} e_7^m [TFPI : E_{10}] + k_{\text{flow}}([TFPI : E_{10}]_{\text{up}} - [TFPI : E_{10}])
\]
\[
-k_5^{\text{TFPI.c10.e7}_m} [TFPI : E_{10}] e_{7}^m
\]
\[
+k_5^{\text{TFPI.c10.e7}_m} [E_{10} : TFPI : E_{7}^m]
\]
\[
-k_5^{\text{C.TFPPI}[TFPI : E_{10}] e_{10} + k_{\text{off}}^{C.TFPPI}[TFPI : E_{10}]
\]

\[
\frac{d}{dt} [TFPI : E_{7}^m] = -k_5^{\text{TFPI.c10.e7}_m} [TFPI : E_{7}^m] + k_5^{\text{TFPI.c10.e7}_m} e_7^m [TFPI : E_{10}] \tag{A.32}
\]
\[
-[TFPI : E_{10} : E_{7}^m] \frac{d}{dt}[PL_s] \frac{1}{p_{\text{PLAS}}}
\]

\[
\frac{d}{dt} [APC] = (k_{\text{cat}}^{e_{7}^m : APC} + k_{\text{cat}}^{-e_{7}^m : APC}) [APC : E_{7}^m] - k_{\text{cat}}^{e_{7}^m : APC} e_7^m \tag{A.33}
\]
\[
+(k_{\text{cat}}^{e_{7}^m : APC} + k_{\text{cat}}^{-e_{7}^m : APC}) [APC : E_{8}^m] - k_{\text{cat}}^{e_{8}^m : APC} e_7^m [APC]
\]
\[
+k_{\text{flow}}([APC]_{\text{up}} - [APC]) - k_{\text{off}}([APC] - [APC_{\text{c5}}])
\]
\[
+(k_{\text{cat}}^{e_{7}^m : APC} + k_{\text{cat}}^{-e_{7}^m : APC}) [APC : E_{5}^m] - k_{\text{cat}}^{e_{7}^m : APC} e_7^m [APC]
\]
\[
+(k_{\text{cat}}^{e_{7}^m : APC} + k_{\text{cat}}^{-e_{7}^m : APC}) [APC : E_{8}^m] - k_{\text{cat}}^{e_{7}^m : APC} e_7^m [APC]
\]
\[
-k_{\text{off}}^{-e_{7}^m : APC} e_7^m [APC] + k_{\text{off}}^{-e_{7}^m : APC} e_7^m [APC]
\]
\[
+k_{\text{cat}}^{e_{7}^m : APC} [APC : E_{5}^m] - k_{\text{cat}}^{e_{7}^m : APC} e_7^m [APC]
\]
\[
+k_{\text{cat}}^{e_{7}^m : APC} [APC : E_{5}^m] + k_{\text{cat}}^{e_{7}^m : APC} [APC : E_{5}^m]
\]

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\[
\frac{d}{dt} [Z_7 : E_2] = k_{\text{flow}}([Z_7 : E_2]_{\text{up}} - [Z_7 : E_2]) + k_{z_7:e_2}^+ e_2 z_7
\]  
\[-(k_{z_7:e_2}^+ + k_{z_7:e_2}^-) [Z_7 : E_2] \tag{A.34}\]

\[
\frac{d}{dt} [Z_7 : E_{10}] = k_{z_7:e_{10}}^+ e_{10} z_7 - (k_{z_7:e_{10}}^+ + k_{z_7:e_{10}}^-) [Z_7 : E_{10}] + k_{\text{flow}}([Z_7 : E_{10}]_{\text{up}} - [Z_7 : E_{10}]) \tag{A.35}\]

\[
\frac{d}{dt} [Z^m_7 : E_{10}] = k_{z_7:e_{10}}^+ e_{10} z_7
\]
\[-(k_{z_7:e_{10}}^+ + k_{z_7:e_{10}}^-) [Z^m_7 : E_{10}] - [Z^m_7 : E_{10}] \frac{d}{dt}[PL_a] \frac{1}{p_{\text{PLAS}}} \tag{A.36}\]

\[
\frac{d}{dt} [Z^m_7 : E_2] = k_{z^m_7:e_2}^+ e_2 z_7
\]
\[-(k_{z^m_7:e_2}^+ + k_{z^m_7:e_2}^-) [Z^m_7 : E_2] - [Z^m_7 : E_2] \frac{d}{dt}[PL_a] \frac{1}{p_{\text{PLAS}}} \tag{A.37}\]

\[
\frac{d}{dt} [Z_{10} : E^m_7] = k_{z_{10}e^m_7}^+ e^m_7 z_{10} - (k_{z_{10}e^m_7}^+ + k_{z_{10}e^m_7}^-) [Z_{10} : E^m_7]
\]
\[-[Z_{10} : E^m_7] \frac{d}{dt}[PL_a] \frac{1}{p_{\text{PLAS}}} \tag{A.38}\]

\[
\frac{d}{dt} [Z^m_{10} : T\text{EN}] = k_{z^m_{10}e_{10}}^+ [T\text{EN}]_{10} - (k_{z^m_{10}e_{10}}^+ + k_{z^m_{10}e_{10}}^-) [Z^m_{10} : T\text{EN}] \tag{A.39}\]

\[
\frac{d}{dt} [Z_5 : E_2] = k_{z_5:e_2}^+ e_2 z_5
\]
\[-(k_{z_5:e_2}^+ + k_{z_5:e_2}^-) [Z_5 : E_2] + k_{\text{flow}}([Z_5 : E_2]_{\text{up}} - [Z_5 : E_2]) \tag{A.40}\]

\[
\frac{d}{dt} [Z^m_5 : e_{10}^m] = k_{z^m_5:e_{10}^m}^+ e_{10}^m z_5 - (k_{z^m_5:e_{10}^m}^+ + k_{z^m_5:e_{10}^m}^-) [Z^m_5 : E_{10}] \tag{A.41}\]

\[
\frac{d}{dt} [Z^m_5 : E_2] = k_{z^m_5:e_2}^+ e_2 z_5
\]
\[-(k_{z^m_5:e_2}^+ + k_{z^m_5:e_2}^-) [Z^m_5 : E_2] \tag{A.42}\]

\[
\frac{d}{dt} [Z^m_9 : E_{10}] = k_{z^m_9:e_{10}^m}^+ e_{10}^m z_9 - (k_{z^m_9:e_{10}^m}^+ + k_{z^m_9:e_{10}^m}^-) [Z^m_9 : E_{10}] \tag{A.43}\]

\[
\frac{d}{dt} [Z^m_8 : E_2] = k_{z^m_8:e_2}^+ e_2 z_8
\]
\[-(k_{z^m_8:e_2}^+ + k_{z^m_8:e_2}^-) [Z^m_8 : E_2] \tag{A.44}\]

\[
\frac{d}{dt} [Z^m_8 : E_2] = k_{z^m_8:e_2}^+ e_2 z_8
\]
\[-(k_{z^m_8:e_2}^+ + k_{z^m_8:e_2}^-) [Z^m_8 : E_2] \tag{A.45}\]

\[
\frac{d}{dt} [APC : E^m_8] = k_{e_8^m:APC^m_8}^+ [APC] - (k_{e_8^m:APC}^+ + k_{e_8^m:APC}^-) [APC : E^m_8] \tag{A.46}\]

\[
\frac{d}{dt} [Z_9 : E^m_7] = k_{z_9:e^m_7}^+ e^m_7 z_9 - (k_{z_9:e^m_7}^+ + k_{z_9:e^m_7}^-) [Z_9 : E^m_7]
\]
\[-[Z_9 : E^m_7] \frac{d}{dt}[PL_a] \frac{1}{p_{\text{PLAS}}} \tag{A.47}\]

\[
\frac{d}{dt} [Z^m_2 : PRO] = k_{z^m_2:PRO^m_2}^+ [PRO] - (k_{z^m_2:PRO}^+ + k_{z^m_2:PRO}^-) [Z^m_2 : PRO] \tag{A.48}\]

\[
\frac{d}{dt} [APC : E^m_5] = k_{e_5^m:APC^m_5}^+ [APC] - (k_{e_5^m:APC}^+ + k_{e_5^m:APC}^-) [APC : E^m_5] \tag{A.49}\]

\[
\frac{d}{dt} [TF] = -[TF] \frac{d}{dt}[PL_a] \frac{1}{p_{\text{PLAS}}} \tag{A.50}\]

\[
\frac{d}{dt} [Z_7 : E_9] = k_{z_7:e_9}^+ e_9 z_7 - (k_{z_7:e_9}^+ + k_{z_7:e_9}^-) [Z_7 : E_9] \tag{A.51}\]
\[
\begin{align*}
\frac{d}{dt}[Z_{10}^m : E_9] &= k_{z_{10}^m, e_9} z_{10}^m - (k_{z_{10}^m, e_9} + k_{z_{10}^m, e_9}) [Z_{10}^m : E_9] \\
&\quad - [Z_{10}^m : E_9] \frac{d}{dt}[PLA] \frac{1}{p_{PLA}} \\
\frac{d}{dt} e^m_{9s} &= k_{on}^s p_{9s, avail} e_9 - k_{off}^s e^m_{9s} + k_{e^m_{9s}, e_9} [TEN^s] \\
&\quad - k_{e^m_{9s}, e_9} e^m_{9s} e_9 - k_{AT, e_9} e^m_{9s} [AT] \\
&\quad - k_{AT, e_9} e^m_{9s} [AT : Hep] \\
\frac{d}{dt}[TEN^s] &= -k_{z_{10}^m, e_9} [TEN^s] + k_{e^m_{9s}, e_9} e^m_{9s} \\
&\quad + (k_{z_{10}^m, TEN} + k_{z_{10}^m, TEN}) [Z_{10}^m : TEN^s] \\
&\quad + k_{z_{10}^m, TEN}[TEN^s] z_{10}^m \\
\frac{d}{dt}[Z_{10}^m : TEN^s] &= k_{z_{10}^m, TEN}[TEN^s] z_{10} - (k_{z_{10}^m, TEN} + k_{z_{10}^m, TEN}) [Z_{10}^m : TEN^s] \\
\frac{d}{dt} e^{cc}_{2} &= k_{diff}(e_2 - e^{cc}_2) + k_{flow}(e^{cc, up}_2 - e^{cc}_2) \\
&\quad - k_{AT, e_2} e^{cc}_2 \\
&\quad - k_{TM, e^c_2}[TM]_{available} + k_{off}[TM : E^{cc}_2] - k_{e^c_2} e^{cc}_2 [AT] \\
\frac{d}{dt}[APC^{cc}] &= k_{flow}([APC]^{up} - [APC^{cc}]) + k_{diff}([APC] - [APC^{cc}]) \\
&\quad + k_{PC:TM:e_2}[TM : E^{cc}_2 : APC] \\
\frac{d}{dt}[TM : E^{cc}_2] &= k_{TM}[E^{cc}_2](1 - [TM : E^{cc}_2] - [TM : E^{cc}_2 : APC]) \\
&\quad - k_{TM}[TM : E^{cc}_2] - k_{PC:TM:e_2}[TM : E^{cc}_2] \\
&\quad + (k_{PC:TM:e_2} + k_{PC:TM:e_2})[TM : E^{cc}_2 : APC] \\
\frac{d}{dt}[TM : E^{cc}_2 : APC] &= k_{PC:TM:e_2}[TM : E^{cc}_2] \\
&\quad - k_{PC:TM:e_2} + k_{PC:TM:e_2}[TM : E^{cc}_2 : APC] \\
\frac{d}{dt} e^{cc}_{9} &= k_{diff}(e_9 - e^{cc}_9) + k_{flow}(e^{up}_9 - e^{cc}_9) \\
&\quad - k_{AT, e_9} e^{cc}_9 - k_{AT, e_9} e^{cc}[AT] \\
\frac{d}{dt} e^{cc}_{10} &= k_{diff}(e_{10} - e^{cc}_{10}) + k_{flow}(e^{up}_{10} - e^{cc}_{10}) \\
&\quad - k_{AT, e_{10}} e^{cc}_{10} - k_{AT, e_{10}} e^{cc}[AT] \\
\frac{d}{dt}[APC : E_5] &= -(k_{e_5 : APC} + k_{e_5 : APC})[APC : E_5] + k_{e_5 : APC} e_5 [APC] \\
\frac{d}{dt}[APC : E_8] &= -(k_{e_8 : APC} + k_{e_8 : APC})[APC : E_8] + k_{e_8 : APC} e_8 [APC] \\
\frac{d}{dt} z_{11} &= k_{flow}(z_{11}^{up} - z_{11}) - k_{on} z_{11} p_{z_{11}}^{available} \\
&\quad - k_{off, z_{11}} z_{11} - k_{k_{z_{11}, e_{11}}} z_{11} e_{11} \\
&\quad - k_{z_{11}, e_{11}} [Z_{11} : E_{11}] - k_{z_{11}, e_{11}} z_{11} e_{11} \\
&\quad - k_{z_{11}, e_{11}} [Z_{11} : E_{11}] - k_{z_{11}, e_{11}} z_{11} e_{2} \\
&\quad - k_{z_{11}, e_{11}} [Z_{11} : E_{2}]
\end{align*}
\]
\[
\begin{align*}
\frac{d}{dt}e_{11} & = k_{\text{flow}}(e_{11}^{up} - e_{11}) - k_{11}^{m, e}e_{11} p_{11}^{\text{avail}} + k_{11}^{g, e}e_{11}^{ms} \tag{A.65} \\
& - k_{z_9, e_{11}}^{+} z_9 e_{11} + (k_{z_9, e_{11}}^{-} + k_{\text{cat}, e_{11}}^{+}) [Z_9 : E_{11}] \\
& - k_{z_{11}, e_{11}}^{-} z_{11} e_{11} + (k_{z_{11}, e_{11}}^{+} + k_{\text{cat}, e_{11}}^{-}) [Z_{11} : E_{11}] \\
& + k_{e_{11}, e_{11}}^{+} [E_{11}^h : E_{11}^{h}] - k_{e_{11}, e_{11}}^{+} [E_{11}^{h} : E_{11}] \\
& + k_{e_{11}, e_{2}}^{+} [E_{11} : E_{2}] \\
& - k_{e_{11}, e_{11}}^{AT} [AT] \\
& - k_{e_{11}, e_{11}}^{AT, H} [AT : Hep] \\
\end{align*}
\]

\[
\begin{align*}
\frac{d}{dt}z_{11}^{m} & = k_{z_{11}, p_{11}}^{m, g, \text{avail}} - k_{z_{11}, e_{11}}^{m} z_{11}^{m} \tag{A.66} \\
& - k_{z_{11}, e_{11}}^{m, h, m} z_{11}^{m, h, m} e_{11} + k_{z_{11}, e_{11}}^{m} [Z_{11}^{m} : E_{11}] \\
& - k_{z_{11}, e_{11}}^{m, h, m} z_{11}^{m, h, m} e_{11} + k_{z_{11}, e_{11}}^{m, h, m} [Z_{11}^{m} : E_{11}] \\
& - k_{z_{11}, e_{11}}^{m, h, m} z_{11}^{m, h, m} e_{11} + k_{z_{11}, e_{11}}^{m, h, m} [Z_{11}^{m} : E_{11}] \\
& - k_{z_{11}, e_{11}}^{m, h, m} z_{11}^{m, h, m} e_{11} + k_{z_{11}, e_{11}}^{m, h, m} [Z_{11}^{m} : E_{11}] \\
\end{align*}
\]

\[
\begin{align*}
\frac{d}{dt}e_{11}^{ms} & = k_{11}^{m, e} e_{11} p_{11}^{\text{avail}} - k_{11}^{g, e} e_{11}^{ms} \tag{A.67} \\
& - k_{z_9, e_{11}}^{m, e} e_{11}^{ms} z_9 \\
& + (k_{z_9, e_{11}}^{-} + k_{\text{cat}, e_{11}}^{+}) [Z_{9}^{m} : E_{11}] \\
& - k_{z_{11}, e_{11}}^{-} z_{11} e_{11}^{ms} + k_{z_{11}, e_{11}}^{+} [Z_{11}^{m} : E_{11}] \\
& + k_{e_{11}, e_{11}}^{+} [E_{11}^{h, m} : E_{11}] \\
& + k_{e_{11}, e_{11}}^{+} [E_{11}^{h, m} : E_{11}] \\
& + k_{e_{11}, e_{11}}^{+} [E_{11}^{h, m} : E_{11}] \\
& - k_{e_{11}, e_{11}}^{+} [E_{11}^{h, m} : E_{11}] \\
& - k_{e_{11}, e_{11}}^{+} [E_{11}^{h, m} : E_{11}] \\
\end{align*}
\]

\[
\begin{align*}
\frac{d}{dt}[Z_{11}^{m} : E_{2}^{m}] & = k_{z_{11}, e_{2}}^{m, e} z_{11}^{m, e} e_{2} - (k_{z_{11}, e_{2}}^{m, e} + k_{\text{cat}, e_{2}}^{+}) [Z_{11}^{m} : E_{2}^{m}] \tag{A.68} \\
& -(k_{z_9, e_{11}}^{m, e} z_9 e_{11}^{ms}) [Z_{9}^{m} : E_{11}] \\
\end{align*}
\]

\[
\begin{align*}
\frac{d}{dt}[Z_{11} : E_{2}] & = k_{\text{flow}} ([Z_{11} : E_{2}]^{up} - [Z_{11} : E_{2}]) + k_{z_{11}, e_{2}}^{+} z_{11} e_{2} \tag{A.70} \\
& - (k_{z_{11}, e_{2}}^{+} + k_{\text{cat}, e_{2}}^{+}) [Z_{11} : E_{2}] \\
\end{align*}
\]

\[
\begin{align*}
\frac{d}{dt}[Z_{9} : E_{11}] & = k_{\text{flow}} ([Z_{9} : E_{11}]^{up} - [Z_{9} : E_{11}]) + k_{z_{9}, e_{11}}^{+} z_{9} e_{11} \tag{A.71} \\
& - (k_{z_{9}, e_{11}}^{+} + k_{\text{cat}, e_{11}}^{+}) [Z_{9} : E_{11}] \\
\end{align*}
\]

\[
\begin{align*}
\frac{d}{dt}[Z_{11} : E_{11}] & = k_{\text{flow}} ([Z_{11} : E_{11}]^{up} - [Z_{11} : E_{11}]) + k_{z_{11}, e_{11}}^{+} z_{11} e_{11} \tag{A.72} \\
& -(k_{z_{11}, e_{11}}^{-} + k_{\text{cat}, e_{11}}^{+}) [Z_{11} : E_{11}] \\
\end{align*}
\]
\[ \frac{d}{dt} e_{11}^h = k_{on,x}^h e_{11}^h p_{11} + k_{off}^h e_{11}^h \]
\[ -k_{on}^h e_{11}^h p_{11} + k_{off}^h e_{11}^h \]
\[ -k_{z_9,x_{11}}^h z_9 e_{11}^h + (k_{x_{11}^z,h,m_{11}}^z + k_{x_{11}^z,h,m}^z) [Z_9 : E_{11}^h] \]
\[ -k_{z_{11}^e_{11} : z_{11}^e_{11}}^h z_{11}^e_{11} + (k_{z_{11}^e_{11} : z_{11}^e_{11}}^z + 2 * k_{z_{11}^e_{11} : z_{11}^e_{11}}^z) [Z_{11} : E_{11}^h] \]
\[ + k_{cat}^z_{z_{11}^e_{11}} [Z_{11} : E_{11}^h] + k_{cat}^{z_{11}^e_{11} : z_{11}^e_{11}} [Z_{11} : E_{2}] \]
\[ -2 * k_{e_{11}^h : e_{11}^h}^z z_{11}^e_{11} \]
\[ + (2 * k_{e_{11}^h : e_{11}^h}^z + k_{cat}^{z_{11}^e_{11} : e_{11}^h}) [E_{11}^h : E_{11}^h] \]
\[ -k_{e_{11}^h : e_{11}^h}^z e_{11}^h + k_{e_{11}^h : e_{11}^h}^z [E_{11}^h : E_{11}^h] \]
\[ -k_{e_{11}^h : e_{11}^h}^z e_{11}^h [E_{11}^h : E_{2}] \]
\[ + k_{flow} (e_{11}^h u_{11}^h - e_{11}^h) \]
\[ -k_{AT}^h e_{11}^h [AT] \]
\[ -k_{AT}^h e_{11}^h [AT : Hep] \]
\[ \frac{d}{dt} e_{11}^{h,m} = k_{on,x}^h e_{11}^h p_{11} - k_{off}^h e_{11}^{h,m} \]
\[ -k_{z_9,x_{11}}^{z_{11}^e_{11}} z_{11}^e_{11} m_{11} e_{11}^{h,m} \]
\[ + (k_{x_{11}^z,h,m_{11}}^z + k_{x_{11}^z,h,m}^z) [Z_9 : E_{11}^{h,m}] \]
\[ -k_{z_{11}^e_{11} : z_{11}^e_{11}}^{z_{11}^e_{11}} z_{11}^e_{11} m_{11} e_{11}^{h,m} \]
\[ + (k_{z_{11}^e_{11} : z_{11}^e_{11}}^{z_{11}^e_{11}} + 2 * k_{z_{11}^e_{11} : z_{11}^e_{11}}^{z_{11}^e_{11}}) [Z_{11}^m : E_{11}^{h,m}] \]
\[ + k_{cat}^z_{z_{11}^e_{11} : z_{11}^e_{11}}^z [Z_{11}^m : E_{11}^{h,m}] + k_{cat}^{z_{11}^e_{11} : z_{11}^e_{11}}^{z_{11}^e_{11}} [Z_{11}^m : E_{2}^m] \]
\[ -k_{e_{11}^h : e_{11}^h}^{z_{11}^e_{11}} z_{11}^e_{11} m_{11} e_{11}^{h,m} \]
\[ + (k_{e_{11}^h : e_{11}^h}^{z_{11}^e_{11}} z_{11}^e_{11} m_{11} e_{11}^{h,m} + k_{cat}^{z_{11}^e_{11} : e_{11}^h}) [E_{11}^{h,m} : E_{11}^{h,m}] \]
\[ -k_{AT}^h e_{11}^{h,m} [AT] \]
\[ -k_{AT}^h e_{11}^{h,m} [AT : Hep] \]
\[ \frac{d}{dt} e_{11}^{h,m,s} = k_{on,x}^{h,m,s} e_{11}^{h,m,s} p_{11} - k_{off}^{h,m,s} e_{11}^{h,m,s} \]
\[ -k_{z_{11}^e_{11} : e_{11}^h}^{z_{11}^e_{11}} e_{11}^{h,m,s} + k_{e_{11}^h : e_{11}^h}^{z_{11}^e_{11}} e_{11}^{h,m,s} [E_{11}^{h,m,s} : E_{11}^{h,m,s}] \]
\[ -k_{z_{11}^e_{11} : e_{11}^h}^{z_{11}^e_{11}} e_{11}^{h,m,s} + k_{e_{11}^h : e_{11}^h}^{z_{11}^e_{11}} e_{11}^{h,m,s} [E_{11}^{h,m,s} : E_{11}^{h,m,s}] \]
\[ -k_{e_{11}^h : e_{11}^h}^{z_{11}^e_{11}} e_{11}^{h,m,s} m_{11} e_{11}^{h,m,s} + k_{e_{11}^h : e_{11}^h}^{z_{11}^e_{11}} e_{11}^{h,m,s} m_{11} e_{11}^{h,m,s} \]
\[ \frac{d}{dt} [Z_9 : E_{11}^h] = k_{flow} ([Z_9 : E_{11}^h] u_{11}^h - [Z_9 : E_{11}^h]) \]
\[ + k_{z_{11}^e_{11}} z_{11}^e_{11} h_{11} - (k_{z_{11}^e_{11}} + k_{z_{11}^e_{11}}^{z_{11}^e_{11}}) [Z_9 : E_{11}^h] \]
\[ \frac{d}{dt} [Z_{11} : E_{11}^{h,m}] = k_{z_{11}^e_{11}} z_{11}^e_{11} m_{11} e_{11}^{h,m} \]
\[ - (k_{z_{11}^e_{11}} + k_{z_{11}^e_{11}}^{z_{11}^e_{11}} m_{11} e_{11}^{h,m}) [Z_{11} : E_{11}^{h,m}] \]
\[
\frac{d}{dt}[Z_{11} : E_{11}^h] = k_{flow}([Z_{11} : E_{11}^h]_{up} - [Z_{11} : E_{11}^h]) \\
+ k_{z_{11} : e_{11}^h} z_{11} \cdot e_{11}^h \\
-k_{z_{11} : e_{11}^h} + k_{cat}\] [Z_{11} : E_{11}^h] \\
(A.78)
\]

\[
\frac{d}{dt}[E_{11}^h : E_{11}^h] = k_{flow}([E_{11}^h : E_{11}^h]_{up} - [E_{11}^h : E_{11}^h]) \\
+ k_{t_{11} : e_{11}^h} e_{11}^h \\
-k_{t_{11} : e_{11}^h} + k_{cat}\] [E_{11}^h : E_{11}^h] \\
(A.79)
\]

\[
\frac{d}{dt}[E_{11}^h : E_{11}] = k_{flow}([E_{11}^h : E_{11}]_{up} - [E_{11}^h : E_{11}]) \\
+ k_{e_{11} : e_{11}} e_{11}^h - (k_{e_{11} : e_{11}} + k_{cat} e_{11}^h) [E_{11}^h : E_{11}] \\
(A.80)
\]

\[
\frac{d}{dt}[E_{11}^h : E_{11}] = k_{flow}([E_{11}^h : E_{11}]_{up} - [E_{11}^h : E_{11}]) \\
+ k_{e_{11} : e_{11}} e_{11}^h - (k_{e_{11} : e_{11}} + k_{cat} e_{11}^h) [E_{11}^h : E_{11}] \\
(A.81)
\]

\[
\frac{d}{dt}[Z_{11}^m : E_{11}^{h,m}] = k_{z_{11}^m : e_{11}^{h,m}} z_{11}^m \cdot e_{11}^{h,m} \\
-k_{z_{11}^m : e_{11}^{h,m}} + k_{cat}\] [Z_{11}^m : E_{11}^{h,m}] \\
(A.82)
\]

\[
\frac{d}{dt}[Z_{11}^m : E_{11}^{m*}] = k_{z_{11}^m : e_{11}^{m*}} z_{11}^m \cdot e_{11}^{m*} \\
-k_{z_{11}^m : e_{11}^{m*}} + k_{cat}\] [Z_{11}^m : E_{11}^{m*}] \\
(A.83)
\]

\[
\frac{d}{dt}[E_{11}^{h,m} : E_{11}^h] = k_{e_{11} : e_{11}^h} e_{11}^h \\
-k_{e_{11} : e_{11}^h} + k_{cat}\] [E_{11}^{h,m} : E_{11}^h] \\
(A.84)
\]

\[
\frac{d}{dt}[E_{11}^{m*} : E_{11}^h] = k_{e_{11} : e_{11}^h} e_{11}^h \\
-k_{e_{11} : e_{11}^h} + k_{cat}\] [E_{11}^{m*} : E_{11}^h] \\
(A.85)
\]

\[
\frac{d}{dt}[E_{11}^{h,m} : E_{11}^{m*}] = k_{e_{11} : e_{11}^{m*}} e_{11}^h \\
-k_{e_{11} : e_{11}^{m*}} + k_{cat}\] [E_{11}^{h,m} : E_{11}^{m*}] \\
(A.86)
\]

\[
\frac{d}{dt}[E_{11}^{h,m} : E_{11}^{m*}] = k_{e_{11} : e_{11}^{m*}} e_{11}^h \\
-k_{e_{11} : e_{11}^{m*}} + k_{cat}\] [E_{11}^{h,m} : E_{11}^{m*}] \\
(A.87)
\]
\[
\frac{d}{dt} e^h_{c^5} = -k_{5om} e^h_{c^5} p_{avail} + k_{off} e^h_{c^5} + k_{f}(e^u_{c^5} - e^r_{c^5}) + (1 - f_5)e_{h5} - p
\]
\[
- k_{5t\epsilon_2} e^h_{c^5} + k_{5t\epsilon_2} [E^h_5 : E_2]
\]
\[
- k_{5t\epsilon_2} [APC : e^h_{c^5} + k_{5t\epsilon_2} [APC : E^h_5]
\]
\[
- k_{TFPI : c^5} e^h_{c^5} [TFPI : E^h_5]
\]
\[
+ k_{TFPI : c^5} [TFPI : E^h_5]
\]
\[
- k_{TFPI : e_{10} : c^5} [TFPI : E_{10}] e^h_{c^5}
\]
\[
+ k_{TFPI : c_{10} : e^h_{c^5}} [E_{10} : TFPI : E^h_5]
\]
\[
- k_{TFPI : e_{10} : c^5} [TFPI : E_{10} e^h_{c^5}]
\]
\[
+ k_{TFPI : e_{10} : c^5} [E_{10} : TFPI : E^h_5]
\]
\[
- k_{c_{10} : c^5} [C : TFPI] e^h_{c^5} + k_{c_{10} : c^5} [C : TFPI : E^h_5]
\]
\[
\frac{d}{dt} PRO^h = + k_{e_{c^5} c_{10} : e_{10}} e^h_{c^5} m_{e_{c^5} c_{10}} - k_{e_{c^5} c_{10} : e_{10}} PRO^h
\]
\[
- k_{Z_{c^5} : PRO^h} PRO^h Z_{c^5} + k_{Z_{c^5} : PRO^h} [Z_{c^5} : PRO^h]
\]
\[
+ k_{Z_{c^5} : PRO^h} [Z_{c^5} : PRO^h]
\]
\[
- k_{TFPI : PRO^h : e_{10}} PRO^h TFPI + k_{TFPI : PRO^h : e_{10}} [TFPI : PRO^h : e_{10}]
\]
\[
- k_{TFPI : PRO^h : e_{10}} [TFPI : PRO^h : e_{10}]
\]
\[
+ k_{TFPI : PRO^h : e_{10}} [TFPI : PRO^h : e_{10}]
\]
\[
+ k_{TFPI : PRO^h : e_{10}} [PRO^h : E^h_5]
\]
\[
- k_{TFPI : PRO^h : e_{10}} [PRO^h : E^h_5]
\]
\[
- k_{c_{10} : PRO^h} [C : TFPI] PRO^h + k_{c_{10} : PRO^h} [C : TFPI : PRO^h]
\]
\[
\frac{d}{dt} [Z_{c^5} : PRO^h] = + k_{Z_{c^5} : PRO^h} PRO^h Z_{c^5} - k_{Z_{c^5} : PRO^h} [Z_{c^5} : PRO^h]
\]
\[
+ k_{Z_{c^5} : PRO^h} [Z_{c^5} : PRO^h]
\]
\[
\frac{d}{dt} [E_{c^5 : E^h_5}] = + k_{e_{c^5} e_{c^5} : e_{10}} e^h_{c^5} - k_{e_{c^5} e_{c^5} : e_{10}} [E_{c^5 : E^h_5} : E^h_5]
\]
\[
- k_{e_{c^5} e_{c^5} : e_{10}} [E_{c^5 : E^h_5} : E^h_5]
\]
\[
\frac{d}{dt} [E_{c^5 : E^h_5}] = + k_{e_{c^5} e_{c^5} : e_{10}} e^h_{c^5} - k_{e_{c^5} e_{c^5} : e_{10}} [E_{c^5 : E^h_5} : E^h_5]
\]
\[
- k_{e_{c^5} e_{c^5} : e_{10}} [E_{c^5 : E^h_5} : E^h_5]
\]
\[
\frac{d}{dt} [TFPI : E^h_5] = + k_{TFPI : e_{10} : e_{c^5} : e_{10} : E^h_5} [TFPI : E^h_5] e^h_{c^5}
\]
\[
- k_{TFPI : e_{10} : e_{c^5} : e_{10} : E^h_5} [TFPI : E^h_5] e^h_{c^5}
\]
\[
+ k_{TFPI : e_{10} : e_{c^5} : e_{10} : E^h_5} [TFPI : PRO^h : e_{10}]
\]
\[
+ k_{TFPI : e_{10} : e_{c^5} : e_{10} : E^h_5} [TFPI : PRO^h : e_{10}]
\]
\[
- k_{c_{10} : c^5} [C : TFPI] PRO^h + k_{c_{10} : c^5} [C : TFPI : PRO^h]
\]
\[
\frac{d}{dt}[APC : E_{5}^{hm}] = +k_{e_{5}^{hm}:APC}^{+}E_{5}^{hm}APC - k_{e_{5}^{hm}:APC}^{-}[APC : E_{5}^{hm}] + k_{e_{5}^{hm}:APC}^{+}APC[APC : E_{5}^{hm}] \tag{A.94}
\]

\[
\frac{d}{dt}[APC : E_{5}^{h}] = +k_{e_{5}^{h}:APC}^{+}APC - k_{e_{5}^{h}:APC}^{-}[APC : E_{5}^{h}] - k_{e_{5}^{h}:APC}^{+}APC[APC : E_{5}^{h}] + k_{flow}([APC : E_{5}^{h}]_{up} - [APC : E_{5}^{h}]) \tag{A.95}
\]

\[
\frac{d}{dt}[TFPI : E_{5}^{h}] = +k_{TFPI:e_{5}^{h}:APC}^{+}TFPI - k_{kTFPI:e_{5}^{h}}^{-}[TFPI : E_{5}^{h}] + k_{flow}([TFPI : E_{5}^{h}]_{up} - [TFPI : E_{5}^{h}]) - k_{TFPI:e_{5}^{h}:e_{10}}^{-}[TFPI : E_{5}^{h}] + k_{TFPI:e_{5}^{h}:e_{10}}^{+}E_{10} : TFPI : E_{5}^{h} + k_{\text{on}}^5[TFPI : E_{5}^{h}]_{\text{on}}^5 + k_{\text{off}}^5[TFPI : E_{5}^{hm}] - k_{C:e_{5}^{h}}^{+}C[TFPI : E_{5}^{h}] + k_{C:e_{5}^{h}}^{-}C : TFPI : E_{5}^{h} \tag{A.96}
\]

\[
\frac{d}{dt}[TFPI : E_{10}^{m}] = +k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{+}TFPI - k_{kTFPI:e_{10}^{m}}^{-}[TFPI : E_{10}^{m}] - k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{-}[TFPI : E_{10}^{m}] + k_{\text{on}}^{10}[TFPI : E_{10}^{m}]_{\text{on}}^{10} + k_{\text{off}}^{10}[TFPI : E_{10}^{m}] - k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{+}[TFPI : E_{10}^{m}] + k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{-}[TFPI : PRO_{h}]_{10}^{10} - k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{-}[TFPI : PRO_{h}] + k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{+}[TFPI : E_{10}^{m}]_{10}^{m} \tag{A.97}
\]

\[
\frac{d}{dt}[TFPI : PRO_{h}]_{10}^{10} = +k_{TFPI:PRO_{h}:v_{10}}^{+}PRO_{h}TFPI - k_{TFPI:PRO_{h}:v_{10}}^{-}[TFPI : PRO_{h}]_{10}^{10} + k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{+}[TFPI : E_{10}^{p}] [TFPI : E_{10}^{m}]_{10}^{m} + k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{-}[TFPI : PRO_{h}]_{10}^{10} \tag{A.98}
\]

\[
\frac{d}{dt}[TFPI : PRO_{h}]_{e_{5}}^{e_{5}} = +k_{TFPI:PRO_{h}:v_{5}}^{+}PRO_{h}TFPI - k_{TFPI:PRO_{h}:v_{5}}^{-}[TFPI : PRO_{h}]_{e_{5}}^{e_{5}} + k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{+}[TFPI : E_{5}^{hm}]_{e_{10}}^{m} - k_{TFPI:e_{10}^{m}:e_{10}^{m}}^{-}[TFPI : PRO_{h}]_{e_{5}}^{e_{5}} - k_{C:proh}^{+}[TFPI : PRO_{h}]_{e_{5}}^{e_{5}} + k_{C:proh}^{-}[C : TFPI : PRO_{h}] \tag{A.99}
\]
\[
\frac{d}{dt}[E_0 \cdot TFPI : E_5^{hm}] = +k_{TFPI,e_10,e_5}^+ [TFPI : E_0^{hm}]e_5^{hm} \\
- k_{TFPI,e_10,e_5}^- [E_0^{hm}] [E_0^{hm}] \\
+ k_{TPFPI,e_5^{hm},e_10}^+ [TFPI : E_5^{hm}]e_10 \\
- k_{TPFPI,e_5^{hm},e_10}^- [E_0^{hm}] [E_0^{hm}] \\
+ k_{ont}^+ [E_0^{hm}] [P_{off}^{avail}] + k_{off}^{+} [C : TFPI : PRO^h]
\]

(A.100)

\[
\frac{d}{dt}[E_0 \cdot TFPI : E_5^h] = +k_{flow}([E_0^{hm}]_{up} - [E_0^{hm}]) \\
+ k_{TFPI,e_10,e_5}^+ [TFPI : E_0^{hm}]e_5^{hm} \\
- k_{TFPI,e_10,e_5}^- [E_0^{hm}] [E_0^{hm}] \\
+ k_{TPFPI,e_5^{hm},e_10}^+ [TFPI : E_5^{hm}]e_10 \\
- k_{TPFPI,e_5^{hm},e_10}^- [E_0^{hm}] [E_0^{hm}] \\
- k_{ont}^+ [E_0^{hm}] [P_{off}^{avail}] + k_{off}^{+} [C : TFPI : PRO^h]
\]

(A.101)

\[
\frac{d}{dt}[E_0 \cdot TFPI : E_5^{hm}] = +k_{ont}^+ [E_0^{hm}] [P_{off}^{avail}] \\
- k_{off}^{+} [E_0^{hm}] [P_{off}^{avail}] \\
- k_{ont}^+ [E_0^{hm}] [P_{off}^{avail}] \\
+ k_{off}^{+} [E_0^{hm}] [P_{off}^{avail}] \\
+ k_{TPFPI,e_5^{hm},e_10}^+ [TFPI : E_5^{hm}]e_10 \\
- k_{TPFPI,e_5^{hm},e_10}^- [E_0^{hm}] [E_0^{hm}] \\
- k_{off}^{+} [E_0^{hm}] [P_{off}^{avail}] \\
- k_{TPFPI,e_5^{hm},e_10}^- [E_0^{hm}] [E_0^{hm}]
\]

(A.102)

\[
\frac{d}{dt}[E_0 \cdot TFPI : E_5^h] = +k_{ont}^+ [E_0^{hm}] [P_{off}^{avail}] \\
- k_{off}^{+} [E_0^{hm}] [P_{off}^{avail}] \\
- k_{ont}^+ [E_0^{hm}] [P_{off}^{avail}] \\
+ k_{off}^{+} [E_0^{hm}] [P_{off}^{avail}] \\
+ k_{TPFPI,e_5^{hm},e_10}^+ [TFPI : E_5^{hm}]e_10 \\
- k_{TPFPI,e_5^{hm},e_10}^- [E_0^{hm}] [E_0^{hm}] \\
- k_{off}^{+} [E_0^{hm}] [P_{off}^{avail}] \\
- k_{TPFPI,e_5^{hm},e_10}^- [E_0^{hm}] [E_0^{hm}]
\]

(A.103)

\[
\frac{d}{dt}[PRO^h : E_2^m] = +k_{PRO^h,e_2}^+ [PRO^hE_2^m] - k_{PRO^h,e_2}^- [PRO^h : E_2^m] \\
- k_{cat}^+ [PRO^h : E_2^m]
\]

(A.104)
\[
\frac{d}{dt}[E_9 : AT] = -k_{o9} \rho_9^{\text{avai}}[E_9 : AT] \\
+ k_{o9}^{\text{off}}[E_9^m : AT] + k_{e9} e_9[AT] \\
+ k_{\text{flow}}([E_9 : AT]_{\text{up}} - [E_9 : AT]) \\
- k_{o9}^{\text{on}} \rho_{91}^{\text{avai}}[E_9 : AT] + k_{o9}^{\text{off}}[E_9^{m*} : AT] \\
\]

\[
\frac{d}{dt}[E_9^m : AT] = +k_{e9}^AT e_9[AT] \\
- k_{o9}^{\text{off}}[E_9^m : AT] + k_{o9}^{\text{on}} \rho_9^{\text{avai}}[E_9 : AT] \\
\]

\[
\frac{d}{dt}[E_9^{m*} : AT] = +k_{c9}^AT e_9^{m*}[AT] \\
- k_{o9}^{\text{off}}[E_9^{m*} : AT] + k_{o9}^{\text{on}} \rho_{91}^{\text{avai}}[E_9 : AT] \\
\]

\[
\frac{d}{dt}[E_{10} : AT] = +k_{e10}^AT e_{10}[AT] \\
+ k_{\text{flow}}([E_{10} : AT]_{\text{up}} - [E_{10} : AT]) \\
+ k_{10} [E_{10}^{m*} : AT] - k_{10}^{\text{on}} \rho_{10}^{\text{avai}}[E_{10} : AT] \\
\]

\[
\frac{d}{dt}[E_{10}^{m*} : AT] = +k_{c10}^AT e_{10}^{m*}[AT] \\
- k_{10}^{\text{off}}[E_{10}^{m*} : AT] + k_{10}^{\text{on}} \rho_{10}^{\text{avai}}[E_{10} : AT] \\
\]

\[
\frac{d}{dt}[E_2 : AT] = +k_{e2}^{A} e_2^{m*}[AT] \\
- k_{e2}^{A} e_2^{m}[AT] \\
+ k_{e2}^{A} e_2^{m*}[AT] - k_{e2}^{A} e_2^{m}[AT] \\
+ k_{\text{flow}}([E_2 : AT]_{\text{up}} - [E_2 : AT]) \\
\]

\[
\frac{d}{dt}[E_2^{m*} : AT] = +k_{e2}^{A} e_2^{m*}[AT] \\
- k_{e2}^{A} e_2^{m}[AT] \\
+ k_{e2}^{A} e_2^{m*}[AT] + k_{e2}^{A} e_2^{m}[AT] \\
\]

\[
\frac{d}{dt}[E_{11} : AT] = +k_{e11}^{A} e_{11}[AT] \\
- k_{e11}^{A} e_{11}^{m*}[AT] \\
+ k_{e11}^{A} e_{11}^{m}[AT] \\
+ k_{e11}^{A} e_{11}^{m*}[AT] - k_{e11}^{A} e_{11}^{m}[AT] \\
+ k_{e11}^{A} e_{11}^{m*}[AT] - k_{e11}^{A} e_{11}^{m}[AT] \\
+ k_{e11}^{A} e_{11}^{m*}[AT] + k_{e11}^{A} e_{11}^{m}[AT] \\
\]

\[
\frac{d}{dt}[AT : E_{11} : AT] = +k_{e11}^{A} [E_{11} : AT][AT] \\
\]

\[
\frac{d}{dt}[E_{11}^{m*} : AT] = +k_{e11}^{A} e_{11}^{m*}[AT] \\
- k_{11}^{A} e_{11}^{m*}[AT] + k_{11}^{A} e_{11}^{m}[AT] \\
\]

\[
\frac{d}{dt}[E_{11}^{m} : AT] = +k_{e11}^{A} e_{11}^{m}[AT] \\
+ k_{11}^{A} e_{11}^{m}[AT] - k_{11}^{A} e_{11}^{m*}[AT] \\
+ k_{11}^{A} e_{11}^{m}[AT] - k_{11}^{A} e_{11}^{m*}[AT] \\
\]

\[
\frac{d}{dt}[E_{11}^{h} : AT] = +k_{e11}^{A} e_{11}^{h}[AT] \\
- k_{11}^{A} e_{11}^{h}[AT] \\
- k_{11}^{A} e_{11}^{h}[AT] + k_{11}^{A} e_{11}^{h}[AT] \\
\]
\[
\frac{d}{dt}[AT] = -k^{AT}_{c_0} e_{9}[AT] - k^{AT}_{c_0} e_{0}^n [AT] + k^{AT}_{c_2} e_{3}^m [AT] - k^{AT}_{c_1} e_{10}[AT] + k^{AT}_{e_{10}} e_{10}^m [AT] - k^{AT}_{c_2} e_{2}[AT] - k^{AT}_{c_2} e_{2}^m [AT] - k^{AT}_{c_1} e_{11}[AT] - k^{AT}_{c_1} e_{11}^m [AT] - k^{AT}_{c_1} e_{11}^h [AT] - k^{AT}_{c_1} e_{11} [AT] + k_{flow} ([AT]_{up} - [AT]) - k^{[AT:Hep]}_{[AT:Hep]} [Hep][AT] + k^{[AT:Hep]}_{[AT:HeP]} [AT : Hep] \quad (A.117)
\]

\[
\frac{d}{dt}[HeP] = -k^{[AT:Hep]}_{[AT:Hep]} [HeP][AT] + k^{[AT:Hep]}_{[AT:HeP]} [AT : Hep] \quad (A.118)
\]

\[
\frac{d}{dt}[AT : Hep] = +k^{[AT:Hep]}_{[AT:Hep]} [HeP][AT] - k^{[AT:Hep]}_{[AT:HeP]} [AT : Hep] - k^{ATH}_{c_{10}} e_{10}[AT : Hep] - k^{ATH}_{e_{10}} e_{10}^m [AT : Hep] - k^{ATH}_{c_2} e_{2}[AT : Hep] - k^{ATH}_{c_2} e_{2}^m [AT : Hep] - k^{ATH}_{c_0} e_{9}[AT : Hep] - k^{ATH}_{c_0} e_{9}^m [AT : Hep] - k^{ATH}_{c_0} e_{9}^h [AT : Hep] - k^{ATH}_{c_1} e_{11}[AT : Hep] - k^{ATH}_{c_1} e_{11}^m [AT : Hep] - k^{ATH}_{c_1} e_{11}^h [AT : Hep] - k^{ATH}_{c_1} e_{11} [AT : Hep] - k^{ATH}_{c_1} e_{11} [AT : Hep] (A.119)
\]

\[
\frac{d}{dt}[E_{10} : ATH] = +k^{ATH}_{c_{10}} e_{10}[AT : Hep] + k_{flow} ([E_{10} : ATH]_{up} - [E_{10} : ATH]) - k^{ATH}_{e_{10}} p_{10} [E_{10} : ATH] + k^{ATH}_{e_{10}} p_{10}^m [E_{10} : ATH] \quad (A.120)
\]

\[
\frac{d}{dt}[E_{10}^m : ATH] = +k^{ATH}_{e_{10}} e_{10}^m [AT : Hep] + k^{ATH}_{e_{10}} e_{10}^m [AT : Hep] - k^{ATH}_{e_{2}} p_{2} [E_{2} : ATH] - k^{ATH}_{e_{2}} p_{2}^m [E_{2} : ATH] \quad (A.121)
\]

\[
\frac{d}{dt}[E_{2} : ATH] = +k^{ATH}_{c_2} e_{2}[AT : Hep] + k_{flow} ([E_{2} : ATH]_{up} - [E_{2} : ATH]) - k^{ATH}_{e_{2}} p_{2}^m [E_{2} : ATH] \quad (A.122)
\]

\[
\frac{d}{dt}[E_{2}^m : ATH] = +k^{ATH}_{e_{2}} e_{2}^m [AT : Hep] + k^{ATH}_{e_{2}} e_{2}^m [AT : Hep] \quad (A.123)
\]

\[
\frac{d}{dt}[E_{9} : ATH] = +k^{ATH}_{c_0} e_{9}[AT : Hep] + k_{flow} ([E_{9} : ATH]_{up} - [E_{9} : ATH]) - k^{ATH}_{e_{9}} p_{9} [E_{9} : ATH] + k^{ATH}_{e_{9}} p_{9}^m [E_{9} : ATH] - k^{ATH}_{e_{9}} p_{9}^h [E_{9} : ATH] + k^{ATH}_{e_{9}} p_{9}^h [E_{9} : ATH] \quad (A.124)
\]

\[
\frac{d}{dt}[E_{9}^m : ATH] = +k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] + k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] - k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] + k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] \quad (A.125)
\]

\[
\frac{d}{dt}[E_{9}^m : ATH] = +k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] + k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] - k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] + k^{ATH}_{e_{9}} e_{9}^m [AT : Hep] \quad (A.126)
\]
\[
\frac{d}{dt} [ATH : E_{11} : ATH] = +k_{c_{11}}^{ATH}[AT : Hep] [E_{11} : ATH] + k_{\text{flow}}([ATH : E_{11} : ATH]_{\text{up}} - [ATH : E_{11} : ATH])
\]
(A.127)

\[
\frac{d}{dt} [E_{11}^{\text{ms}} : ATH] = +k_{c_{11}}^{ATH}e_{11}^{\text{ms}}[AT : Hep] + k_{e_{11}}^{\text{on}}p_{111}^{\text{avail}}[E_{11} : ATH] - k_{e_{11}}^{\text{off}}[E_{11}^\text{ms} : ATH]
\]
(A.128)

\[
\frac{d}{dt} [E_{11}^h : ATH] = +k_{c_{11}}^{ATH}e_{11}^h[AT : Hep] + k_{\text{flow}}([E_{11}^h : ATH]_{\text{up}} - [E_{11}^h : ATH]) + k_{e_{11}}^{\text{off}}[E_{11}^h : ATH] - k_{e_{11}}^{\text{on}}p_{111}^{\text{avail}}[E_{11}^h : ATH]
\]
(A.129)

\[
\frac{d}{dt} [E_{11}^\text{hm} : ATH] = +k_{c_{11}}^{ATH}e_{11}^\text{hm}[AT : Hep] - k_{e_{11}}^{\text{off}}[E_{11}^\text{hm} : ATH] + k_{e_{11}}^{\text{on}}p_{111}^{\text{avail}}[E_{11}^h : ATH]
\]
(A.130)

\[
\frac{d}{dt} C = -k_+^{C : \text{TFPI}} C[\text{TFPI}] + k_+^{C : \text{TFPI}}[C : \text{TFPI}]
\]
(A.131)

\[
\frac{d}{dt} [C : \text{TFPI}] = +k_+^{C : \text{TFPI}} C[\text{TFPI}] - k_-^{C : \text{TFPI}}[C : \text{TFPI}]
\]
(A.132)

\[
\frac{d}{dt} [\text{TFPI} : C : \text{TFPI}] = +k_+^{\text{TFPI} : C : \text{TFPI}}[\text{TFPI}][C : \text{TFPI}] + k_+^{\text{TFPI} : C : \text{TFPI}}[C : \text{TFPI}][C : \text{TFPI}]
\]
(A.133)

\[
\frac{d}{dt} [C : \text{TFPI} : E_{5}^{h\text{m}}] = +k_+^{C : e_{5}^{h\text{m}}}[C : \text{TFPI} : E_{5}^{h\text{m}}] - k_-^{C : e_{5}^{h\text{m}}}[C : \text{TFPI} : E_{5}^{h\text{m}}]
\]
(A.134)

\[
\frac{d}{dt} [C : \text{TFPI} : E_{5}^{h\text{m}}] = +k_+^{C : e_{5}^{h\text{m}}}[C : \text{TFPI} : E_{5}^{h\text{m}}] - k_-^{C : e_{5}^{h\text{m}}}[C : \text{TFPI} : E_{5}^{h\text{m}}]
\]
(A.135)

\[
\frac{d}{dt} [C : \text{TFPI} : PRO^h] = +k_+^{C : \text{PRO}^h}[C : \text{TFPI} : PRO^h] - k_-^{C : \text{PRO}^h}[C : \text{TFPI} : PRO^h]
\]
(A.136)
## A.3 TABLES

### A.3.1 INITIAL PLASMA LEVELS.

Descriptions, notation and labels for each parameter associated with initial plasma levels are listed. The value of each parameter is found in the corresponding table listed above.

<table>
<thead>
<tr>
<th>Description</th>
<th>Notation</th>
<th>Label</th>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>$z_2$</td>
<td>$Z_2$</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Factor V</td>
<td>$z_5$</td>
<td>$Z_5$</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Factor VII</td>
<td>$z_7$</td>
<td>$Z_7$</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Factor VII</td>
<td>$z_8$</td>
<td>$Z_8$</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Factor IX</td>
<td>$z_9$</td>
<td>$Z_9$</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Factor X</td>
<td>$z_{10}$</td>
<td>$Z_{10}$</td>
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<tr>
<td>Factor XI</td>
<td>$z_{11}$</td>
<td>$Z_{11}$</td>
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<td>TFPI</td>
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<td>AT</td>
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<td>AT</td>
<td>A.3.9</td>
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<tr>
<td>Hep</td>
<td>[Hep]</td>
<td>Hep</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Concizumab</td>
<td>C</td>
<td>C</td>
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</tr>
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### A.3.2 PLATELET CHARACTERISTICS.

Descriptions, notation and labels for each parameter associated with platelet characteristics are listed. The value of each parameter is found in the corresponding table listed above.

<table>
<thead>
<tr>
<th>Description</th>
<th>Notation</th>
<th>Label</th>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>PL$^{up}$</td>
<td>PLup</td>
<td>A.3.9</td>
</tr>
<tr>
<td>Binding site number for II</td>
<td>$N_2$</td>
<td>N2</td>
<td>A.3.9</td>
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<tr>
<td>Binding site number for IIa</td>
<td>$N_2^*$</td>
<td>N2$^*$</td>
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</tr>
<tr>
<td>Binding site number for V/Vh/Va</td>
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<td>N5</td>
<td>A.3.9</td>
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<tr>
<td>Binding site number for VIII/VIIIa</td>
<td>$N_8$</td>
<td>N8</td>
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</tr>
<tr>
<td>Binding site number for IX</td>
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<td>Binding site number for IXa</td>
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<td>N9$^*$</td>
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<td>Binding site number for X/Xa</td>
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<td>Binding site number for XI</td>
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<td>Binding site number for Xla</td>
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<td>Rate of unactivated platelets adhering to SE</td>
<td>$k_{adh}^{ad}$</td>
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<tr>
<td>Rate of activated platelets adhering to SE</td>
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<tr>
<td>Rate of platelet activation by platelet in solution</td>
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<td>A.3.15</td>
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<tr>
<td>Rate of platelet activation on SE</td>
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<td>kact$_{plt}$</td>
<td>A.3.15</td>
</tr>
<tr>
<td>Rate of platelet activation by thrombin</td>
<td>$k_{e2}$</td>
<td>kact$_{e2}$</td>
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</table>
A.3.3 KINETIC RATE CONSTANTS.

Descriptions, notation and labels for each parameter associated with kinetic rate constants are listed. The value of each parameter is found in the corresponding table listed above.

<table>
<thead>
<tr>
<th>Description</th>
<th>Notation</th>
<th>Label</th>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates of activation of TF:VII by fX</td>
<td>$K_M$, $k_{cat}^{7:10}$, $k_{20:26}$</td>
<td>KZ7mE10M, KZ7mE10CAT, KZ7mE10MI</td>
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</tr>
<tr>
<td>Rates of activation of fX by TF:VIIa</td>
<td>$K_M$, $k_{cat}^{10:7}$, $k_{26:20}$</td>
<td>KZ10E7mM, KZ10E7mCAT, KZ10E7mMI</td>
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</tr>
<tr>
<td>Rates of activation of fIX by TF:VIIa</td>
<td>$K_M$, $k_{cat}^{7:10}$, $k_{20:26}$</td>
<td>KZ9E7mM, KZ9E7mCAT, KZ9E7mMI</td>
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</tr>
<tr>
<td>Rates of binding of fVII/fVIIa to TF</td>
<td>$k_{on}$, $k_{off}$</td>
<td>K7ON, K7OFF</td>
<td>A.3.11</td>
</tr>
<tr>
<td>Rates of activation of TF:VII by fXa</td>
<td>$K_M$, $k_{cat}^{7:10}$, $k_{20:26}$</td>
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<td>A.3.11</td>
</tr>
<tr>
<td>Rates of activation of TF:VII by fIIa</td>
<td>$K_M$, $k_{cat}^{7:10}$, $k_{20:26}$</td>
<td>KZ7E2M, KZ7E2CAT</td>
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</tr>
<tr>
<td>Rates of activation of fIX by fXIa-fXIa</td>
<td>$k_{+}^{9:11}$, $k_{cat}^{11:9}$, $k_{off}^{11:9}$</td>
<td>K9E11P, K9E11CAT</td>
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</tr>
<tr>
<td>Rates of binding of fXII/fXIIa to plt. surface</td>
<td>$k_{on}$, $k_{off}$, $k_{11}$</td>
<td>K10ON, K10OFF</td>
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<td>Rates of binding of fIX/fIXa to plt. surface</td>
<td>$k_{on}$, $k_{off}$</td>
<td>K5ON, K5OFF</td>
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<td>Rates of binding of fIXII/fIXIIa to plt. surface</td>
<td>$k_{on}$, $k_{off}$</td>
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<td>Rates of binding of fXI/fXIa to plt. surface</td>
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<td>Rates of binding of fII/fIIa to plt. surface</td>
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<tr>
<td>Rates of binding of fXIIf/fXIIfa to plt. surface</td>
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<td>K11ON, K11SON, K11OFF, K11SOFF</td>
<td>A.3.12</td>
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A.3.4 KINETIC RATE CONSTANTS.

Descriptions, notation and labels for each parameter associated with kinetic rate constants are listed. The value of each parameter is found in the corresponding table listed above.

<table>
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<th>Description</th>
<th>Notation</th>
<th>Label</th>
<th>Table</th>
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</thead>
<tbody>
<tr>
<td>Rates of activation of fV by fXa on plt. surface</td>
<td>$K_M$</td>
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<td>$k_{cat}$</td>
<td>KZ5mE10mCAT</td>
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</tr>
<tr>
<td></td>
<td>$k_0^e_{m10}$</td>
<td>KZ5mE10mMI</td>
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<td>Rates of activation of fV by fIIa on plt. surface</td>
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</tr>
<tr>
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<td>$k_{0}^e_{m2}$</td>
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<td>Rates of activation of fVIII by fXa on plt. surface</td>
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<tr>
<td></td>
<td>$k_0^e_{m10}$</td>
<td>KZ8mE10mMI</td>
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<tr>
<td>Rates of activation of fVIII by fIIa on plt. surface</td>
<td>$K_M$</td>
<td>KZ8mE2mM</td>
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<tr>
<td></td>
<td>$k_{cat}$</td>
<td>KZ8mE2mCAT</td>
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</tr>
<tr>
<td></td>
<td>$k_0^e_{m2}$</td>
<td>KZ8mE2mMI</td>
<td>A.3.13</td>
</tr>
<tr>
<td>Rates of activation of fX by TEN on plt. surface</td>
<td>$K_M$</td>
<td>KZ10mTENm</td>
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<td>$k_{cat}$</td>
<td>KZ10mTENCAT</td>
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<tr>
<td>Rates of activation of fII by PRO on plt. surface</td>
<td>$K_M$</td>
<td>KZ2mPROM</td>
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<td>KZ2mPROCAT</td>
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<tr>
<td>Rates of activation of fXI by fIIa on plt. surfaces</td>
<td>$k_0^e_{m11}$</td>
<td>KZ11mE2mP</td>
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<tr>
<td></td>
<td>$k_{cat}$</td>
<td>KZ11mE2mCAT</td>
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</tr>
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<td>$k_0^e_{m11}$</td>
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<tr>
<td>Rates of activation of fIX by fXIa-fXIa on plt. surface</td>
<td>$K_M$</td>
<td>KZ9mE11mP</td>
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<tr>
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<td>$k_{cat}$</td>
<td>KZ9mE11mCAT</td>
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</tr>
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<td>$k_0^e_{m11}$</td>
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<tr>
<td>Rates of formation of TEN on plt. surface</td>
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<tr>
<td>Rates of formation of PRO on plt. surface</td>
<td>$k_0^e_{m11}$</td>
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<td>KE5mE10mMI</td>
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</tr>
<tr>
<td>Rates of inhibition of fXa by TFPI</td>
<td>$k_{0}^e_{m10}$</td>
<td>KTFPIE10_P</td>
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<tr>
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<td>$k_{cat}$</td>
<td>KTFPIE10_M</td>
<td>A.3.14</td>
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<tr>
<td>Rates of inhibition of TF:VIIa by TFPIa</td>
<td>$k_{0}^e_{m10}$</td>
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<td>$k_{cat}$</td>
<td>KTFPIaE7m_M</td>
<td>A.3.14</td>
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<tr>
<td>Rates of inhibition of fVa by APC on plt. surface</td>
<td>$K_M$</td>
<td>KE5mAPCM</td>
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<td>$k_{cat}$</td>
<td>KE5mAPCCAT</td>
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<tr>
<td>Rates of inhibition of fVIIIa by APC on plt. surface</td>
<td>$K_M$</td>
<td>KE8mAPCM</td>
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<td>$k_{cat}$</td>
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<tr>
<td>Rates of inhibition of fIIa by TM on plt. surface</td>
<td>$k_{0}^e_{m10}$</td>
<td>KTMM</td>
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### A.3.5 NEW KINETIC RATE CONSTANTS ADDED IN TFPI EXTENSION.

Descriptions, notation and labels for each parameter associated with kinetic rate constants are listed.

The value of each parameter is found in the corresponding table listed above.

<table>
<thead>
<tr>
<th>Description</th>
<th>Notation</th>
<th>Label</th>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates of binding of fV-h by fXa on plt. surface</td>
<td>( k_{+}^{Xa})</td>
<td>KE5HME10MP</td>
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</tr>
<tr>
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<td>( k_{-}^{Xa})</td>
<td>KE5HME10MMI</td>
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<td>Rates of activation of II by PROh on plt. surface</td>
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<tr>
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<td>( k_{cat}^{II} )</td>
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<td>A.3.10</td>
</tr>
<tr>
<td></td>
<td>( k_{-}^{II} )</td>
<td>KE5HME2MMI</td>
<td>A.3.13</td>
</tr>
<tr>
<td>Rates of activation of fV-h by IIa on plt. surface</td>
<td>( K_{M}^{V} )</td>
<td>KE5HME2MM</td>
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</tr>
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<td>( k_{cat}^{V} )</td>
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<tr>
<td></td>
<td>( k_{-}^{V} )</td>
<td>KE5HME2MMI</td>
<td>A.3.13</td>
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<td>Rates of activation of fV-h by IIa in fluid</td>
<td>( K_{M}^{V} )</td>
<td>KE5HE2M</td>
<td>A.3.13</td>
</tr>
<tr>
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<td>( k_{cat}^{V} )</td>
<td>KE5HE2CAT</td>
<td>A.3.13</td>
</tr>
<tr>
<td></td>
<td>( k_{-}^{V} )</td>
<td>KE5HE2M</td>
<td>A.3.13</td>
</tr>
<tr>
<td>Rates of binding of fV-h by TFPI on plt. surface</td>
<td>( k_{+}^{TFPI} )</td>
<td>KTFPIE5HMP</td>
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</tr>
<tr>
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<td>( k_{-}^{TFPI} )</td>
<td>KTFPIE5HMI</td>
<td>A.3.14</td>
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<tr>
<td>Rates of binding of fV-h by TFPI in fluid</td>
<td>( k_{+}^{TFPI} )</td>
<td>KTFPIE5HP</td>
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<td>( k_{-}^{TFPI} )</td>
<td>KTFPIE5HMI</td>
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</tr>
<tr>
<td>Rates of binding of fXa by TFPI on plt. surface</td>
<td>( k_{+}^{TFPI} )</td>
<td>KTFPI_E10M_P</td>
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</tr>
<tr>
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<td>( k_{-}^{TFPI} )</td>
<td>KTFPI_E10M_MI</td>
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</tr>
<tr>
<td>Rates of binding of PROh by TFPI on plt. surface by binding fXa</td>
<td>( k_{+}^{TFPI} )</td>
<td>KTFPIPRoHV10P</td>
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<tr>
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<td>( k_{-}^{TFPI} )</td>
<td>KTFPIPRoHV10M</td>
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<tr>
<td>Rates of binding of PROh by TFPI on plt. surface by binding fV-h</td>
<td>( k_{+}^{TFPI} )</td>
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<tr>
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<td>( k_{-}^{TFPI} )</td>
<td>KTFPIPRoHV5MI</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of fV-h by APC on plt. surface</td>
<td>( K_{M}^{V} )</td>
<td>KE5HMAPCM</td>
<td>A.3.10</td>
</tr>
<tr>
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<td>( k_{cat}^{V} )</td>
<td>KE5HMAPCCAT</td>
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</tr>
<tr>
<td></td>
<td>( k_{-}^{V} )</td>
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<tr>
<td>Rates of inactivation of fV-h by APC in fluid</td>
<td>( K_{M}^{V} )</td>
<td>KE5HAPCM</td>
<td>A.3.10</td>
</tr>
<tr>
<td></td>
<td>( k_{cat}^{V} )</td>
<td>KE5HAPCCAT</td>
<td>A.3.14</td>
</tr>
<tr>
<td></td>
<td>( k_{-}^{V} )</td>
<td>KE5HAPCMI</td>
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A.3.6 NEW KINETIC RATE CONSTANTS ADDED IN AT EXTENSION.

Descriptions, notation and labels for each parameter associated with kinetic rate constants are listed. The value of each parameter is found in the corresponding table listed above.

<table>
<thead>
<tr>
<th>Description</th>
<th>Notation</th>
<th>Label</th>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates of inactivation of fIXa by AT on plt. surface</td>
<td>$k_{e9}$</td>
<td>KE9MATIII</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of fXa by AT on plt. surface</td>
<td>$k_{e10}$</td>
<td>KE10MATIII</td>
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</tr>
<tr>
<td>Rates of inactivation of IIa by AT on plt. surface</td>
<td>$k_{e2}$</td>
<td>KE2MATIII</td>
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<tr>
<td>Rates of inactivation of fXIIIa by AT on plt. surface</td>
<td>$k_{e11}$</td>
<td>KE11MATIII</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of fIXa by AT in fluid</td>
<td>$k_{e9}$</td>
<td>KE9ATIII</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of fXa by AT in fluid</td>
<td>$k_{e10}$</td>
<td>KE10ATIII</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of IIa by AT in fluid</td>
<td>$k_{e2}$</td>
<td>KE2ATIII</td>
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</tr>
<tr>
<td>Rates of inactivation of fXIIIa by AT in fluid</td>
<td>$k_{e11}$</td>
<td>KE11ATIII</td>
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</tr>
<tr>
<td>Rates of binding of AT by Heparin on plt. surface</td>
<td>$k_{AT:Hep}^{+}$</td>
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<tr>
<td>Rates of binding of AT by Heparin on plt. surface</td>
<td>$k_{AT:Hep}^{-}$</td>
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<tr>
<td>Rates of inactivation of fIXa by ATH on plt. surface</td>
<td>$k_{e9}$</td>
<td>KE9MATH</td>
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</tr>
<tr>
<td>Rates of inactivation of fXa by ATH on plt. surface</td>
<td>$k_{e10}$</td>
<td>KE10MATH</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of IIa by ATH on plt. surface</td>
<td>$k_{e2}$</td>
<td>KE2MATH</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of fXIIIa by ATH on plt. surface</td>
<td>$k_{e11}$</td>
<td>KE11MATH</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of inactivation of fIXa by ATH in fluid</td>
<td>$k_{e9}$</td>
<td>KE9ATH</td>
<td>A.3.14</td>
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<tr>
<td>Rates of inactivation of fXa by ATH in fluid</td>
<td>$k_{e10}$</td>
<td>KE10ATH</td>
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<tr>
<td>Rates of inactivation of IIa by ATH in fluid</td>
<td>$k_{e2}$</td>
<td>KE2ATH</td>
<td>A.3.14</td>
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<tr>
<td>Rates of inactivation of fXIIIa by ATH in fluid</td>
<td>$k_{e11}$</td>
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## A.3.7 NEW KINETIC RATE CONSTANTS ADDED IN CONCIZUMAB EXTENSION.

Descriptions, notation and labels for each parameter associated with kinetic rate constants are listed. The value of each parameter is found in the corresponding table listed above.

<table>
<thead>
<tr>
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<th>Label</th>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates of binding of concizumab to TFPI</td>
<td>$k^+_{CTFPPI}$</td>
<td>KCCMBTFPIP</td>
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</tr>
<tr>
<td></td>
<td>$k^-_{CTFPPI}$</td>
<td>KCCMBTFPIIMI</td>
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</tr>
<tr>
<td>Rates of binding of concizumab:TFPI complex to TFPI</td>
<td>$k^+_{TFPI:C:TFPI}$</td>
<td>KTFPICTFPPIP</td>
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<tr>
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<td>$k^-_{TFPI:C:TFPI}$</td>
<td>KTFPICTFPPIIMI</td>
<td>A.3.14</td>
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<tr>
<td>Rates of binding of concizumab:TFPI complex to fV-h</td>
<td>$k^+_{ct:e^h}$</td>
<td>KCTFPIBE5HP</td>
<td>A.3.14</td>
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<tr>
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<td>$k^-_{ct:e^h}$</td>
<td>KCTFPIBE5HMI</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of binding of concizumab to TFPI:fV-h complex</td>
<td>$k^+_{C:te^h}$</td>
<td>KCBTFPIE5HP</td>
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</tr>
<tr>
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<td>$k^-_{C:te^h}$</td>
<td>KCBTFPIE5HMI</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of binding of concizumab:TFPI complex to fV-h on platelet</td>
<td>$k^+_{ct:e^hm}$</td>
<td>KCTFPIBE5HP</td>
<td>A.3.14</td>
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<tr>
<td></td>
<td>$k^-_{ct:e^hm}$</td>
<td>KCTFPIBE5HMI</td>
<td>A.3.14</td>
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<tr>
<td>Rates of binding of concizumab to TFPI:fV-h complex on platelet</td>
<td>$k^+_{C:te^hm}$</td>
<td>KCBTFPIE5HP</td>
<td>A.3.14</td>
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<td></td>
<td>$k^-_{C:te^hm}$</td>
<td>KCBTFPIE5HMI</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of binding of concizumab:TFPI complex to PROh</td>
<td>$k^+_{ct:PROh}$</td>
<td>KCTFP1BPROHP</td>
<td>A.3.14</td>
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<tr>
<td></td>
<td>$k^-_{ct:PROh}$</td>
<td>KCTFP1BPROHP</td>
<td>A.3.14</td>
</tr>
<tr>
<td>Rates of binding of concizumab to TFPI:PROh complex</td>
<td>$k^+_{C:tproh}$</td>
<td>KCBTFPIPROHP</td>
<td>A.3.14</td>
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<tr>
<td></td>
<td>$k^-_{C:tproh}$</td>
<td>KCBTFPIPROHMI</td>
<td>A.3.14</td>
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</table>
Kinetic and Physical Parameter Values:

A.3.8 DIFFUSION COEFFICIENTS FOR PLATELETS AND FLUID-PHASE CHEMICAL SPECIES

(a) From [153]. (b) From [154].

<table>
<thead>
<tr>
<th></th>
<th>Diffusion Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>$2.5 \times 10^{-7}$ cm$^2$/s</td>
</tr>
<tr>
<td>Proteins</td>
<td>$5 \times 10^{-7}$ cm$^2$/s</td>
</tr>
</tbody>
</table>

A.3.9 NORMAL CONCENTRATIONS AND SURFACE BINDING SITE NUMBERS

(a) From [155]. (b) From [156]. (c) [157] suggests that normal plasma concentration of fVIIa is about 1% of the normal fVII concentration. (d) From [158]. (e) (f) From [159]. (g) Estimated as described in the text of the Supplementary Information. (h) From [160]. (i) From [161]. (j) From [162]. (k) From [163]. (l) From [164, 165]. (m) Number of fV molecules released per activated platelet [166]. (n) Maximum concentration of platelets in a 2 µm high reaction zone assuming that 20 platelets can cover a 10µm-by-10µm injured surface [167]. (o) From [111]. (p) Refer to heparin dosage calculation in later section of supplemental material. (q) Varied based on 'steady state' model.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Value</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>1.4 µM</td>
<td>a</td>
</tr>
<tr>
<td>Factor V</td>
<td>0.01 µM</td>
<td>b</td>
</tr>
<tr>
<td>Factor VII</td>
<td>0.01 µM</td>
<td>a</td>
</tr>
<tr>
<td>Factor VIIa</td>
<td>0.1 nM</td>
<td>c</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>1.0 nM</td>
<td>a</td>
</tr>
<tr>
<td>Factor IX</td>
<td>0.09 µM</td>
<td>a</td>
</tr>
<tr>
<td>Factor X</td>
<td>0.17 µM</td>
<td>a</td>
</tr>
<tr>
<td>Factor XI</td>
<td>30.0 nM</td>
<td>a</td>
</tr>
<tr>
<td>TFPI</td>
<td>0.5 nM</td>
<td>d</td>
</tr>
<tr>
<td>Protein C</td>
<td>65 nM</td>
<td>e</td>
</tr>
<tr>
<td>Platelet count</td>
<td>$2.5(10)^5$/µl</td>
<td>f</td>
</tr>
<tr>
<td>$N_2$</td>
<td>1000/plt</td>
<td>g</td>
</tr>
<tr>
<td>$N_2^*$</td>
<td>1000/plt</td>
<td>g</td>
</tr>
<tr>
<td>$N_5$</td>
<td>3000/plt</td>
<td>h</td>
</tr>
<tr>
<td>$N_8$</td>
<td>450/plt</td>
<td>i</td>
</tr>
<tr>
<td>$N_9$</td>
<td>250/plt</td>
<td>j</td>
</tr>
<tr>
<td>$N_9^*$</td>
<td>250/plt</td>
<td>j</td>
</tr>
<tr>
<td>$N_{10}$</td>
<td>2700/plt</td>
<td>k</td>
</tr>
<tr>
<td>$N_{11}$</td>
<td>1500/plt</td>
<td>l</td>
</tr>
<tr>
<td>$N_{11}^*$</td>
<td>250/plt</td>
<td>l</td>
</tr>
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<td>$n_5$</td>
<td>3000/plt</td>
<td>m</td>
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<td>$pPLAS$</td>
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<td>n</td>
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<td>o</td>
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<td>LMWH</td>
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<tr>
<td>Concizumab</td>
<td>Varied</td>
<td>q</td>
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</table>
A.3.10 REACTIONS ON SUBENDOTHELIUM

(a) $k_{z_{10}^{7}}^{\text{cat}} = 5.0 \text{ sec}^{-1}$ and $K_M = 1.2 \cdot 10^{-6} \text{ M}$ [168]. (b) $k_{z_{2}^{7}}^{\text{cat}} = 6.1 \cdot 10^{-2} \text{ sec}^{-1}$ and $K_M = 2.7 \cdot 10^{-6} \text{ M}$ [168]. (d) $k_{z_{10}^{7}}^{\text{cat}} = 1.15 \text{ sec}^{-1}$ and $K_M = 4.5 \cdot 10^{-7} \text{ M}$ [155]. (e) $k_{z_{9}^{7}}^{\text{cat}} = 1.15 \text{ sec}^{-1}$ and $K_M = 2.4 \cdot 10^{-7} \text{ M}$ [169]. (e) $K_d = 1.0 \cdot 10^{-10} \text{ M}$ [170].

| Activation (of - by -) | (TF:VII,fIIa) $E_{10}^{m}, Z_{7}^{m}$ | $k_{z_{10}^{7}}^{\text{on}} = 5.0 \cdot 10^{7}$ | $k_{z_{10}^{7}}^{\text{off}} = 1.0$ | $k_{z_{10}^{7}}^{\text{cat}} = 5.0$ | a \\
| | (TF:VII, fIIa) $E_{2}^{m}, Z_{7}^{m}$ | $E_{2}^{m} k_{z_{10}^{7}}^{\text{on}} = 3.92 \cdot 10^{5}$ | $k_{z_{10}^{7}}^{\text{off}} = 1.0$ | $k_{z_{10}^{7}}^{\text{cat}} = 6.1 \cdot 10^{-2}$ | b \\
| | (IX, TF:VIIa) $E_{2}^{m}, Z_{10}^{m}$ | $E_{10}^{m} k_{z_{20}^{m}}^{\text{on}} = 5.0 \cdot 10^{6}$ | $k_{z_{20}^{m}}^{\text{off}} = 1.0$ | $k_{z_{20}^{m}}^{\text{cat}} = 1.15$ | c \\
| | (IX, TF:VIIa) $E_{2}^{m}, Z_{9}^{m}$ | $E_{9}^{m} k_{z_{20}^{m}}^{\text{on}} = 9.4 \cdot 10^{6}$ | $k_{z_{20}^{m}}^{\text{off}} = 1.0$ | $k_{z_{20}^{m}}^{\text{cat}} = 1.15$ | d \\
| Binding (of -, with -) | (VII, TF) $Z_{7}, TF$ | $Z_{7}^{m} k_{z_{7}^{m}}^{\text{on}} = 5.0 \cdot 10^{7}$ | $k_{z_{7}^{m}}^{\text{off}} = 5.0 \cdot 10^{-3}$ | e \\
| | (VIIa, TF) $E_{7}, TF$ | $E_{7}^{m} k_{z_{7}^{m}}^{\text{on}} = 5.0 \cdot 10^{7}$ | $k_{z_{7}^{m}}^{\text{off}} = 5.0 \cdot 10^{-3}$ | e |
A.3.11 REACTIONS IN THE PLASMA

(a) $k_{z7:e10}^{\text{cat}} = 5.0 \text{ sec}^{-1}$ and $K_M = 1.2 \cdot 10^{-6} \text{ M}$ [168]. (b) $k_{z7:e2}^{\text{cat}} = 6.1 \cdot 10^{-2} \text{ sec}^{-1}$ and $K_M = 2.7 \cdot 10^{-6} \text{ M}$ [168] (c) $k_{z8:e2}^{\text{cat}} = 0.23 \text{ sec}^{-1}$ and $K_M = 7.17 \cdot 10^{-8} \text{ M}$ [171]. (d) $k_{z8:e2}^{\text{cat}} = 0.9 \text{ sec}^{-1}$ [172] and $K_M = 2 \cdot 10^{-7} \text{ M}$ [173]. (e) $k_{z11:e2}^{\text{cat}} = 1.3 \cdot 10^{-4}$, $K_M = 50\text{nM}$ [174]. Rate constants apply also for thrombin-activation of Xla-XI. (f) $k_{z9:e11}^{h} = 0.21$, $K_M = 0.2 \mu\text{M}$ [175, 176]. Rate constants apply also for activation of IX by Xla-Xla.

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<th>Product</th>
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<th>$\text{sec}^{-1}$</th>
<th>$\text{sec}^{-1}$</th>
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<td>$E_7$</td>
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<tr>
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A.3.12 BINDING TO PLATELET SURFACES

(a) For fIX binding to platelets, \( K_d = 2.5 \cdot 10^{-9} \) M [162], and for fX binding to platelets, \( K_d \) has approximately the same value [160]. For fX binding to PCPS vesicles, the on-rate is about \( 10^7 \) M\(^{-1}\)sec\(^{-1}\) and the off-rate is about 1.0 sec\(^{-1}\) [177] giving a dissociation constant of about \( 10^{-7} \) M. To estimate on- and off-rates for the higher-affinity binding of fX to platelets, we keep the on-rate the same as for vesicles and adjust the off-rate to give the correct dissociation constant. The rates for fIX binding with platelets are taken to be the same as for fX binding. (b) We assume binding constants for fIXa binding to the specific fIXa binding sites are the same as for shared sites. (c) fV binds with high-affinity to phospholipids (PCPS) [177] and we use the same rate constants reported there to describe fV binding to platelets. (d) The \( K_d \) for fVIII binding with platelets is taken from [161]. We set the off-rate \( k_{2\text{off}} \) for fVIII binding to platelets equal to that for fV binding to platelets, and calculate the on-rate \( k_{2\text{on}} \). (e) For prothrombin interactions with platelets, \( K_d \) is reported to be \( 5.9 \cdot 10^{-7} \) M [178]. We choose \( k_{2\text{off}} \) and set \( k_{2\text{on}} = k_{2\text{off}} / K_d \). (f) Estimated as described in the text of the Supplementary Information. (g) \( K_d = 10 \) nM [179]. (h) \( K_d = 1.7 \) nM [165].

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<th>( \text{sec}^{-1} )</th>
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A.3.13 REACTIONS ON PLATELET SURFACES

(a) $k_{Z_2 \cdot X_{10}}^{cat} = 0.046 \text{ sec}^{-1}$ and $K_M = 10.4 \cdot 10^{-9} \text{ M} [180]$. (b) The rate constants for thrombin activation of fV on platelets are assumed to be the same as in plasma. (c) $k_{Z_2 \cdot X_{10}}^{cat} = 0.023 \text{ sec}^{-1}$ and $K_M = 2.0 \cdot 10^{-8} \text{ M} [173]$. (d) The rate constants for thrombin activation of fVIII on platelets are assumed to be the same as in plasma. (e) The formation of the tenase and prothrombinase complexes is assumed to be very fast with $K_d = 1.0 \cdot 10^{-10} \text{ M} [181]$. (f) $k_{Z_2 \cdot X_{10} \cdot \text{ten}}^{cat} = 20 \text{ sec}^{-1}$ and $K_M = 1.6 \cdot 10^{-7} \text{ M} [182]$. (g) $k_{Z_2 \cdot \text{pro}}^{cat} = 30 \text{ sec}^{-1}$ and $K_M = 3.0 \cdot 10^{-7} \text{ M} [183]$. (h) $k_{Z_2 \cdot X_{11} \cdot \text{pro}}^{cat} = 1.3 \cdot 10^{-4}$, $K_M = 50 \text{ nM} [174]$. Rate constants apply also for thrombin-activation of Plt-XIa-XI. (i) $k_{Z_2 \cdot X_{9} \cdot \text{pro}}^{cat} = 0.21$, $K_M = 0.2 \mu \text{M} [175, 176]$. Rate constants apply also for activation of platelet-bound IX by Plt-XIa-XIa.

<table>
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<th>Reactants</th>
<th>Complex</th>
<th>Product</th>
<th>$M^{-1}\text{sec}^{-1}$</th>
<th>$k_{\text{sec}^{-1}}$</th>
<th>$k_{\text{sec}^{-1}}$</th>
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A.3.14 INHIBITION REACTIONS

(a) From [111]. (b) From [117]. (c) For inhibition of fVa by APC, $k_{cat, APC}^{c_{h}} = 0.5 \text{ sec}^{-1}$ and $K_M = 12.5 \cdot 10^{-9}$ [184]. We assume the same reaction rates for the inhibition of fVIIIa by APC. (d) From [91]. (e) From [30]. (f) From [185]. (g) $K_d = 0.5 \text{ nM}$ and $[PC] = 65 \text{ nM}$ [186]. (h) From [113]. (i) From [63]. (j) $k_{PC:TM; c}^{ec} = 0.167 \text{ sec}^{-1}$, $K_M = 0.7 \cdot 10^{-6} \text{ M}$ [187].
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<td>$k_{PC,TM:E^{sec}_2}^{PC,TM} = 1.7 \cdot 10^6$</td>
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A.3.15 PLATELET TRANSITIONS

(a) Estimated from data in [188, 189] as described in [190]. (b) Estimated from data in [191] as described in [190]. SE=subendothelium.

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<th>Reactants</th>
<th>Reactants</th>
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<td>Platelet activation by thrombin</td>
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<td>$k_{act} = 0.50$</td>
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</table>
A.4 Conversion of Heparin Potency to Molar Concentration

Based on the recommended dosage of heparin treatment (0.3-0.7 U/ml) [125], we use the value of 0.5 U/ml. Based on the information from second international standard for heparin by WHO, the conversion factor will be 130 U/mg [192]. By using the mean molecular weight of heparin as 15kDa [193], we can get:

\[ 0.5 \text{ U/ml} \times \frac{1}{130} \text{ mg/U} = 0.0038 \text{ mg/ml} \]

\[ 0.0038 \text{ g/L} \times \frac{1}{15000} \text{ mol/g} = 2.53 \times 10^{-7} \text{ M} = 253 \text{ nM} \]

For the LMWH, since the molecular weight of LMWH varies based on different product [194], we use 5kDa as its molecular weight. Therefore, the concentration of heparin at 100% is determined by:

\[ 0.0038 \text{ g/L} \times \frac{1}{5000} \text{ mol/g} = 2.53 \times 10^{-7} \text{ M} = 759 \text{ nM} \]

Both concentrations were set as 100% baseline dosage concentration. For example, when we use 50% concentration of UFH and LMWH, we are using 126.5 nM and 379.5 nM respectively.

A.5 Local Sensitivity Analysis - Method

As in our previous work [61], we again focus on the sensitivity of three special thrombin metrics:

1. Lag time: A measure of how fast the system is turned on, defined as the amount of time required for thrombin to reach 1 nM.

2. Maximum relative rate: A measure of how fast thrombin is produced once the system is turned on, defined as

\[ \max_{t > 1 \text{ nM}} \left( \frac{d[\text{thrombin}]}{dt} / [\text{thrombin}] \right) \]

3. Final concentration: The thrombin concentration after 20 minutes of clotting activity.

We examine the sensitivity of these metrics to two types of parameter variations: (i) the plasma levels of seven zymogens and two inhibitors, and (ii) the values of 24 new kinetic parameters that are related to TFPI reactions. We used a derivative-based approach to quantify the sensitivity of each metric with respect to centered difference in the parameters in a range of values (50%, 75%, 100%, 125% and 150% for the plasma level parameters, and 90%, 95%, 100%, 105% and 110% for kinetic parameters). The standard values for each plasma level parameters were set to the initial conditions. Similar to our previous SA results [61], we found that each of the metrics behaved monotonically with respect to varying each plasma level from 50% to 150% of the standard values, as shown in Fig. (Figure A.2A-C) and the kinetic parameters from 90% to 110% as shown in Fig. (Figure A.3A-C). The min/max values of these monotonic curves shows the change in the thrombin metric due to the factor change. Clotting factor variations had a significant effect on all three thrombin metrics but the largest change in the thrombin metrics due to variations in the
kinetic parameters was less than 0.2% and therefore, we did not characterize the sensitivity of these parameters further. For the clotting factors and inhibitors, we quantified their sensitivity by the absolute difference they produced in each metric when considering their extremal values (i.e., 50% and 150%). For each metric, we ranked the parameters by considering their relative absolute difference. We define $x = (x_1, x_2, ..., x_p)$ to be the standard model parameter values and $m_i(x_{j,y\%})$ to be the values of the $i$-th metric when parameter $j$ is chosen to be $y\%$ of its standard value and all other parameters are chosen to be at their standard value. The local sensitivity of the $i$-th metric to the $j$-th parameter is then:

$$LS_j^i = \frac{|m_i(x_{j,150\%}) - m_i(x_{j,50\%})|}{\max_k(|m_i(x_{k,150\%}) - m_i(x_{k,50\%})|)}$$

Each sensitivity score, LS, is then a number between 0 and 1 and we use these values to rank the input sensitivities. In our results, we denote LS scores higher than 0.75 with solid black triangles, LS scores from 0.25 to 0.75 as gray triangles, and for LS scores lower than 0.25 we use open triangles. In addition, because the response of the system outputs was monotonic throughout the entire range, we show separately the change in each metric for the 50% increase with the triangle upward and a 50% decrease with a triangle facing downward. Then the $y$-value of the triangle corresponds to its result on the output.

The local SA results in Fig. (Figure A.2D-I) reveal the most influential clotting factors and inhibitors, when perturbed one at a time for each of the three thrombin metrics. Fig. (Figure A.2D) shows that FVIII and FX have the greatest effect on the lag time, where an increase in either FVIII or FX levels by 50% leads to an approximately 10% decrease in the lag time from baseline. This is seen with the solid black (LS scores above 0.75), upward-facing (increase in factor level) triangles, with $y$-value near -10% showing the decrease in lag time. Comparing with sensitivity results from our old model([61]), we see an increased sensitivity to TFPI, where a decrease/increase by 50% leads to about a 8% decrease/5% increase in the lag time from baseline, respectively, although the TFPI LS score still does not reach 0.75. Fig. (Figure A.2E) shows that variations in FVIII, FIX, and FX have the largest effect on the maximum relative rate of thrombin generation, and this metric still has low sensitivity to TFPI. These findings are the same as in our previous results and make sense since these factors influence the rate of formation of the tenase complex on platelets, which affects the amplification stage of coagulation, and the inhibitory effect by TFPI does not alter such amplification process. It also indicates that new TFPI inhibitory reactions does not have significant influence towards the rate at which thrombin is being made. Fig. (Figure A.2F) shows that the final concentration metric is sensitive only to prothrombin (FII) as was found previously([61]). The corresponding LS scores are shown in Fig. (Figure A.2H-I).

Fig.(Figure A.3) demonstrates the local SA results for each of kinetic parameters that are related to TFPI reactions. Forward and reverse rate for each of the reaction are varied by 10% and change in lag
time, maximum relative rate and final concentrations were observed. The results indicate that slight perturbation in reaction kinetics has minimal effect towards these three thrombin metrics, where none of the kinetic parameter caused more than 1% change from baseline in each cases. Such insensitiveness of the kinetic parameters, however, indicates the tolerance of the model towards the possible error in the kinetic parameters retrieved from experimental design.

A.6 Other Figures

Figure A.2 Local sensitivity analysis of clotting factor levels on thrombin metrics. The initial conditions of clotting factor and inhibitor levels were varied between 50% and 150% of their baseline values. Shown are (A,B,C) the amplitude change in lag time, maximum relative rate, and final thrombin concentration, (D,E,F) the percentage change in each of the metrics, and (G,H,I) the LS scores for each metric and for each species. Solid black triangles represent the species with LS score higher than 0.75, gray triangles for LS scores from 0.25 to 0.75, and open triangles for LS lower than 0.25. The arrow direction indicates if the variable was increased or decreased.
Figure A.3 Local sensitivity analysis of TFPI-related kinetic rates on thrombin metrics. The new kinetic parameters were varied between 90% and 110% of their baseline values. Shown are (A,B,C) the amplitude of the changes in the lag time, maximum relative rate and final thrombin concentration due to the kinetic parameter variations. The plus/minus sign indicates the association/dissociation rate, respectively. Lower case \( m \) represents the components that are bound to platelet surface. The forward slash shows which two components are interacting each other, while the "ter" and "term" indicates interactions that involve a ternary complex and whether the species is in plasma or bound to the platelet surface, respectively. For example: term/FXa (+) indicates the rate of association between the platelet-bound TFPI:FV-h complex and the fluid phase FXa to form the ternary complex FXa:TFPI:FV-h.

Figure A.4 Thrombin generation time courses under different TFPI levels (0 nM and 0.5 nM) plotted in linear scale. TF level is varied by 2.5 fmol/cm\(^2\) (A) and 10 fmol/cm\(^2\) (B). Shear rate is fixed at 100/s.
Figure A.5 FXa concentration in the presence of LMWH (A) or UFH (B), FIXa concentration in the presence of LMWH (C) or UFH (D), FX:TF:VIIa concentration in the presence of LMWH (E) or UFH (F), and FX:tenase concentration in the presence of LMWH (G) or UFH (H). The time course is obtained from simulations in which we turn off all the AT-mediated inactivation reactions and then allow inhibition of FXa, FIXa, FXIa and thrombin, individually and one by one. Each curve thus shows thrombin/tenase generation when there is either no or only one inactivation reaction that exists in the system. TF density was set to 15 fmol/cm² and shear rate was set to 100/s. Heparin concentration is fixed to 100% of the standard therapeutic concentration.
Figure A.6 Subendothelium-attached platelet count (PLAS) and platelet-attached-activated platelet count (PLAV) time course with varied LMWH treatment (A-C) or UFH treatment (D-F). TF level is fixed to 6 fmol/cm². We examined how heparin in the system might affect platelet deposition. We specifically looked at two types of platelets: those that are activated and bound to subendothelium (PLAS), and those that are activated and bound to deposited platelets (PLAV), and their sum. The platelets accumulate on the subendothelium (SE) and PLAV eventually plateaus due to the limited space at the SE, whereas platelets above the injury site will continue to grow. Increasing the heparin concentrations led to decreases in both platelet species through time. This is because by increasing amount of heparin, it can greatly reduce the thrombin in the reaction zone, which leads to reduced amount of platelet to be activated by thrombin. Such a reduction can cause a shift from platelet-bound platelet to subendothelium-bound platelet. The increase in subendothelium-bound platelet will physically cover up the surface, which can negatively influence the initiation phase of coagulation.
Figure A.7 Concentration time course of platelet surface bound FXa, FV-h, and their complexes with TFPI. TF level is varied by 2.5 fmol/cm$^2$ (A,C,E,G) and 10 fmol/cm$^2$ (B,D,F,H). Under each TF level, TFPI level is varied by 0.5 nM and 2.5 nM, and shear rate is varied by 100/s, 500/s and 1500/s.
Figure A.8 Instantaneous generation and removal of TAT (A,B), and accumulative concentration of TAT in plasma (C) and on the platelet membrane (D). TF level is fixed to 5 fmol/cm², and shear rate is fixed to 100/s.
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2. Inhibition of platelet-surface-bound proteins during coagulation under flow II: Antithrombin and heparin

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Best,

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