1 Materials and Methods

Title: Synergy Between Tissue Factor and Exogenous Factor XIa in Initiating Coagulation
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1.1 Experiments

Materials
Normal pooled plasma (NPP, VisuCon-F® Coag Screen N) and Factor XI affinity depleted plasma (FXI-DP) were obtained from Affinity Biologicals (Ancaster, Ontario, Canada). Apheresis platelets from normal donors collected using an MCS + LN 9000 (Haemonetics, Braintree, MA) in 1:10 of acid citrate dextrose (ACD) to blood ratio were obtained from the Department of Transfusion Medicine, National Institute of Health (Bethesda, Maryland, USA) as described in [1]. The fluorogenic substrate used in the thrombin generation test, Z-Gly-Gly-Arg-AMC, was from Bachem Americas (King of Prussia, Pennsylvania, USA). Phospholipid vesicles were obtained from Rossix (Molndal, Sweden). Lipidated human recombinant tissue factor (TF, Recombiplastin®) was from Instrumentation Laboratory (Bedford, Massachusetts, USA). Corn trypsin inhibitor (CTI) was from Haematologic Technologies (Essex Junction, Vermont, USA). Calcium chloride ($CaCl_2$) and prostaglandin E1 were from Sigma-Aldrich (St. Louis, USA). Trisodium citrate dehydrate was from Mallinckrodt Baker (Phillipsburg, New Jersey, USA). Sodium chloride was from Fisher Scientific (Fair Lawn, New Jersey, USA). Thrombin calibrator (Thrombinoscope brand) was from Stago US (Parsippany, New Jersey, USA). Activated Factor XI (FXIa) was from the National Institute for Biological Standards and Control (an international reference reagent for FXIa, NIBSC code 11/236, Potters Bar, United Kingdom).

Automated TG/FG assay
A dilution series of FXIa was prepared in Tris-BSA buffer using VIAFLO II electronic pipettes with the VIAFLO ASSIST robotic pipetting assistant (INTEGRA Biosciences, Hudson, NH, USA). The mixture of calcium chloride (10 mM final concentration), the fluorogenic substrate Z-Gly-Gly-Arg-AMC (800 μM final concentration), and Tris-BSA buffer (pH 7.4, Aniara, West Chester, Ohio, USA) was then added to FXIa wells, and these mixtures were then transferred onto a half-area 96-well flat bottom plate. Human plasma (50% vol/vol in the final reaction) was mixed with CTI (53-79 μg/ml final concentration). PGE1 (1 μM) was added to platelets to inhibit aggregation; these platelets were then centrifuged for 25 min at 1000 x g. The supernatant was pipetted off and the pellet was resuspended in an approximately equal volume (5 mL) of NPP + CTI as described above. This platelet-rich plasma (PRP) resuspension was shaken for at least 30 min at room temperature for the PGE1 inhibition to reverse, and platelets were counted by CELL-DYN 3700 (Abbott Laboratories, Abbott Park, Illinois, USA). NPP and PRP were mixed to give a platelet concentration of approximately 250,000 platelets per μL final concentration. Tris-BSA buffer or citrate-buffered saline (10 mM trisodium citrate, 150

mM sodium chloride, pH 7.4) was added to this plasma/platelet/CTI mixture as needed. In platelet-free experiments, Tris-BSA buffer and phospholipid vesicles (4 μM final concentration) were added to the plasma/CTI mixture. A dilution series of TF was prepared in Tris-BSA buffer using VIAFLO II electronic pipettes with the VIAFLO ASSIST pipetting assistant. This mixture was transferred to a 96-well round bottom plate; Thrombinoscope thrombin calibrator was also added in place of TF in two wells. The plasma/CTI/platelet or plasma/CTI/buffer/phospholipid mixture was then added to these wells. Clotting was initiated by transferring the plasma mixture onto a half-area 96-well flat bottom plate containing the FXIa/ calcium chloride/substrate mixture using a 96-channel pipettor (Hydra Liquid Handling System, Thermo Scientific, Pittsburgh, USA or VIAFLO 96, INTEGRA Biosciences) to ensure rapid and simultaneous recalcification in all wells of a microplate. The flat bottom plate, containing the activated reaction mixture, was transferred to an Infinite F500 (Tecan, Mnnedorf, Switzerland) or Synergy H4 (BioTek Instruments, Winooski, Vermont, USA) microplate reader regulated at 37°C. Fluoresence (360-380 nm excitation and 430-460 nm emission) and absorbance (499-492 nm) were recorded for at least two hours. To account for location-based microplate artifacts, each FXIa and TF condition was tested in two wells positioned symmetrically with respect of center of the microplate as described in [2].

TG/FG curve processing software
Data processing was performed using an OriginPro (OriginLab, Northampton, MA) software package described previously [3]; the package is available from us upon request). Thrombin activity was calculated from the fluorogenic substrate conversion rates using a plot of fluorescence vs. change in fluorescence (an internal calibrator approach) as described in [4]. Thrombin generation data in duplicate wells were analyzed independently.

1.2 Mathematical Model

The simulations performed for this paper were carried out using the mathematical model described in [5]. Here we summarize the model and its assumptions and a complete list of the reactions treated in the model and the values of the kinetic parameters associated with these reactions are given in Tables M-I - M-VIII below. More details on the model can be found in [5, 6, 7].

The mathematical model simulates the clotting response due to a small injury to a vessel wall. The response is monitored in a small reaction zone above a region where tissue factor in the subendothelium (SE) is exposed to flowing blood (see Fig. M-IB). Within the reaction zone, platelet and clotting factor concentrations are assumed to change due to transport in and out of the zone and due to their involvement in the coagulation reactions depicted in Fig. M-IC. Each species in the reaction zone is assumed to be uniformly distributed ('well-mixed') and is described by its concentration, whose dynamics are tracked through an ordinary differential equation. Adjacent to the reaction zone, in the direction perpendicular to the flow, is an endothelial zone (Fig. M-IC) with height equal to that of the reaction zone and width dependent on the flow shear rate and protein diffusion coefficients [7].

Thrombin can diffuse from the reaction zone into the endothelial zone, bind to thrombomodulin (TM), and produce activated protein C (APC), which may then diffuse into the reaction zone. Each species in the endothelial zone is also assumed to be well-mixed.

Platelets are either (i) unactivated, unattached, and so free to move with the fluid, or (ii) activated, bound to the SE or to other activated platelets, and therefore stationary. Platelet activation occurs by contact with the SE, by exposure to thrombin, or by contact with other APs. The last of these is used as a surrogate for activation by platelet-released ADP which we do not explicitly track in this model.

Protein species are characterized not only by their chemical identity but also by whether they are in the fluid, bound to the SE, or bound to an activated platelet surface (APS). Proteins bound to a surface are stationary whereas proteins in the plasma move with the fluid. During a transition from SE to APS, or vice versa, a protein is subjected to flow and thus might be carried downstream.

Our assumptions about protein interactions follow, and further discussion of them including citations to the literature can be found in [6]:

1. FVII and FVIIa can bind to TF in the SE. FXa can activate FVII in plasma and when FVII is bound to TF. FXa can bind to the TF:VII complex directly from plasma without having to first bind the SE.

2. FIX and FX can be activated by the TF:VIIa complex on the SE. They attach to TF:VIIa directly from plasma. FX can also be activated by the VIIIa:IXa ('tenase') complex on an APS.

3. FV and FVIII can be activated by thrombin in plasma and by thrombin and FXa on an APS.

4. FIX can be activated by FXIa in plasma and on an APS. FXI can be activated by thrombin in plasma and on an APS.

5. The chemical inhibitors that we include in the model are antithrombin (AT), APC, and TFPI. Since the concentration of AT is high in plasma, we assume it acts in a first order manner to inactivate plasma FIXa, FXa, FXIa, and thrombin. APC can bind to fluid-phase and platelet-bound FVa and FVIIIa to permanently inactivate them with second-order kinetics, but cannot bind to FVIIIa in a tenase complex or to FVa in a prothrombinase complex. APC is produced in the endothelial zone by a complex of thrombomodulin and thrombin. TFPI present in the plasma must first bind to FXa and then the complex TFPI:Xa must bind to the TF:VIIa complex to inhibit it.

6. The activity of the TF:VIIa complex decreases as platelet deposition on the injured tissue increases, *i.e.,* we assume that a platelet that adheres to the SE physically blocks the activity of the TF:VIIa complexes on the patch of SE to which the platelet has adhered.

We wrote a FORTRAN program to set up the system of differential equations, to set parameter values, and to run the simulation. This program uses well-accepted methods, as implemented in the software package DLSODE [8], to solve this system of differential equations. Graphical processing of

simulation results was done with MATLAB. A complete listing of the model's differential equations and of the base parameter values used in the simulations can be found in [5].

For each simulation, we specify the initial plasma concentrations of platelet and protein species, the rate constants for all reactions, the numbers of specific binding sites for coagulation factors on each APS, the dimensions of the injury, the flow velocity near the injured wall, the diffusion coefficients for all fluid-phase species, and the density of exposed TF. The outputs of the simulation are the concentration of every protein species in the reaction zone at each instant of time from initiation of the injury until the completion of the simulation, and the concentrations of platelets attached either directly to the SE or to other platelets.

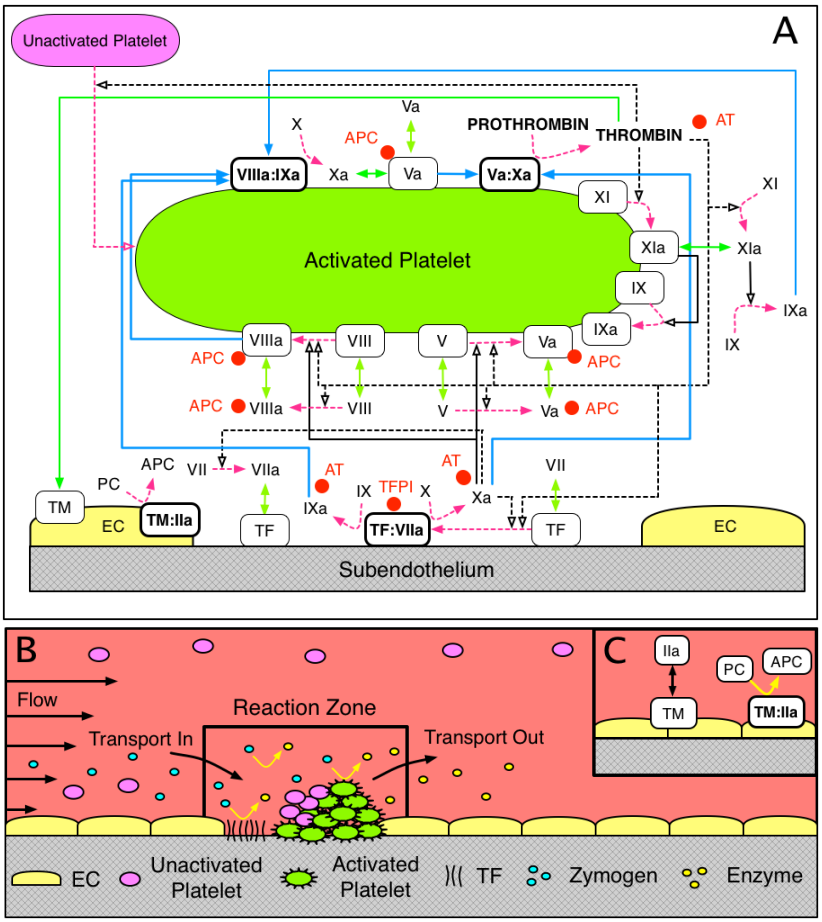

Figure M-I: Schematic of (A) coagulation reactions included in our model. Dashed magenta arrows show cellular or chemical activation processes. Blue arrows show chemical transport in the fluid or on a surface. Green segments with two arrowheads depict binding and unbinding from a surface. Rectangular boxes denote surface-bound species. Solid black lines with open arrows show enzyme action in a forward direction, while dashed black lines with open arrows show feedback action of enzymes. Red disks show chemical inhibitors. Schematic of (B) reaction zone and (C) endothelial zone.

Kinetic and Physical Parameters:

Platelets	2.5×10^{-7} cm^2/s	a
Proteins	5×10^{-7} cm^2/s	b

Table M-I: DIFFUSION COEFFICIENTS FOR PLATELETS AND FLUID-PHASE CHEMICAL SPECIES (a) From [9]. (b) From [10].

Prothrombin	1.4 μM	a
Factor V	0.01 μM	b
Factor VII	0.01 μM	a
Factor VIIa	0.1 nM	c
Factor VIII	1.0 nM	a
Factor IX	0.09 μM	a
Factor X	0.17 μM	a
Factor XI	30.0 nM	a
TFPI	2.5 nM	d
Protein C	65 nM	e
Platelet count	$2.5(10)^5/\mu$l	f
N_2	1000/plt	g
N_2^*	1000/plt	g
N_5	3000/plt	h
N_8	450/plt	i
N_9	250/plt	j
N_9^*	250/plt	j
N_{10}	2700/plt	k
N_{11}	1500/plt	l
N_{11}^*	250/plt	l
n_5	3000/plt	m
p_{PLAS}	0.167 nM	n

Table M-II: NORMAL CONCENTRATIONS AND SURFACE BINDING SITE NUMBERS (a) From [11]. (b) From [12]. (c) [13] suggests that normal plasma concentration of fVIIa is about 1% of the normal fVII concentration. (d) From [14]. (e) (f) From [15]. (g) Estimated as described in the text of the Supplementary Information. (h) From [16]. (i) From [17]. (j) From [18]. (k) From [19]. (l) From [20, 21]. (m) Number of fV molecules released per activated platelet [22]. (n) Maximum concentration of platelets in a 2 μm high reaction zone assuming that 20 platelets can cover a 10μm-by-10μm injured surface [23].

For the following tables, the basic reaction nomenclature used is:

Binding/Unbinding

$$\mathrm{S} + \mathrm{B} \underset{k^{\mathrm{off}}}{\overset{k^{\mathrm{on}}}{\rightleftharpoons}} \mathrm{S{:}B}$$

Enzymatic Activation

$$\mathrm{S} + \mathrm{E} \underset{k^{-}}{\overset{k^{+}}{\rightleftharpoons}} \mathrm{S{:}E} \xrightarrow{k^{\mathrm{cat}}} \mathrm{P} + \mathrm{E}.$$

Reaction	$M^{-1}sec^{-1}$	sec^{-1}	sec^{-1}	Note
Activation (of -, by -)				
(TF:FVII,FXa)	$k^{+}_{z_7^m:e_{10}}$=$5.0 \cdot 10^6$	$k^{-}_{z_7^m:e_{10}}$=1.0	$k^{\text{cat}}_{z_7^m:e_{10}}$=5.0	a
(TF:FVII, FIIa)	$k^{+}_{z_7^m:e_2}$=$3.92 \cdot 10^5$	$k^{-}_{z_7^m:e_2}$=1.0	$k^{\text{cat}}_{z_7^m:e_2}$=$6.1 \cdot 10^{-2}$	b
(FX, TF:FVIIa)	$k^{+}_{z_{10}:e_7^m}$=$5.0 \cdot 10^6$	$k^{-}_{z_{10}:e_7^m}$=1.0	$k^{\text{cat}}_{z_{10}:e_7^m}$=1.15	c
(FIX, TF:FVIIa)	$k^{+}_{z_9:e_7^m}$=$9.4 \cdot 10^6$	$k^{-}_{z_9:e_7^m}$=1.0	$k^{\text{cat}}_{z_9:e_7^m}$=1.15	d
Binding (of -, with -)				
(FVII, TF)	$k_7^{\text{on}} = 5.0 \cdot 10^7$	k_7^{off}=$5.0 \cdot 10^{-3}$		e
(FVIIa, TF)	k_7^{on}=$5.0 \cdot 10^7$	k_7^{off}=$5.0 \cdot 10^{-3}$		e

Table M-III: REACTIONS ON SUBENDOTHELIUM (a) $k^{\text{cat}}_{z_7^m:e_{10}} = 5.0$ sec^{-1} and $K_M = 1.2 \cdot 10^{-6}$ M [24]. (b) $k^{\text{cat}}_{z_7^m:e_2} = 6.1 \cdot 10^{-2}$ sec^{-1} and $K_M = 2.7 \cdot 10^{-6}$ M [24]. (d) $k^{\text{cat}}_{z_{10}:e_7^m} = 1.15$ sec^{-1} and $K_M = 4.5 \cdot 10^{-7}$ M [11]. (d) $k^{\text{cat}}_{z_9:e_7^m} = 1.15$ sec^{-1} and $K_M = 2.4 \cdot 10^{-7}$ M [25]. (e) $K_d = 1.0 \cdot 10^{-10}$ M [26].

Reaction	$M^{-1}sec^{-1}$	sec^{-1}	sec^{-1}	Note
Activation (of -, by -)				
(FVII, FXa)	$k^{+}_{z_7:e_{10}}$=5 $\cdot 10^6$	$k^{-}_{z_7:e_{10}}$=1.0	$k^{\rm cat}_{z_7:e_{10}}$=5.0	a
(FVII, FIIa)	$k^{+}_{z_7:e_2}$=3.92 $\cdot 10^5$	$k^{-}_{z_7:e_2}$=1.0	$k^{\rm cat}_{z_7:e_2}$=6.1 $\cdot 10^{-2}$	b
(FV, FIIa)	$k^{+}_{z_5:e_2}$= $1.73 \cdot 10^7$	$k^{-}_{z_5:e_2}$=1.0	$k^{\rm cat}_{z_5:e_2}$=0.23	c
(FVIII, FIIa)	$k^{+}_{z_8:e_2}$=$2.64 \cdot 10^7$	$k^{-}_{z_8:e_2}$=1.0	$k^{\rm cat}_{z_8:e_2}$=0.9	d
(FXI-FXI, FIIa)	$k^{+}_{z_{11}:e_2} = 2.0 \cdot 10^7$	$k^{-}_{z_{11}:e_2} = 1.0$	$k^{\rm cat}_{z_{11}:e_2} = 1.3 \cdot 10^{-4}$	e
(FIX, FXIa)	$k^{+}_{z_9:e^h_{11}} = 0.6 \cdot (10)^7$	$k^{-}_{z_9:e^h_{11}} = 1.0$	$k^{\rm cat}_{z_9:e^h_{11}} = 0.21$	f

Table M-IV: REACTIONS IN THE PLASMA (a) $k^{\rm cat}_{z_7:e_{10}} = 5.0$ sec^{-1} and $K_M = 1.2 \cdot 10^{-6}$ M [24]. (b) $k^{\rm cat}_{z_7:e_2} = 6.1 \cdot 10^{-2}$ sec^{-1} and $K_M = 2.7 \cdot 10^{-6}$ M [24] (c) $k^{\rm cat}_{z_5:e_2} = 0.23$ sec^{-1} and $K_M = 7.17 \cdot 10^{-8}$ M [27]. (d) $k^{\rm cat}_{z_8:e_2} = 0.9$ sec^{-1} [28] and $K_M = 2 \cdot 10^{-7}$ M [29]. (e) $k^{\rm cat}_{z_{11}:e_2} = 1.3 \cdot 10^{-4}$, $K_M = 50$nM [30]. Rate constants apply also for thrombin-activation of FXIa-FXI. (f) $k^{\rm cat}_{z_9:e^h_{11}} = 0.21$, $K_M = 0.2\mu$M [31, 32]. Rate constants apply also for activation of FIX by FXIa-FXIa.

Reaction	$M^{-1}sec^{-1}$	sec^{-1}	Note
Binding (of -, to -)			
(Factor IX,Plt)	k_9^{on}=$1.0 \cdot 10^7$	k_9^{off}=$2.5 \cdot 10^{-2}$	a
(Factor IXa,Plt)	k_9^{on}=$1.0 \cdot 10^7$	k_9^{off}=$2.5 \cdot 10^{-2}$	a
(Factor IXa,Plt)	k_9^{on}=$1.0 \cdot 10^7$	k_9^{off}=$2.5 \cdot 10^{-2}$	b
(Factor X,Plt)	k_{10}^{on}=$1.0 \cdot 10^7$	k_{10}^{off}=$2.5 \cdot 10^{-2}$	a
(Factor Xa,Plt)	k_{10}^{on}=$1.0 \cdot 10^7$	k_{10}^{off}=$2.5 \cdot 10^{-2}$	a
(Factor V,Plt)	k_5^{on}=$5.7 \cdot 10^7$	k_5^{off}=0.17	c
(Factor Va,Plt)	$k_5^{on} = 5.7 \cdot 10^7$	k_5^{off}=0.17	c
(Factor VII,Plt)I	$k_8^{on} = 5.0 \cdot 10^7$	k_8^{off}=0.17	d
(Factor VIIIa,Plt)	$k_8^{on} = 5.0 \cdot 10^7$	k_8^{off}=0.17	d
(Factor II,Plt)	$k_2^{on} = 1.0 \cdot 10^7$	k_2^{off}=5.9	e
(Factor IIa,Plt)	$k_2^{*,on} = 1.0 \cdot 10^7$	$k_2^{*,off} = 0.2$	f
(Factor XI,Plt)	$k_{z_{11}}^{on} = 1.0 \cdot 10^7$	$k_{z_{11}}^{off}$=0.1	g
(Factor XIa,Plt)	$k_{e_{11}}^{on} = 1.0 \cdot 10^7$	$k_{e_{11}}^{off}$=0.017	h

Table M-V: BINDING TO PLATELET SURFACES (a) For FIX binding to platelets, $K_d = 2.5 \cdot 10^{-9}$ M [18], and for FX binding to platelets, K_d has approximately the same value [16]. 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} [33] 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) [33] 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 [17]. We set the off-rate k_8^{off} for FVIII binding to platelets equal to that for FV binding to platelets, and calculate the on-rate k_8^{on}. (e) For prothrombin interactions with platelets, K_d is reported to be $5.9 \cdot 10^{-7}$ M [34]. We choose k_2^{off} and set $k_2^{on} = k_2^{off}/K_d$. (f) Estimated as described in [5] (g) $K_d = 10$ nM [35]. (h) $K_d = 1.7$ nM [21].

Reaction	$M^{-1}sec^{-1}$	sec^{-1}	sec^{-1}	Note
Activation (of -, by -)				
(FV, FXa)	$k^{+}_{z_5^m:e_{10}^m}$=$1.0 \cdot 10^8$	$k^{-}_{z_5^m:e_{10}^m}$=1.0	$k^{\mathrm{cat}}_{z_5^m:e_{10}^m}$=$4.6 \cdot 10^{-2}$	a
(V, IIa)	$k^{+}_{z_5^m:e_2^m}$=$1.73 \cdot 10^7$	$k^{-}_{z_5^m:e_2^m}$=1.0	$k^{\mathrm{cat}}_{z_5^m:e_2^m}$= 0.23	b
(FVIII, FXa)	$k^{+}_{z_8^m:e_{10}^m}$=$5.1 \cdot 10^7$	$k^{-}_{z_8^m:e_{10}^m}$=1.0	$k^{\mathrm{cat}}_{z_8^m:e_{10}^m}$=$2.3 \cdot 10^{-2}$	c
(FVIII, FIIa)	$k^{+}_{z_8^m:e_2^m}$=$2.64 \cdot 10^7$	$k^{-}_{z_8^m:e_2^m}$=1.0	$k^{\mathrm{cat}}_{z_8^m:e_2^m}$= 0.9	d
(FX, FVIIIa:FIXa)	$k^{+}_{z_{10}^m:ten}$=$1.31 \cdot 10^8$	$k^{-}_{z_{10}^m:ten}$=1.0	$k^{\mathrm{cat}}_{z_{10}^m:ten}$=20.0	f
(FX, FVIIIa:FIXa*)	$k^{+}_{z_{10}^m:ten}$=$1.31 \cdot 10^8$	$k^{-}_{z_{10}^m:ten}$=1.0	$k^{\mathrm{cat}}_{z_{10}^m:ten}$=20.0	f
(FII, FVa:FXa)	$k^{+}_{z_2^m:pro}$= $1.03 \cdot 10^8$	$k^{-}_{z_2^m:pro}$=1.0	$k^{\mathrm{cat}}_{z_2^m:pro}$=30.0	g
(FXI-FXI, FIIa)	$k^{+}_{z_{11}^m:e_2^m}$ = $2.0 \cdot 10^7$	$k^{-}_{z_{11}^m:e_2^m}$ = 1.0	$k^{\mathrm{cat}}_{z_{11}^m:e_2^m}$ = $1.3 \cdot 10^{-4}$	h
(FX, FXIa)	$k^{+}_{z_9^m:e_{11}^m}$ = $0.6 \cdot 10^7$	$k^{-}_{z_9^m:e_{11}^m}$ = 1.0	$k^{\mathrm{cat}}_{z_9^m:e_{11}^m}$ = 0.21	i
Binding (of -, with -)				
(FVIIIa, FIXa)	k^{+}_{ten}=$1.0 \cdot 10^8$	k^{-}_{ten}=0.01		e
(FVIIIa, FIXa*)	k^{+}_{ten}=$1.0 \cdot 10^8$	k^{-}_{ten}=0.01		e
(FVa, FXa)	k^{+}_{pro}=$1.0 \cdot 10^8$	k^{-}_{pro}=0.01		e

Table M-VI: REACTIONS ON PLATELET SURFACES (a) $k^{\mathrm{cat}}_{z_5^m:e_{10}^m} = 0.046\,\mathrm{sec}^{-1}$ and $K_M = 10.4{\cdot}10^{-9}$ M [36]. (b) The rate constants for thrombin activation of FV on platelets are assumed to be the same as in plasma. (c) $k^{\mathrm{cat}}_{z_8^m:e_{10}^m} = 0.023$ sec^{-1} and $K_M = 2.0 \cdot 10^{-8}$ M [29]. (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 $K_d = 1.0 \cdot 10^{-10}$ M [37]. (f) $k^{\mathrm{cat}}_{z_{10}^m:ten} = 20$ sec^{-1} and $K_M = 1.6 \cdot 10^{-7}$ M [38]. (g) $k^{\mathrm{cat}}_{z_2^m:pro} = 30$ sec^{-1} and $K_M = 3.0 \cdot 10^{-7}$ M [39]. (h) $k^{\mathrm{cat}}_{z_{11}^m:e_2^m} = 1.3 \cdot 10^{-4}$, $K_M = 50$ nM [30]. Rate constants apply also for thrombin-activation of Plt-FXIa-FXI. (i) $k^{\mathrm{cat}}_{z_9^m:e_{11}^{h,m}} = 0.21$, $K_M = 0.2\mu$M [31, 32]. Rate constants apply also for activation of platelet-bound FIX by Plt-FXIa-FXIa.

Reaction	$M^{-1}sec^{-1}$	sec^{-1}	sec^{-1}	Note
Inactivation (of -, by -)				
(FIXa, AT)		$k^{in}_{AT:e_9}$=0.1		a
(FXa, AT)		$k^{in}_{AT:e_{10}}$=0.1		a
(FIIa, AT)		$k^{in}_{AT:e_2}$=0.2		a
(FXIa, AT)		$k^{in}_{AT:e_{11}}$=0.2		a
(APC, FVa)	$k^{+}_{e^m_5:APC} = 1.2 \cdot 10^8$	$k^{-}_{e^m_5:APC} = 1.0$	$k^{\text{cat}}_{e^m_5:APC} = 0.5$	b
(APC, FVIIIa)	$k^{+}_{e^m_8:APC} = 1.2 \cdot 10^8$	$k^{-}_{e^m_8:APC} = 1.0$	$k^{\text{cat}}_{e^m_8:APC} = 0.5$	b
Binding (of -, with -)				
(TFPI, FXa)	$k^{+}_{tfpia:e_{10}}$=$1.6 \cdot 10^7$	$k^{-}_{tfpia:e_{10}}$=$3.3 \cdot 10^{-4}$		c
(TFPIa, TF:FVIIa)	$k^{+}_{tfpia:e^m_7}$=$1.0 \cdot 10^7$	$k^{-}_{tfpia:e^m_7}$=$1.1 \cdot 10^{-3}$		c
(TM, Thrombin)	$k^{\text{on}}_{TM} = 1.0 \cdot 10^8$	$k^{\text{off}}_{TM} = 5.0 \cdot 10^{-2}$		d
Activation (of -, by -)				
(PC, TM:Thrombin)	$k^{+}_{PC:TM:e^{ec}_2} = 1.7 \cdot 10^6$	$k^{-}_{PC:TM:e^{ec}_2} = 1.0$	$k^{\text{cat}}_{PC:TM:e^{ec}_2} = 0.16$	e

Table M-VII: INHIBITION REACTIONS (a) We estimate these parameters based on the half-lives of Factors FIXa, FXa, FIIa in plasma [40] and assume that the rate of FXIa inactivation is the same as that of FXa and thrombin. (b) For inhibition of FVa by APC, $k^{\text{cat}}_{e^m_5:APC} = 0.5$ sec^{-1} and $K_M = 12.5 \cdot 10^{-9}$ [41]. We assume the same reaction rates for the inhibition of FVIIIa by APC. (c) From [42]. (d) $K_d = 0.5$ nM and $[PC] = 65$ nM [43]. (e) $k_{PC:TM:e^{ec}_2} = 0.167$ sec^{-1} , $K_M = 0.7 \cdot 10^{-6}$ M [44].

Reactants	$M^{-1}sec^{-1}$	sec^{-1}	Note
Unactivated platelet adhering to SE	k^{+}_{adh}=2 $\cdot 10^{10}$	k^{-}_{adh}=0	a
Activated platelet adhering to SE	k^{+}_{adh}=2 $\cdot 10^{10}$	k^{-}_{adh}=0	a
Platelet activation by platelet in solution	$k^{\text{act}}_{plt} = 3 \cdot 10^{8}$		b
Platelet activation on SE	$k^{\text{act}}_{plt} = 3 \cdot 10^{8}$		b
Platelet activation by thrombin		$k^{\text{act}}_{e_2}$=0.50	b

Table M-VIII: PLATELET TRANSITIONS (a) Estimated from data in [45, 46] as described in [6]. (b) Estimated from data in [47] as described in [6]. SE=subendothelium.

References

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