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Supporting Material

Task-oriented modular decomposition of biological networks: trigger mechanism in blood coagulation

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Supporting Information

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1. Model Description

Notation

The following notation is used throughout the text. Concentration designations: x_j, concentration of the j-th active coagulation factor; y_j, concentration of the inactive precursor of the j-th coagulation factor; i_j, concentration of the j-th inhibitor; $x_{i\text{-}j}$, concentration of the complex of the i-th and j-th factors; x_j^F, concentration of the free j-th factor (not bound to another factor or phospholipid membrane); x_j^{BF}, concentration of the free j-th factor on the membrane (not in complex with another factor on the same membrane). Parameter designations: k_j, kinetic constant or forward rate constant of the j-th reaction; $k_{\text{-}j}$, backward rate constant of the j-th reaction; K_j, the Michaelis or equilibrium constant of the j-th reaction; n_j, number of membrane binding sites for the j-th factor; h_j, rate constant of the j-th inhibition reaction. Initial concentrations and rate constants are summarized in Table S1.

Model equations

1. Clotting initiation:

VIIa–TF: $$\frac{dx_{7\text{–}3}}{dt} = \left(k_1 \cdot x_7 \cdot x_3 - k_{\text{–}1} \cdot x_{7\text{–}3}{}^F\right) + k_2 \cdot y_{7\text{–}3} \cdot x_2{}^F + k_3 \cdot y_{7\text{–}3} \cdot x_{10}{}^F - h_1 \cdot x_{7\text{–}3}{}^F \cdot i_3 - h_2 \cdot x_{10\text{–}7\text{–}3} \cdot i_2 \quad \text{(S1)}$$

VII–TF: $$\frac{dy_{7\text{–}3}}{dt} = \left(k_1 \cdot y_7 \cdot x_3 - k_{\text{–}1} \cdot y_{7\text{–}3}\right) - k_2 \cdot y_{7\text{–}3} \cdot x_2{}^F - k_3 \cdot y_{7\text{–}3} \cdot x_{10}{}^F \quad \text{(S2)}$$

TF: $$\frac{dx_3}{dt} = -\left(k_1 \cdot x_7 \cdot x_3 - k_{\text{–}1} \cdot x_{7\text{–}3}{}^F\right) - \left(k_1 \cdot y_7 \cdot x_3 - k_{\text{–}1} \cdot y_{7\text{–}3}\right) \quad \text{(S3)}$$

VIIa: $$\frac{dx_7}{dt} = -\left(k_1 \cdot x_7 \cdot x_3 - k_{\text{–}1} \cdot x_{7\text{–}3}{}^F\right) + k_2 \cdot y_7 \cdot x_2{}^F \quad \text{(S4)}$$

VII: $$\frac{dy_7}{dt} = -\left(k_1 \cdot y_7 \cdot x_3 - k_{\text{–}1} \cdot y_{7\text{–}3}\right) - k_2 \cdot y_7 \cdot x_2{}^F \quad \text{(S5)}$$

2. The cascade backbone:

IXa: $$\frac{dx_9}{dt} = \frac{k_4}{K_4} \cdot y_9 \cdot x_{7\text{–}3}{}^F + \frac{k_5 \cdot y_9 \cdot x_{11}}{K_5 + y_9} - h_3 \cdot i_1 \cdot x_9 \quad \text{(S6)}$$

IX: $$\frac{dy_9}{dt} = -\frac{k_4}{K_4} \cdot y_9 \cdot x_{7\text{–}3}{}^F - \frac{k_5 \cdot y_9 \cdot x_{11}}{K_5 + y_9} \quad \text{(S7)}$$

Xa: $$\frac{dx_{10}}{dt} = \frac{k_6}{K_6} \cdot y_{10} \cdot x_{7\text{–}3}{}^F + \frac{k_7 \cdot x_9{}^{BF} \cdot y_{10}{}^B}{p \cdot K_7} + \frac{k_8 \cdot x_9{}^{BF} \cdot x_8{}^{BF} \cdot y_{10}{}^B}{p^2 \cdot K_9 \cdot K_8} - \left(k_{11} \cdot x_{10}{}^F \cdot i_2 - k_{\text{–}11} \cdot i_3\right) -$$

$$-\left(h_4 \cdot i_1 + h_5 \cdot i_6 + h_6 \cdot i_7 + h_7 \cdot i_{10}\right) \cdot x_{10}{}^F - h_8 \cdot i_1 \cdot x_{10\text{–}5}{}^B \quad \text{(S8)}$$

X: $$\frac{dy_{10}}{dt} = -\frac{k_6}{K_6}\cdot y_{10}\cdot x_{7-3}^{\ F} - \frac{k_7\cdot x_9^{\ BF}\cdot y_{10}^{\ B}}{p\cdot K_7} - \frac{k_8\cdot x_9^{\ BF}\cdot x_8^{\ BF}\cdot y_{10}^{\ B}}{p^2\cdot K_9\cdot K_8} \quad \text{(S9)}$$

IIa: $$\frac{dx_2}{dt} = k_{12}\cdot p\cdot x_{10}^{\ F}\cdot y_2 + \frac{k_{13}\cdot x_{10-5}^{\ B}\cdot y_2^{\ B}}{p} - \left(h_9\cdot i_1 + h_{10}\cdot i_6 + h_{11}\cdot i_7 + h_{12}\cdot i_{10} + h_{13}\cdot i_9\right)\cdot x_2^{\ F} \quad \text{(S10)}$$

II: $$\frac{dy_2}{dt} = -k_{12}\cdot p\cdot x_{10}^{\ F}\cdot y_2 - \frac{k_{13}\cdot x_{10-5}^{\ B}\cdot y_2^{\ B}}{p} \quad \text{(S11)}$$

Fibrin: $$\frac{dx_1}{dt} = \frac{k_{14}}{K_{14}}\cdot y_1\cdot x_2^{\ F} \quad \text{(S12)}$$

Fibrinogen: $$\frac{dy_1}{dt} = -\frac{k_{14}}{K_{14}}\cdot y_1\cdot x_2^{\ F} \quad \text{(S13)}$$

3. Major positive feedback loops:

VIIIa: $$\frac{dx_8}{dt} = \frac{k_{15}\cdot y_8\cdot x_2^{\ F}}{K_{15} + x_2^{\ F}} - h_{14}\cdot x_8 \quad \text{(S14)}$$

VIII: $$\frac{dy_8}{dt} = -\frac{k_{15}\cdot y_8\cdot x_2^{\ F}}{K_{15} + x_2^{\ F}} \quad \text{(S15)}$$

Va: $$\frac{dx_5}{dt} = \frac{k_{16}\cdot y_5\cdot x_2^{\ F}}{K_{16} + x_2^{\ F}} - h_{15}\cdot i_4\cdot x_5^{\ BF} \quad \text{(S16)}$$

V: $$\frac{dy_5}{dt} = -\frac{k_{16}\cdot y_5\cdot x_2^{\ F}}{K_{16} + x_2^{\ F}} \quad \text{(S17)}$$

XIa: $$\frac{dx_{11}}{dt} = k_{17}\cdot p\cdot y_{11}\cdot x_2^{\ F} - \left(h_{16}\cdot i_1 + h_{17}\cdot i_8 + h_{18}\cdot i_7 + h_{19}\cdot i_{10} + h_{20}\cdot i_{11}\right)\cdot x_{11} \quad \text{(S18)}$$

XI: $$\frac{dy_{11}}{dt} = -k_{17}\cdot p\cdot y_{11}\cdot x_2^{\ F} \quad \text{(S19)}$$

4. Principal inhibition reactions:

AT–III: $$\frac{di_1}{dt} = -\left(h_3\cdot x_9 + h_4\cdot x_{10}^{\ F} + h_8\cdot x_{10-5}^{\ B} + h_9\cdot x_2^{\ F} + h_{16}\cdot x_{11}\right)\cdot i_1 \quad \text{(S20)}$$

TFPI: $$\frac{di_2}{dt} = -\left(k_{11}\cdot x_{10}^{\ F}\cdot i_2 - k_{-11}\cdot i_3\right) - h_2\cdot x_{10-7-3}\cdot i_2 \quad \text{(S21)}$$

Xa–TFPI: $$\frac{di_3}{dt} = \left(k_{11}\cdot x_{10}^{\ F}\cdot i_2 - k_{-11}\cdot i_3\right) - h_1\cdot x_{7-3}^{\ F}\cdot i_3 \quad \text{(S22)}$$

APC: $$\frac{di_4}{dt} = k_{18}\cdot i_5\cdot x_2^{\ F} - \left(h_{21}\cdot i_6 + h_{22}\cdot i_8 + h_{23}\cdot i_7 + h_{24}\cdot i_{10}\right)\cdot i_4 \quad \text{(S23)}$$

PC: $\frac{di_5}{dt} = -\, k_{18} \cdot i_5 \cdot {x_2}^F$ (S24)

5. The compact notation for the concentrations of free/bound/in-complex factors in the sections 1-4 above should be read as follows:

VIIa–TF^F: $x_{7-3}^F = \frac{x_{7-3}}{1 + \frac{y_9}{K_4} + \frac{y_{10}}{K_6}}$ (S25)

Xa–VIIa–TF: $x_{10-7-3} = \frac{k_6}{K_6 \cdot k_{-19}} \cdot y_{10} \cdot x_{7-3}^F$ (S26)

IXa^{BF} : ${x_9}^{BF} = \frac{x_9 \cdot p \cdot n_{20}}{K_{20} + x_9}$ (S27)

VIIIa^{BF} : ${x_8}^{BF} = \frac{x_8 \cdot p \cdot n_{21}}{\left(K_{21} + x_8\right) \cdot \left(1 + \frac{{y_{10}}^B}{p \cdot K_{10}}\right) \cdot \left(1 + \frac{i_{12}}{K_{22}}\right)}$ (S28)

Xa–Va^B: ${x_{10-5}}^B = \frac{x_{10} \cdot {x_5}^B}{K_{23} \cdot \left(1 + \frac{i_{12}}{K_{24}} + \frac{x_{10}}{K_{23}}\right) + {x_5}^B}$ (S29)

Xa^F: ${x_{10}}^F = x_{10} - {x_{10-5}}^B$ (S30)

X^B: ${y_{10}}^B = \frac{y_{10} \cdot p \cdot n_{25}}{K_{25} \cdot \left(1 + \frac{y_{10}}{K_{25}} + \frac{y_2}{K_{26}}\right)}$ (S31)

IIa^F: ${x_2}^F = \frac{x_2}{1 + \frac{x_1 + y_1}{K_{14}}}$ (S32)

II^B: ${y_2}^B = \frac{y_2 \cdot p \cdot n_{25}}{K_{26} \cdot \left(1 + \frac{y_{10}}{K_{25}} + \frac{y_2}{K_{26}}\right)}$ (S33)

Va^B: ${x_5}^B = \frac{x_5 \cdot p \cdot n_{27}}{K_{27} + x_5}$ (S34)

Va^{BF} : ${x_5}^{BF} = {x_5}^B - {x_{10-5}}^B$ (S35)

Table S1. Model parameters

Parameter	Initial value of the concentration variable[a]	Factor
$x_{7\text{-}3}$	0	VIIa–TF
$y_{7\text{-}3}$	0	VII–TF
x_3	special[b]	TF
x_7	0.1	VIIa
y_7	10	VII
x_9	0	IXa
y_9	90	IX
x_{10}	0	Xa
y_{10}	170	X
x_2	0	IIa
y_2	1400	II
x_1	0	fibrin
y_1	7600	fibrinogen
x_8	0	VIIIa
y_8	0.7	VIII
x_5	0	Va
y_5	20	V
x_{11}	0	XIa
y_{11}	30	XI
i_1	3400	AT-III
i_2	2.5	TFPI
i_3	0	Xa–TFPI
i_4	0	APC
i_5	60	PC
Parameter	**Value of the concentration constant[a]**	**Factor**

i_6	3000	α_2-macroglobulin
i_7	40000	α_1-antitrypsin
i_8	1100	α_2-antiplasmin
i_9	1400	heparin cofactor II
i_{10}	88	protein C inhibitor
i_{11}	1700	C1 inhibitor
i_{12}	346	protein S
p	$7.5 \cdot 10^{-5}$ [c]	phospholipid

Parameter	**Value of the rate constant**	**Reference**
k_1, k_{-1}	4.2 $nM^{-1}min^{-1}$, 1.1 min^{-1}	(1,2) [d]
k_2	0.0014 $nM^{-1}min^{-1}$	(3)
k_3	0.4 $nM^{-1}min^{-1}$	(4)
k_4, K_4	15 min^{-1}, 210 nM	(5)
k_5, K_5	5.8 min^{-1}, 200 nM	(6)
k_6, K_6	435 min^{-1}, 238 nM	(7)
k_7, K_7	0.06 min^{-1}, 230 molecules/platelet	(8,9) [d]
k_8	6350 min^{-1}	(10)
K_8	1216 molecules/platelet	(10)
K_9	278 molecules/platelet	(10)
K_{10}	1655 molecules/platelet	(10)
k_{11}, k_{-11}	0.052 $nM^{-1}min^{-1}$, 0.02 min^{-1}	(11)
k_{12}	45 $nM^{-2}min^{-1}$	(12,13) [d]
k_{13}	1.44 min^{-1}	(14) [d]
k_{14}, K_{14}	5040 min^{-1}, 7200 nM	(15)
k_{15}, K_{15}	54 min^{-1}, 147 nM	(16)
k_{16}, K_{16}	14 min^{-1}, 71.7 nM	(17)
k_{17}	0.03 $nM^{-2}min^{-1}$	(18) [d]
k_{18}	0.00002 $min^{-1}nM^{-1}$	(19)
k_{-19}	770 min^{-1}	(20)
n_{20}, K_{20}	260 sites/platelet, 2.57 nM	(21)
n_{21}, K_{21}	750 sites/platelet, 1.5 nM	(22)
K_{22}	150 nM	(23)

K_{23}	0.118 nM	(12)
K_{24}	200 nM	(24) [d]
n_{25}, K_{25}, K_{26}	16000 sites/platelet, 320 nM, 470 nM	(25)
n_{27}, K_{27}	2700 sites/platelet, 2.9 nM	(12,26)
h_1	0.44 $nM^{-1}min^{-1}$	(11)
h_2	6 $nM^{-1}min^{-1}$	(20)
h_3	0.0000082 $nM^{-1}min^{-1}$	(27)
h_4	0.00015 $nM^{-1}min^{-1}$	(28)
h_5	0.00004 $nM^{-1}min^{-1}$	(29)
h_6	0.0000136 $nM^{-1}min^{-1}$	(29)
h_7	0.0012 $nM^{-1}min^{-1}$	(30)
h_8	0.000022 $nM^{-1}min^{-1}$	(31)
h_9	0.00041 $nM^{-1}min^{-1}$	(28)
h_{10}	0.0001 $nM^{-1}min^{-1}$	(32)
h_{11}	0.000003 $nM^{-1}min^{-1}$	(33)
h_{12}	0.00037 $nM^{-1}min^{-1}$	(30)
h_{13}	0.000063 $nM^{-1}min^{-1}$	(34)
h_{14}	0.35 min^{-1}	(35)
h_{15}	7.7 $nM^{-1}min^{-1}$	(36) [d]
h_{16}	0.000019 $nM^{-1}min^{-1}$	(37)
h_{17}	0.000026 $nM^{-1}min^{-1}$	(37)
h_{18}	0.000006 $nM^{-1}min^{-1}$	(37)
h_{19}	0.0054 $nM^{-1}min^{-1}$	(30)
h_{20}	0.00014 $nM^{-1}min^{-1}$	(38)
h_{21}	0.000006 $nM^{-1}min^{-1}$	(39)
h_{22}	0.000006 $nM^{-1}min^{-1}$	(39)
h_{23}	0.0000007 $nM^{-1}min^{-1}$	(33)
h_{24}	0.00039 $nM^{-1}min^{-1}$	(30)

[a] Concentrations are from (40,41).

[b] Concentrations are specified in figure legends for each numerical experiment.

[c] Activity of procoagulant surface in platelet-free plasma as expressed in activated platelet equivalents (42).

[d] Estimated on the basis of experimental data.

Model assumptions and modification

Principal model assumptions and their substantiation can be found in (42). Briefly, binding of all factors to phospholipid membranes was assumed to be rapid within the fibrin formation timescale. In contrast, consumption of α_2-macroglobulin, α_1-antitrypsin and some other inhibitors (Table S1) was assumed to be negligible over the total course of clotting. Concentration of phospholipid membranes available for binding of coagulation factors in platelet-free plasma and assumed to be mainly provided by platelet-derived microparticles was expressed in activated platelet equivalents (42). The model of membrane-dependent factor X activation by intrinsic tenase was incorporated from (10); the model of factor X activation by extrinsic tenase and of the TFPI pathway was from (20).

Of particular importance to this study is assumption that fibrin clot optical density (the system outcome observed experimentally) is directly related to the concentration of generated fibrin (the system outcome obtained from the model). This assumption is substantiated by the reports that fibrin polymerization is rapid (43), that clot optical density is proportional to fibrin concentration (44), and, in contrast to fibrin clot architecture, does not depend on polymerization kinetics or thrombin concentration (45). However, a care should be exercised when comparing computer simulation results with experiments at very low fibrin concentrations that might be below the minimal concentration required for polymerization (46).

For the purposes of this study, the model of (42) was modified as follows: a) thrombomodulin and platelets were removed from the model, because these components were absent in our experimental design; b) several kinetic constants were changed to bring the model in accordance with the used experimental conditions, such as rate constants for the factor X activation by extrinsic tenase assembled on recombinant TF instead of fibroblasts; c) ordinary differential equations were used instead of partial ones, because of the homogeneous experimental design instead of the reaction-diffusion system.

It is essential to note that this model has already been subjected to some explicit and implicit reduction. Formation of all enzyme-substrate complexes (a total of 16) was assumed to be rapid within the characteristic timescale of fibrin clot formation, as well as platelet binding of proteins (a total of 13); concentrations of all stoichiometric inhibitors except for AT-III and TFPI (a total of 7) were assumed to be well in excess and thus constant (42). Some components and processes (thrombomodulin, platelet activation, and others) not present in the experimental system studied in this work were excluded. Thus, the actual initial number of species in the network approached one hundred.

2. Modular Decomposition

Task-oriented sensitivity and necessity analysis

In order to identify the minimal set of components necessary and sufficient for initial stages of clotting, the effect of one-factor-at-a-time removal of each variable on clot time t_{clot} was estimated using computer simulations. This process henceforth called necessity analysis was performed as follows. In order to remove a reactant from the system, we assumed its initial concentration and production rate to be fixed to zero. Relative change of clot time Δt_{clot} was calculated for each reactant R as follows:

$$\Delta t_{clot} = \frac{t_{clot}^{0} - t_{clot}\big|_{R=0}}{t_{clot}^{0}}, \tag{S36}$$

where t_{clot}^{0} is clot time of the unperturbed system (3.376 min), and $t_{clot}\big|_{R=0}$ is clot time with removed reactant R. In order to estimate local system sensitivity to perturbations in the reactant initial concentration, this concentration was changed by +1% and –1%, and the task-specific control coefficient was estimated for each reactant R using the approximate formula:

$$C_{t_{clot}} = \frac{t_{clot}\big|_{R=101\%} - t_{clot}\big|_{R=99\%}}{0.02 \cdot t_{clot}^{0}}, \tag{S37}$$

Both the necessity and sensitivity analyzes must be performed because reactions with low control coefficients can be necessary, and vice versa: a traditional example is a flat metabolic pathway in a steady state, wherein flux is completely controlled by the slowest reaction, but no reaction can be removed (47). Although the term 'sensitivity analysis' is not limited to derivative-based sensitivies and can include exploration of the complete space of model inputs (48) thus encompassing our 'necessity analysis', we shall use both terms to clearly distinguish necessary components from the controlling ones.

Table S2. Relative importance of coagulation system components

Notation: n.c., no clotting; n.a., not applicable. The components were assumed to be necessary if there was no clotting in their absence; to be essential if $|\Delta t_{clot}|$ exceeded 0.10; to be controlling if $|C_{t_{clot}}|$ exceeded 0.10.

Parameter	Factor	Δt_{clot}	$C_{t_{clot}}$	Necessary	Essential	Controlling
$x_{7\text{-}3}$	VIIa–TF	n.c.	n.a.	**Yes**	**Yes**	
$y_{7\text{-}3}$	VII–TF	n.c.	n.a.	**Yes**	**Yes**	
x_3	TF	n.c.	-0.55	**Yes**	**Yes**	**Yes**
x_7	VIIa	n.c.	-0.52	**Yes**	**Yes**	**Yes**
y_7	VII	-0.68	0.49	No	Yes	Yes

x_9	IXa	0.00	n.a.	No	No	
y_9	IX	-0.01	0.01	No	No	No
x_{10}	Xa	n.c.	n.a.	**Yes**	**Yes**	
y_{10}	X	n.c.	-0.07	**Yes**	**Yes**	**No**
x_2	IIa	n.c.	n.a.	**Yes**	**Yes**	
y_2	II	n.c.	-0.25	**Yes**	**Yes**	**Yes**
x_1	Fibrin	n.c.	n.a.	**Yes**	**Yes**	
y_1	Fibrinogen	n.c.	-0.01	**Yes**	**Yes**	**No**
x_8	VIIIa	0.00	n.a.	No	No	
y_8	VIII	0.00	-0.01	No	No	No
x_5	Va	n.c.	n.a.	**Yes**	**Yes**	
y_5	V	n.c.	-0.37	**Yes**	**Yes**	**Yes**
x_{11}	XIa	0.00	n.a.	No	No	
y_{11}	XI	0.00	0.00	No	No	No
i_1	AT–III	-0.20	0.25	**No**	**Yes**	**Yes**
i_2	TFPI	-0.41	0.44	**No**	**Yes**	**Yes**
i_3	Xa–TFPI	0.00	n.a.	No	No	
i_4	APC	0.00	n.a.	No	No	
i_5	PC	0.00	0.00	No	No	No

Parameter	Reaction	Δt_{clot}	$C_{t_{clot}}$	Necessary	Essential	Controlling
k_1	factor VIIa/VII association with TF	n.c.	-0.01	**Yes**	**Yes**	No
k_{-1}	factor VIIa–TF complex dissociation	n.c.	-0.27	**Yes**	**Yes**	**Yes**
k_2	factor VII (VII–TF) activation by factor IIa	0.00	-0.01	No	No	No
k_3	factor VII–TF activation by factor Xa	0.02	-0.01	No	No	No
k_4	factor IX activation by factor VIIa–TF	0.00	-0.01	No	No	No
k_5	factor IX activation by factor XIa	0.00	0.00	No	No	No
k_6	factor X activation by factor VIIa–TF	11.18	-0.13	No	**Yes**	**Yes**
k_7	factor X activation by factor IXa	0.00	0.00	No	No	No
k_8	factor X activation by factor IXa–VIIIa	0.00	-0.01	No	No	No
k_{11}	factor Xa inhibition by TFPI	-0.04	0.04	No	No	No
k_{-11}	factor Xa–TFPI complex dissociation	0.00	0.00	No	No	No
k_{12}	factor II activation by factor Xa	n.c.	-0.10	**Yes**	**Yes**	**Yes**
k_{13}	factor II activation by factor Xa–Va	n.c.	-0.43	**Yes**	**Yes**	**Yes**
k_{14}	fibrinogen cleavage by factor IIa	n.c.	-0.16	**Yes**	**Yes**	**Yes**

k_{15}	factor VIII activation by factor IIa	0.00	-0.01	No	No	No
k_{16}	factor V activation by factor IIa	n.c.	-0.37	**Yes**	**Yes**	**Yes**
k_{17}	factor XI activation by factor IIa	0.00	0.00	No	No	No
k_{18}	Protein C activation by factor IIa	0.00	0.00	No	No	No
k_{-19}	factor VIIa–TF–Xa complex dissociation	n.c.	-0.41	**Yes**	**Yes**	**Yes**
n_{20}	factor IXa binding to lipids	0.00	-0.01	No	No	No
n_{21}	factor VIIIa binding to lipids	0.00	-0.01	No	No	No
n_{25}	factor X and II binding to lipids	n.c.	-0.43	**Yes**	**Yes**	**Yes**
n_{27}	factor Va binding to lipids	n.c.	-0.41	**Yes**	**Yes**	**Yes**
h_1	factor VIIa–TF inhibition by factor Xa-TFPI	0.00	0.00	No	No	No
h_2	factor VIIa–TF–Xa inhibition by TFPI	-0.40	0.40	No	**Yes**	**Yes**
h_3	factor IXa inhibition by AT-III	0.00	0.00	No	No	No
h_4	factor Xa inhibition by AT-III	-0.14	0.13	**Yes**	**Yes**	**Yes**
h_5	factor Xa inhibition by α_2-macroglobulin	-0.03	0.04	No	No	No
h_6	factor Xa inhibition by α_1-antitrypsin	-0.15	0.16	**Yes**	**Yes**	**Yes**
h_7	factor Xa inhibition by protein C inhibitor	-0.03	0.03	No	No	No
h_8	factor Xa–Va inhibition by AT-III	0.00	0.00	No	No	No
h_9	factor IIa inhibition by AT-III	-0.09	0.10	No	No	**Yes**
h_{10}	factor IIa inhibition by α_2-macroglobulin	-0.02	0.01	No	No	No
h_{11}	factor IIa inhibition by α_1-antitrypsin	-0.01	0.01	No	No	No
h_{12}	factor IIa inhibition by protein C inhibitor	0.00	0.00	No	No	No
h_{13}	factor IIa inhibition by heparin cofactor-II	-0.01	0.01	No	No	No
h_{14}	factor VIIIa dissociation	0.00	0.00	No	No	No
h_{15}	factor Va degradation by APC	0.00	0.00	No	No	No
h_{16}	factor XIa inhibition by AT-III	0.00	0.00	No	No	No

h_{17}	factor XIa inhibition by α_2-antiplasmin	0.00	0.00	No	No	No
h_{18}	factor XIa inhibition by α_1-antitrypsin	0.00	0.00	No	No	No
h_{19}	factor XIa inhibition by protein C inhibitor	0.00	0.00	No	No	No
h_{20}	factor XIa inhibition by C1 inhibitor	0.00	0.00	No	No	No
h_{21}	APC inhibition by α_2-macroglobulin	0.00	0.00	No	No	No
h_{22}	APC inhibition by α_2-antiplasmin	0.00	0.00	No	No	No
h_{23}	APC inhibition by α_1-antitrypsin	0.00	0.00	No	No	No
h_{24}	APC inhibition by protein C inhibitor	0.00	0.00	No	No	No

The results of these simulations are summarized in Table S2. When interpreting these data, it should be kept in mind that t_{clot} is inversely related to clotting efficiency, and thus a positive control coefficient $C_{t_{clot}}$ means inhibition of clotting. The data show that, within the coagulation network, it is possible to identify a smaller subsystem that is necessary and sufficient for the regulation of clot time. These principal variables include initial TF pathway components (TF, VIIa–TF, VII–TF, VII), the lower backbone of the clotting cascade (factors Xa/X, IIa/II, fibrin/fibrinogen), one positive feedback loop (factor Va/V), and two inhibitors (AT–III and TFPI).

The composition of this subsystem agrees well with previous experimental reports with regard to action of coagulation system components over the course of blood coagulation in homogeneous and reaction-diffusion systems. Factors VII, X, and V are known to be essential for regulation of the clotting lag time (44,49). In contrast, the negative feedback of the protein C pathway has no effect of clotting initiation in agreement with the previous experimental reports (42,50). Contribution of another negative feedback, the Xa–TFPI complex in the two-step TFPI mechanism of action, is negligible as well, in line with experimental data (11). Small contributions of the positive feedbacks involving factors VIII and XI also find support in known data (44,51). Zymogen factor VII inhibits clotting (the Δt_{clot} is negative and the $C_{t_{clot}}$ is positive) due to competition effects, which has also been established (52). Comparison of local sensitivity and necessity coefficients in Table S2 illustrates that sensitivity analysis alone would be insufficient for the task of module identification. For example, fibrinogen and factor X are absolutely necessary in order for any clotting to occur, but their concentrations are not controlling ($C_{t_{clot}}$ is –0.07 and -0.01, respectively).

Subsequently, necessity of, and system sensitivity to, individual reactions were estimated by the analogy: by either setting to zero or slightly perturbing their principal constants. The results are also listed in Table S2. The

general picture of important reactions and pathways corresponds closely to that obtained with reactants: there is no contribution of the PC and intrinsic pathways. Formation of extrinsic tenase (the k_1 constant) provides yet another example of a necessary process that is not controlling ($C_{t_{clot}}$ =–0.01).

However, one prediction of the reaction significance analysis is unexpected: that the positive feedback of factor VII activation by either thrombin (k_2, Δt_{clot}=0.00, $C_{t_{clot}}$ =–0.01) or factor Xa (k_3, Δt_{clot}=0.02, $C_{t_{clot}}$ =–0.01) has no effect on t_{clot}. This feedback loop is the most immediate and short one in the cascade, and could be expected to contribute substantially to the initiation reaction (53). There is no existing experimental evidence to validate this result, but possible reasons for it will be theoretically examined below.

As an additional control, we carried out sensitivity analysis simulations using 10% and 50% changes instead of 1%; these gave comparable results (data not shown).

Sensitivity and necessity: possible errors in their estimation

One-at-a-time variation of parameters in a complex system may not always be sufficient for module identification. Although a full multiparameter variation is not always technically feasible, it is important to check the impact of the most significant parameters on the results of the analyisis.

Importantly, there are at least two cases when necessity and sensitivity coefficients Δt_{clot} and $C_{t_{clot}}$ can be misleading. First, contribution of different reactions might depend upon conditions, first of all the level of activation that can vary greatly in coagulation depending on the degree of vascular damage. A good example is one of the most important inhibitors of coagulation, AT–III. Table S2 suggests that its influence on the initial stages of coagulation is not very significant (the absolute values of both Δt_{clot} and $C_{t_{clot}}$ are ~0.2, which is on the verge of our assumed significance boundary). However, if coagulation is activated by smaller TF concentrations, these values are greatly increased: Δt_{clot} reaches its maximal possible amplitude of –1 (Fig. S1A), and $C_{t_{clot}}$ increases up to 10 and higher (Fig. S1B). Thus, for the purposes of this study, which focuses on trigger properties of clotting at low TF concentrations, AT–III is indispensable.

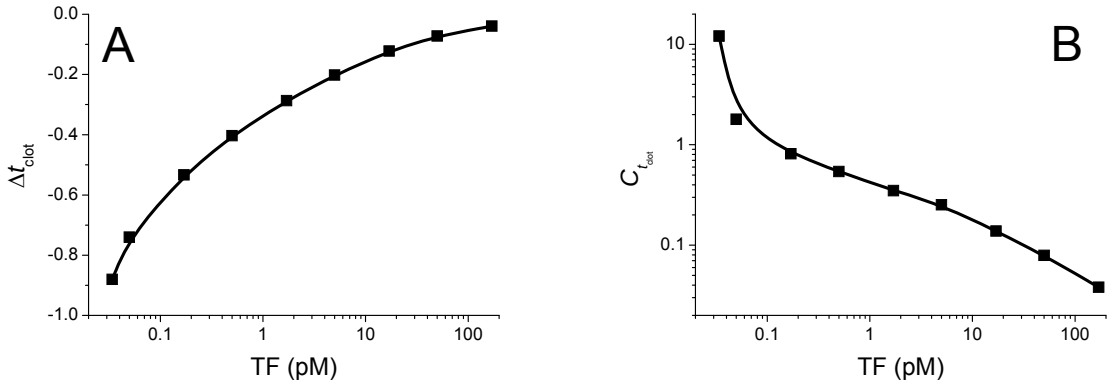


Fig. S1. Importance of AT–III as a function of activation. Necessity (A) and sensitivity (B) coefficients for AT–III were calculated as described in the legend to Table S2 except that simulations were performed for a wide range of TF concentrations. The parameters Δt_{clot} (■) and $C_{t_{clot}}$ (□) are shown as functions of TF.

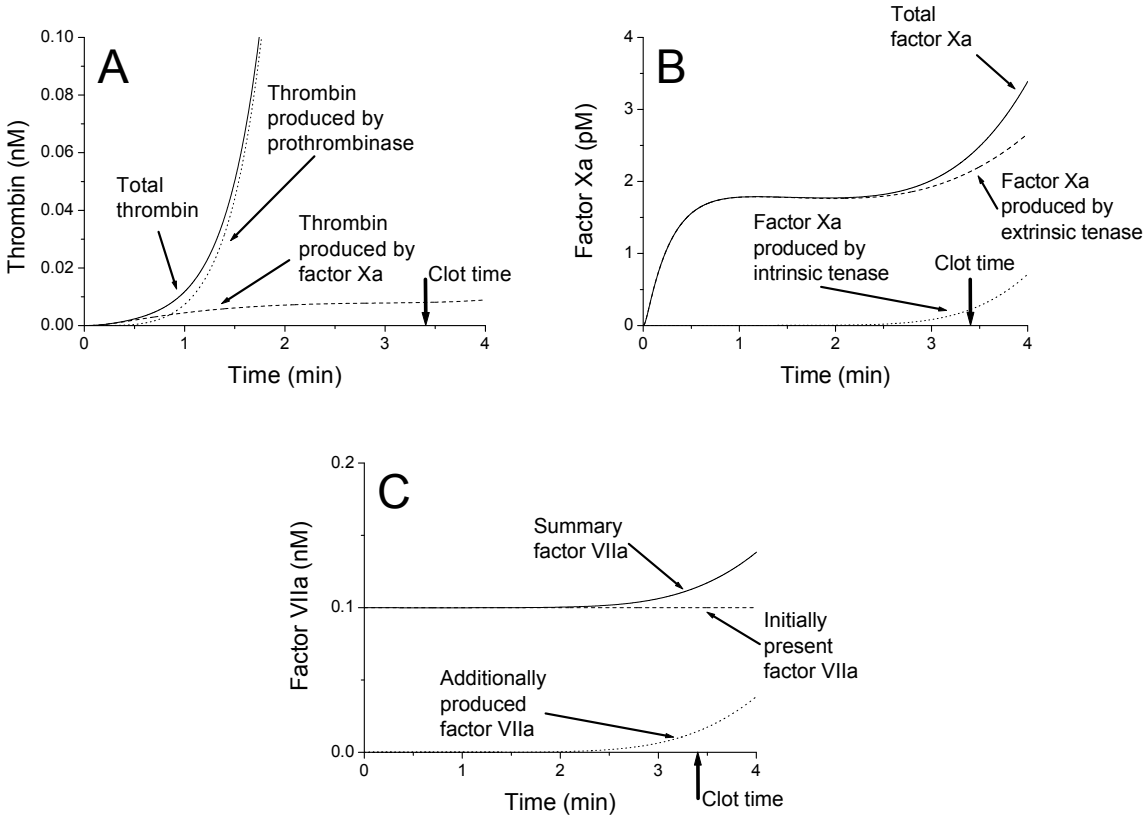
Another possible error can be caused by that several reactions may independently function to achieve the same goal. Each such reaction could be easily dispensable individually, but an attempt to remove all these "insignificant" reactions simultaneously could result in erroneous result. A simple example is inhibition of factor Xa carried out by numerous inhibitors. As can be seen from Table S2, each of these reactions has little control alone: AT-III, 0.13; α_2-macroglobulin, 0.04; α_1-antitrypsin, 0.16; protein C inhibitor, 0.03. Yet, together they form a major regulation.

Comparison of the positive feedback loops of coagulation

An essential issue to address is difference between contributions of positive feedback loops. Among the four major positive feedbacks of the clotting system (that is, activation of factors V, VII, VIII, and XI), factor V activation is the only one playing any role in the regulation of the initial stages of coagulation. While necessity/sensitivity analysis provides this result, additional steps should be taken to explain it.

In order to gain insight into the mechanisms of positive feedback action, we simulated experiments wherein contributions of feedbacks to the production of their target enzyme by the time of clotting were calculated. Thrombin kinetics in Fig. S2A shows that quantity of thrombin produced by prothrombinase by the clot time vastly exceeds that produced by factor Xa alone (their ratio is ~500). In contrast, contribution of intrinsic tenase to factor Xa production is detectable only at later stages, when the clot is already formed (panel B); this fact also explains inefficiency of the factor XI-dependent feedback, whose effect is mediated by intrinsic tenase. Finally, the same is true for factor VII feedback activation, which (until long after clot time) contributes much less factor VIIa than it is initially present in plasma.

Fig. S2. Comparison of positive feedbacks in coagulation. The panels show kinetics of the rate-limiting coagulation enzymes factors IIa (A), Xa (B), and VIIa (C) and relative contributions of positive feedbacks into their formation. (A) Thrombin concentration as a function of time: total (solid), produced by factor Xa alone (dash), or by the factor Xa–Va complex (dot). (B) Factor Xa concentration as a function of time: total (solid), produced by the VIIa–TF complex (dash), or by the factor IXa–VIIIa complex (dot). (B) Factor VIIa concentration as a function of time: total (solid), present initially in plasma (dash), or produced via feedback activation by factor Xa and thrombin (dot). The calculations were performed as described in the legend to Table S2. It can be seen that the contribution of the factor V-dependent positive feedback to thrombin concentration by the time of clot formation is great (A). In contrast, feedbacks contribute negligibly to the formation of factors Xa (B) and VIIa (C) by this time.



These data indicate that difference in the contributions of positive feedbacks is due solely to their kinetic properties, and not to the network structure. If the time required for a feedback to make a detectable contribution is smaller than the clot time then this feedback would be important for clotting.

3. Model Reduction

Step A. Removal of non-essential variables and reactions

In order to analyze the subtask of the activating signal recognition, reduction was aimed to retain quantitative behavior at short time intervals (faster than, or equal to, clot time) and low activator concentrations (below than, or equal to, those required to achieve clot time).

As a first stage, we removed those variables and reactions, which had no significant effect on clot formation as shown by Table S2. The criterion used was that both the Δt_{clot} and $C_{t_{clot}}$ parameters for these reactions should be below 0.1, i.e. their relative contribution should not exceed 10%. If any of those parameters exceeded this value (in other words, if a reaction or a reactant was either controlling or required for clotting), the respective component was retained. The equations below show the model after removal of non-essential components.

1. Clotting initiation:

VIIa–TF: $\frac{dx_{7-3}}{dt} = \left(k_1 \cdot x_7 \cdot x_3 - k_{-1} \cdot {x_{7-3}}^F\right) - h_2 \cdot x_{10-7-3} \cdot i_2$ (S38)

VII–TF: $\frac{dy_{7-3}}{dt} = \left(k_1 \cdot y_7 \cdot x_3 - k_{-1} \cdot y_{7-3}\right)$ (S39)

TF: $\frac{dx_3}{dt} = -\left(k_1 \cdot x_7 \cdot x_3 - k_{-1} \cdot {x_{7-3}}^F\right) - \left(k_1 \cdot y_7 \cdot x_3 - k_{-1} \cdot y_{7-3}\right)$ (S40)

VIIa: $\frac{dx_7}{dt} = -\left(k_1 \cdot x_7 \cdot x_3 - k_{-1} \cdot {x_{7-3}}^F\right)$ (S41)

VII: $\frac{dy_7}{dt} = -\left(k_1 \cdot y_7 \cdot x_3 - k_{-1} \cdot y_{7-3}\right)$ (S42)

2. The cascade backbone:

Xa: $\frac{dx_{10}}{dt} = \frac{k_6}{K_6} \cdot y_{10} \cdot {x_{7-3}}^F - \left(h_4 \cdot i_1 + h_5 \cdot i_6 + h_6 \cdot i_7 + h_7 \cdot i_{10}\right) \cdot {x_{10}}^F - h_8 \cdot i_1 \cdot {x_{10-5}}^B$ (S43)

X: $\frac{dy_{10}}{dt} = -\frac{k_6}{K_6} \cdot y_{10} \cdot {x_{7-3}}^F$ (S44)

IIa: $\frac{dx_2}{dt} = k_{12} \cdot p \cdot {x_{10}}^F \cdot y_2 + \frac{k_{13} \cdot {x_{10-5}}^B \cdot {y_2}^B}{p} - \left(h_9 \cdot i_1 + h_{10} \cdot i_6 + h_{11} \cdot i_7 + h_{12} \cdot i_{10} + h_{13} \cdot i_9\right) \cdot {x_2}^F$ (S45)

II: $\frac{dy_2}{dt} = -k_{12} \cdot p \cdot {x_{10}}^F \cdot y_2 - \frac{k_{13} \cdot {x_{10-5}}^B \cdot {y_2}^B}{p}$ (S46)

Fibrin: $\frac{dx_1}{dt} = \frac{k_{14}}{K_{14}} \cdot y_1 \cdot {x_2}^F$ (S47)

Fibrinogen: $\frac{dy_1}{dt} = -\frac{k_{14}}{K_{14}} \cdot y_1 \cdot {x_2}^F$ (S48)

3. Positive feedback loops:

Va: $\frac{dx_5}{dt} = \frac{k_{16} \cdot y_5 \cdot {x_2}^F}{K_{16} + {x_2}^F}$ (S49)

V: $\frac{dy_5}{dt} = -\frac{k_{16} \cdot y_5 \cdot {x_2}^F}{K_{16} + {x_2}^F}$ (S50)

4. Inhibition reactions:

AT–III: $\frac{di_1}{dt} = -\left(h_3 \cdot x_9 + h_4 \cdot {x_{10}}^F + h_8 \cdot {x_{10-5}}^B + h_9 \cdot {x_2}^F + h_{16} \cdot x_{11}\right) \cdot i_1$ (S51)

TFPI: $\frac{di_2}{dt} = -h_2 \cdot x_{10-7-3} \cdot i_2$ (S52)

5. The compact notation:

VIIa–TF^F: $x_{7-3}^F = \frac{x_{7-3}}{1 + \frac{y_{10}}{K_6}}$ (S53)

Xa–VIIa–TF: $x_{10-7-3} = \frac{k_6}{K_6 \cdot k_{-19}} \cdot y_{10} \cdot x_{7-3}^F$ (S54)

Xa–Va^B: ${x_{10-5}}^B = \frac{x_{10} \cdot {x_5}^B}{K_{23} \cdot \left(1 + \frac{i_{12}}{K_{24}} + \frac{x_{10}}{K_{23}}\right) + {x_5}^B}$ (S55)

Xa^F: ${x_{10}}^F = x_{10} - {x_{10-5}}^B$ (S56)

X^B: ${y_{10}}^B = \frac{y_{10} \cdot p \cdot n_{25}}{K_{25} \cdot \left(1 + \frac{y_{10}}{K_{25}} + \frac{y_2}{K_{26}}\right)}$ (S57)

IIa^F: ${x_2}^F = \frac{x_2}{1 + \frac{x_1 + y_1}{K_{14}}}$ (S58)

II^B: ${y_2}^B = \frac{y_2 \cdot p \cdot n_{25}}{K_{26} \cdot \left(1 + \frac{y_{10}}{K_{25}} + \frac{y_2}{K_{26}}\right)}$ (S59)

Va^B: ${x_5}^B = \frac{x_5 \cdot p \cdot n_{27}}{K_{27} + x_5}$ (S60)

Step B. Temporal hierarchy reduction

In order to identify variables with different timescales and to decipher temporal hierarchy of processes in a complex network, the set of differential equations describing the network has to be made dimensionless. This should be performed so as to allow all variables (including time) to have the same order of magnitude, which is

usually achieved by a normalization by their maximal values thus effectively confining them to an interval from 0 to 1. Maximal values achieved by variables can be estimated from computer simulations, though it should be always kept in mind that these values depend on numerous parameters; and, in our case, the most variable parameter is degree of coagulation activation, i.e. tissue factor concentration. Fortunately, the set of equations to be reduced in this study (S38-S60) is simple enough to allow analytical estimation.

Reduction of initiation reactions. Let us consider eqs. S38-S42 describing interactions of TF and factors VII/VIIa. They are isolated and not affected by the rest of the system. We can reasonably expect that formation of TF-containing complexes is a relatively rapid process, and therefore introduce a new variable:

Total TF: $$\frac{dx_{3p}}{dt} = \frac{dx_{7-3}}{dt} + \frac{dy_{7-3}}{dt} + \frac{dx_3}{dt} = -h_2 \cdot x_{10-7-3} \cdot i_2 \qquad \text{(S61)}$$

This variable is a pool of the old ones, and, as such, can be expected to be more slow. We also note several essential quantitative relationships. Initial tissue factor concentration is usually picomolar or even subpicomolar, and thus is much smaller than that of factor VIIa:

$$x_3\big|_{t=0} << x_7\big|_{t=0} = 100 \text{ pM} \qquad \text{(S62)}$$

The difference between initial concentrations of factors VIIa and VII is also great (Table 1):

$$x_7\big|_{t=0} = 1/100 \cdot y_7\big|_{t=0} << y_7\big|_{t=0} \qquad \text{(S63)}$$

From eq. S54, we also estimate:

$$x_{10-7-3} \approx \kappa \cdot x_{7-3}, \qquad \text{(S64)}$$

where κ is comparable to unit. We finally notice that variables x_{7-3} and y_{7-3} (see eqs. S38 and S39) have comparable inactivation terms, while their production rates differ by a factor of $x_7\big|_{t=0} / y_7\big|_{t=0} = 1/100$:

VIIa–TF: $$\frac{dx_{7-3}}{dt} \sim k_1 \cdot x_7 \cdot x_3 \approx k_1 \cdot x_7\big|_{t=0} \cdot x_3 \qquad \text{(S65)}$$

VII–TF: $$\frac{dy_{7-3}}{dt} \sim k_1 \cdot y_7 \cdot x_3 \approx k_1 \cdot y_7\big|_{t=0} \cdot x_3 \qquad \text{(S66)}$$

In order to make the system dimensionless, let us normalize the variables as follows. For TF, factors VIIa and VII, the maximal values are their initial values. The natural normalization for TF-containing complexes is also initial TF concentration; however, keeping in mind that $x_{7-3} << y_{7-3}$ (S65-S66), it is reasonable to correct normalization of x_{7-3} by a factor of $x_7\big|_{t=0} / y_7\big|_{t=0}$. Thus the new dimensionless variables are:

$$\bar{x}_{3p} = \frac{x_{3p}}{x_3\big|_{t=0}} \qquad \text{(S67)}$$

$$\bar{x}_{7-3} = \frac{x_{7-3}}{x_3\big|_{t=0} \cdot x_7\big|_{t=0} / y_7\big|_{t=0}} \tag{S68}$$

$$\bar{y}_{7-3} = \frac{y_{7-3}}{x_3\big|_{t=0}} \tag{S69}$$

$$\bar{x}_3 = \frac{x_3}{x_3\big|_{t=0}} \tag{S70}$$

$$\bar{x}_7 = \frac{x_7}{x_7\big|_{t=0}} \tag{S71}$$

$$\bar{y}_7 = \frac{y_7}{y_7\big|_{t=0}} \tag{S72}$$

Let us also select variable $\bar{x}_{3p}$ as an internal standard of time for the dimensionless system. In other words, let us make transition to the dimensionless time so as to avoid appearance of either large or small parameters in this equation. This is achieved by defining dimensionless time as:

$$\tau = \frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{y_7\big|_{t=0}} \cdot t \tag{S73}$$

Finally, let us write dimensionless equations in such a form that right parts of the equations would not contain large terms, but only those comparable to unit. We obtain the following dimensionless system for the initiation reactions:

Total TF: $$\frac{d\bar{x}_{3p}}{d\tau} = \varepsilon_0 \frac{d\bar{x}_{3p}}{d\tau} = -\kappa \cdot \bar{x}_{7-3} \tag{S74}$$

VIIa–TF: $$\frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{k_1 \cdot y_7\big|_{t=0} \cdot y_7\big|_{t=0}} \cdot \frac{d\bar{x}_{7-3}}{d\tau} = \varepsilon_1 \cdot \frac{d\bar{x}_{7-3}}{d\tau} = \bar{x}_7 \cdot \bar{x}_3 - \frac{k_{-1} \cdot x_{7-3}^{\ F} - h_2 \cdot x_{10-7-3} \cdot i_2}{k_1 \cdot x_7\big|_{t=0} \cdot x_3\big|_{t=0}} \tag{S75}$$

VII–TF: $$\frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{k_1 \cdot y_7\big|_{t=0} \cdot y_7\big|_{t=0}} \frac{d\bar{y}_{7-3}}{d\tau} = \varepsilon_2 \cdot \frac{d\bar{y}_{7-3}}{d\tau} = \bar{y}_7 \cdot \bar{x}_3 - \frac{k_{-1}}{k_1 \cdot y_7\big|_{t=0}} \cdot \bar{y}_{7-3} \tag{S76}$$

TF: $$\frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{k_1 \cdot y_7\big|_{t=0} \cdot y_7\big|_{t=0}} \cdot \frac{d\bar{x}_3}{d\tau} = \varepsilon_3 \cdot \frac{d\bar{x}_3}{d\tau} = -\bar{y}_7 \cdot \bar{x}_3 + \frac{-k_1 \cdot x_7 \cdot x_3 + k_{-1} \cdot y_{7-3} + k_{-1} \cdot x_{7-3}^{\ F}}{k_1 \cdot y_7\big|_{t=0} \cdot x_3\big|_{t=0}} \tag{S77}$$

VIIa: $$\frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{k_1 \cdot y_7\big|_{t=0} \cdot x_3\big|_{t=0}} \cdot \frac{d\bar{x}_7}{d\tau} = \varepsilon_4 \cdot \frac{d\bar{x}_7}{d\tau} = -\bar{x}_7 \cdot \bar{x}_3 + \frac{k_{-1} \cdot x_{7-3}}{k_1 \cdot x_7\big|_{t=0} \cdot x_3\big|_{t=0}}^{\ F} \tag{S78}$$

VII: $$\frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{k_1 \cdot y_7\big|_{t=0} \cdot x_3\big|_{t=0}} \frac{d\bar{y}_7}{d\tau} = \varepsilon_5 \cdot \frac{d\bar{y}_7}{d\tau} = -\bar{y}_7 \cdot \bar{x}_3 + \frac{k_{-1} \cdot \bar{y}_{7-3}}{k_1 \cdot y_7\big|_{t=0}} \tag{S79}$$

Numerical values of the parameters ε_0-ε_5 are summarized in Table S3.

Table S3. Temporal hierarchy of variables in coagulation

The table shows values of the parameters, which appear as multipliers of the derivatives in the left equation parts of the dimensionless model of coagulation. If this dimensionless parameter was small (ε <<1) the variable was assumed to be rapid compared with $\bar{x}_{3p}$; if it was large (ε >>1) the variable was assumed to be slow; if it was of the order of unit (ε~1) then the variable was of the same temporal order as $\bar{x}_{3p}$.

Factor	Variable	Parameter	Value	Position in the hierarhy
Total TF	$\bar{x}_{3p}$	ε_0	1	**Normal**
VIIa–TF	$\bar{x}_{7-3}$	ε_1	0.0036	Rapid
VII–TF	$\bar{y}_{7-3}$	ε_2	0.0036	Rapid
TF	$\bar{x}_3$	ε_3	0.0036	Rapid
VIIa	$\bar{x}_7$	ε_4	$\frac{0.036\ \text{nM}}{x_3\big\vert_{t=0}}$	Slow (for TF~1 pM and less)
VII	$\bar{y}_7$	ε_5	$\frac{0.036\ \text{nM}}{x_3\big\vert_{t=0}}$	Slow (for TF~1 pM and less)
Xa	$\bar{x}_{10}$	ε_6	0.017	Rapid
X	$\bar{y}_{10}$	ε_7	$\frac{0.96\ \text{nM}}{x_3\big\vert_{t=0}}$	Slow (for TF~1 pM and less)
IIa	$\bar{x}_2$	ε_8	$0.16 \cdot e^{\frac{x_3\vert_{t=0}}{3.7\ fM}}$	Rapid (for TF~10 fM and less) or **normal**
II	$\bar{y}_2$	ε_9	$\frac{1234\ \text{nM}}{x_3\big\vert_{t=0}}$	Slow (for TF~1 pM and less)
Va	$\bar{x}_5$	ε_{10}	$0.02\ \text{nM} \cdot \frac{1-e^{\frac{x_3\vert_{t=0}}{3.7\ fM}}}{x_3\big\vert_{t=0}}$	**Normal**
V	$\bar{y}_5$	ε_{11}	$\frac{0.42\ \text{nM}}{x_3\big\vert_{t=0} \cdot e^{\frac{x_3\vert_{t=0}}{3.7\ fM}}}$	Slow (for TF~1 pM and less)
AT–III	$\bar{i}_1$	ε_{12}	$\frac{201\ \text{nM}}{x_3\big\vert_{t=0} \cdot e^{\frac{x_3\vert_{t=0}}{3.7\ fM}}}$	Slow (for TF~1 pM and less)
TFPI	$\bar{i}_1$	ε_{13}	$\frac{2.5\ \text{nM}}{x_3\big\vert_{t=0}}$	Slow (for TF~1 pM and less)

We can now apply Tikhonov's theorem (54) and see that variables of the system can be segregated into three categories with regard to their timescales. Total TF (x_{3p}) is a "normal" variable, because there are neither small nor large multipliers beside the derivative. TF and two TF-containing complexes (x_3, x_{7-3} and y_{7-3}) have small

multiplier parameters; these variables are rapid, and we can now safely assume equilibrium in complex formation. Finally, factors VIIa and VII (x_7 and y_7) have large parameters, at least at picomolar TF. This means that these variables change extremely slowly, and can be assumed to be constant. This means that, with regard to the timescale determined by the characteristic time $t/\tau = y_7\big|_{t=0} / h_2 \cdot i_2 \cdot x_7\big|_{t=0}$ ~7 min, the set of equations (S38-S42, S61) can be rewritten as follows:

Total TF: $\frac{dx_{3p}}{dt} = - h_2 \cdot x_{10-7-3} \cdot i_2$ (S80)

VIIa–TF: $0 = \left(k_1 \cdot x_7 \cdot x_3 - k_{-1} \cdot x_{7-3}{}^{F}\right) - h_2 \cdot x_{10-7-3} \cdot i_2$ (S81)

VII–TF: $0 = \left(k_1 \cdot y_7 \cdot x_3 - k_{-1} \cdot y_{7-3}\right)$ (S82)

TF: $0 = - \left(k_1 \cdot x_7 \cdot x_3 - k_{-1} \cdot x_{7-3}{}^{F}\right) - \left(k_1 \cdot y_7 \cdot x_3 - k_{-1} \cdot y_{7-3}\right)$ (S83)

VIIa: $\frac{dx_7}{dt} = 0$ (S84)

VII: $\frac{dy_7}{dt} = 0$ (S85)

If we assume that factor X and TFPI are also slow variables (as this will indeed be shown below) then x_{10-7-3} is proportional to x_{7-3}, and this set of equations has a simple exponential solution:

VIIa–TF: $x_{7-3} = x_3\big|_{t=0} \cdot b_1 \cdot e^{-b_2 \cdot t}$ (S86)

b_1=0.00256 b_2=0.009048 min^{-1} (S87)

Reduction of the cascade backbone. In order to analytically estimate maximal values of other model variables, let us assume that all variables representing active factors remaining in the system and having both production and inhibition terms (factors Xa and IIa) are rapid variables, while all remaining zymogens, pro-cofactors and inhibitors (factors X, II, V, AT–III, TFPI) are slow variables. This assumption will give a good upper estimate of their variation with time and allow to test whether they are indeed rapid or slow. Furthermore, we substitute for zero all items if they are less than 10% of a constant item present in the same sum for simplicity. For example, we substitute $\left(1 + i_{12}/K_{24} + x_{10}/K_{23}\right)$ for $\left(1 + i_{12}/K_{24}\right)$ in eq. S55. The remaining equations will then have the form:

Xa: $\frac{dx_{10}}{dt} = a_1 \cdot x_{7-3} - a_2 \cdot x_{10} = 0,$ a_1=181.2 min^{-1}, a_2=1.28 min^{-1} (S88)

IIa: $\frac{dx_2}{dt} = a_3 \cdot x_{10} + a_4 \cdot x_{10} \cdot x_5 - a_5 \cdot x_2 = 0,$ a_3=4.73 min^{-1}, a_4=3298.5 min^{-1}nM^{-1}, a_5=0.94 min^{-1} (S89)

Va: $\frac{dx_5}{dt} = a_6 \cdot x_2$, a_6= 1.96 min^{-1} (S90)

Fibrin: $\frac{dx_1}{dt} = a_7 \cdot x_2$, a_7=2588.1 min^{-1} (S91)

Using eq. S86 we obtain analytical solutions for factors Xa, II, Va, and fibrin:

Xa: $x_{10} = x_3\big|_{t=0} \cdot b_3 \cdot e^{-b_2 \cdot t}$ b_3=0.36 (S92)

IIa: $x_2 = b_4 \cdot x_3\big|_{t=0} \cdot e^{b_5 \cdot x_3|_{t=0} \cdot \left(1-e^{-b_2 \cdot t}\right)} \cdot e^{-b_2 \cdot t}$ b_4=1.82 b_5 = 269288.8 nM^{-1} (S93)

Fibrin: $x_1 = b_6 \cdot \left(e^{b_5 \cdot \varepsilon \cdot \left(1-e^{-b_2 \cdot t}\right)} - 1\right)$ b_6 = 1.94 nM (S94)

Va: $x_5 = b_7 \cdot \left(e^{b_5 \cdot x_3|_{t=0} \cdot \left(1-e^{-b_2 \cdot t}\right)} - 1\right)$ b_7 =0.0015 nM (S95)

Let us now perform normalization of differential equations S43-S52. For factors Xa, IIa, Va, the following maximal values will be used:

Xa: $x_3\big|_{t=0} \cdot b_3$ (S96)

IIa: $b_4 \cdot x_3\big|_{t=0} \cdot e^{b_5 \cdot TF|_{t=0}}$ (S97)

Va: $b_7 \cdot \left(e^{b_5 \cdot x_3|_{t=0}} - 1\right)$ (S98)

For factors X, II, V, TFPI, AT-III, we shall use their initial concentrations. Fibrin is an indicator variable, which does not affect the rest of the system; in addition, it is actually proportional to factor Va. Therefore, it will not be analyzed in detail. The normalization is carried out by analogy with that for the initiation reactions. For short, we shall write down only the final multiplier parameters obtained:

Xa: $\varepsilon_6 = \frac{h_2 \cdot i_2\big|_{t=0} \cdot b_3}{\frac{k_6}{K_6} \cdot y_{10}\big|_{t=0}} = 0.017$ (S99)

X: $\varepsilon_7 = \frac{h_2 \cdot i_2\big|_{t=0}}{x_3\big|_{t=0} \cdot \frac{k_6}{K_6}} = \frac{0.96 \text{ nM}}{x_3\big|_{t=0}}$ (S100)

IIa: $\varepsilon_8 = \frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0} \cdot b_4 \cdot e^{b_5 \cdot x_3|_{t=0}}}{b_3 \cdot y_7\big|_{t=0} \cdot k_{12} \cdot p \cdot y_2\big|_{t=0}} = 0.16 \cdot e^{\frac{x_3|_{t=0}}{3.7 \ fM}}$ (S101)

II: $\varepsilon_9 = \frac{h_2 \cdot i_2\big|_{t=0} \cdot x_7\big|_{t=0}}{x_3\big|_{t=0} \cdot b_3 \cdot y_7\big|_{t=0} \cdot k_{12} \cdot p} = \frac{1234 \text{ nM}}{x_3\big|_{t=0}}$ (S102)

Va: $\varepsilon_{10}=\dfrac{h_2\cdot i_2\big|_{t=0}\cdot x_7\big|_{t=0}\cdot K_{16}\cdot b_7}{b_4\cdot y_7\big|_{t=0}\cdot k_{16}\cdot y_5\big|_{t=0}}\dfrac{1-e^{\frac{x_3|_{t=0}}{3.7\ fM}}}{x_3\big|_{t=0}}=0.02\text{ nM}\cdot\dfrac{1-e^{\frac{x_3|_{t=0}}{3.7\ fM}}}{x_3\big|_{t=0}}$ (S103)

V: $\varepsilon_{11}=\dfrac{h_2\cdot i_2\big|_{t=0}\cdot x_7\big|_{t=0}\cdot K_{16}}{b_4\cdot y_7\big|_{t=0}\cdot k_{16}}\dfrac{1}{x_3\big|_{t=0}\cdot e^{\frac{x_3|_{t=0}}{3.7\ fM}}}=\dfrac{0.42\text{ nM}}{x_3\big|_{t=0}\cdot e^{\frac{x_3|_{t=0}}{3.7\ fM}}}$ (S104)

AT-III: $\varepsilon_{12}=\dfrac{h_2\cdot i_2\big|_{t=0}\cdot x_7\big|_{t=0}}{b_4\cdot y_7\big|_{t=0}\cdot h_9}\cdot\dfrac{1}{x_3\big|_{t=0}\cdot e^{\frac{x_3|_{t=0}}{3.7\ fM}}}=\dfrac{201\text{ nM}}{x_3\big|_{t=0}\cdot e^{\frac{x_3|_{t=0}}{3.7\ fM}}}$ (S105)

TFPI: $\varepsilon_{13}=\dfrac{i_2\big|_{t=0}}{x_3\big|_{t=0}}=\dfrac{2.5\text{ nM}}{x_3\big|_{t=0}}$ (S106)

Comparison of the values of ε_0-ε_{13} (Table S3) shows that majority of the variables in the set are indeed either rapid ($\varepsilon<<1$) or slow ($\varepsilon>>1$) within the timescale considered if TF concentration is in the picomolar range. Exceptions to this rule are concentrations of total TF ($x_{3\text{p}}$), factor Va (x_5), and thrombin (x_2), though at sufficiently low TF (0.01 pM and less) thrombin also becomes a rapid variable. Thus the more general reduced version of the model of blood coagulation initiation has the following form:

$$\begin{cases}\dfrac{dx_{3p}}{dt}=-b_2\cdot x_{3p}\\ \dfrac{dx_2}{dt}=b_3\cdot x_{3p}\cdot\left(a_3+a_4\cdot x_5\right)-a_5\cdot x_2\\ \dfrac{dx_5}{dt}=a_6\cdot x_2\\ x_1=a_7/a_6\cdot x_5\end{cases}\quad\text{(S107)}$$

For very low TF concentrations, thrombin is a rapid variable, and the system has an explicit solution:

$$\begin{cases}x_{7-3}=x_3\big|_{t=0}\cdot b_1\cdot e^{-b_2\cdot t}\\ x_{10}=x_3\big|_{t=0}\cdot b_3\cdot e^{-b_2\cdot t}\\ x_2=x_3\big|_{t=0}\cdot b_4\cdot e^{b_5\cdot x_3|_{t=0}\cdot\left(1-e^{-b_2\cdot t}\right)}\cdot e^{-b_2\cdot t}\\ x_1=b_6\cdot\left(e^{b_5\cdot x_3|_{t=0}\cdot\left(1-e^{-b_2\cdot t}\right)}-1\right)\\ x_5=b_7\cdot\left(e^{b_5\cdot x_3|_{t=0}\cdot\left(1-e^{-b_2\cdot t}\right)}-1\right)\end{cases}\quad\text{(S108)}$$

In order to take into account fibrinogen consumption, the term $a_7\cdot x_2$ in eq. S91 should be transformed into $a_7\cdot x_2\cdot\left(1-\dfrac{x_1}{y_1\big|_{t=0}}\right)$. This gives a more accurate version of the solution for fibrin:

$$x_1 = y_1\big|_{t=0} \cdot \left(1 - e^{-\frac{b_6}{y_1|_{t=0}} \cdot \left(e^{b_5 \cdot x_3|_{t=0} \cdot \left(1-e^{-b_2 \cdot t}\right)} - 1\right)}\right) \quad \text{(S108a)}$$

Reduction without factor V. If we now consider reduction of a model without factor V (which corresponds to the case of factor V-deficient plasma), the result will be qualitatively different. Without this feedback, the equation for thrombin will be:

IIa: $\frac{dx_2}{dt} = a_3 \cdot x_{10} - a_5 \cdot x_2$ (S109)

Thrombin will be a rapid variable independently of TF concentration. Its reduction will be much simpler and will result in an exponential dependence identical to those for the VIIa–TF complex and factor Xa:

IIa: $x_2 = \frac{a_3 \cdot x_{10}}{a_5} = \frac{a_3 \cdot b_3}{a_5} \cdot x_3\big|_{t=0} \cdot e^{-b_2 \cdot t}$ (S110)

Fibrin kinetics then will be:

Fibrin: $x_1 = a_7 \cdot \int_{\tau=0}^{\tau=t} x_2(\tau) \cdot d\tau = \frac{a_7 \cdot a_3 \cdot b_3 \cdot x_3\big|_{t=0}}{a_5} \cdot \int_{\tau=0}^{\tau=t} e^{-b_2 \cdot t} \cdot d\tau = \frac{a_7 \cdot a_3 \cdot b_3 \cdot x_3\big|_{t=0}}{a_5 \cdot b_2} \cdot (1 - e^{-b_2 \cdot t})$ (S111)

This formula is qualitatively different from eq. S108 both at small and large temporal intervals. Instead of exponential growth, it is a linear function of t at $t \to 0$ and a linear function of $TF\big|_{t=0}$ at $t \to \infty$.

By analogy with eq. S108a, a version accounting for fibrinogen consumption is:

$$\left\{ x_1 = y_1\big|_{t=0} \cdot \left(1 - e^{-\frac{1}{y_1|_{t=0}} \cdot \frac{a_7 \cdot a_3 \cdot b_3 \cdot x_3|_{t=0}}{a_5 \cdot b_2} \cdot (1-e^{-b_2 \cdot t})}\right) \right. \quad \text{(S111a)}$$

4. Phase Plane Analysis of the Model

Check for a stable steady state existence and retention during the reduction

The first step in the analysis of a mathematical model is identification of stable steady-state solutions. Examination of the original model (eqs. S1-S35) shows that steady state is possible when, and only when concentrations of all active coagulation factors (except for factors VIIa and Va that do not possess independent activity and do not have first-order inhibition) are zero. Otherwise, they cause irreversible activation of the predecessors, and the reaction rates are not zero. The same holds for the reduced model (eqs. S107). Furthermore, this solution is stable. While it is usually studied by Jacobi matrix analysis (55), a simpler demonstration can suffice for this system. If the model (eqs. S1-S35) is linearized in the vicinity of the stable state, all positive feedback loops except for negligible activation of factors XI and VII would disappear completely; and a proteolytic

cascade with inhibition of all active enzymes is stable (56). Stability of the steady state in a reduced model (eqs. S107) follows from the same considerations indicating that (x_{3p}=0, x_2=0) is the only attractor in this system.

It should be noted though that this attractor is not actually a point, but a line (x_{10}=0, x_2=0, x_5 can be any). This occurs because factor Va is not inhibited in the reduced system, and only accumulates. In the complete model (eqs. S1-S35), it can be inhibited by activated protein C, but this inhibition is second-order. Therefore, it requires a sufficient amount of activated protein C to be produced, and this occurs only when large quantities of thrombin are already available. Thus, contribution of this inhibition to system dynamics was rightly judged to be negligible from Table S2. Because of this, stability of the system (eqs. S107) changes along the line (x_{10}=0, x_2=0, x_5 is arbitrary): much smaller activation is needed for explosive thrombin formation if there is non-zero factor Va present. Thus, while the steady state of the system is stable, it is not robust: small perturbations of factor Va concentration can cause significant changes.

This non-robustness is not an obstacle for the use of the models. They still remains correct with regard to experimental data description and accuracy of reduction. However, for the purpose of dynamic analysis, it is advisable to make the system robust by adding a perturbation in the form of some non-zero, first-order rate of factor Va inhibition. This "robusterization" serves to achieve two ends: 1) to check structural stability, i.e. if this will qualitatively change the behavior of the system; 2) to highlight the mechanisms of the system dynamics, which are usually more convenient to observe in a robust system. While no specific inhibitor of factor Va except for activated protein C is known, it is likely that the life-time of this active cofactor in plasma is limited due to degradation by non-specific proteases. Therefore, introduction of a first-order inhibition of this factor in the system is a reasonable assumption and a good test of structural stability of the system.

Analysis of the reduced "robusterized" model

In order to analyze system dynamics qualitatively, let us make the reduced model (eqs. S107) structurally robust by adding an arbitrarily small term for factor Va inhibition as discussed in the previous section:

$$\frac{dx_5}{dt} = a_6 \cdot x_2 - h_{x_5} \cdot x_5 \tag{S112}$$

Assuming this variable to be rapid it is possible to re-write eqs. S107 in the following form:

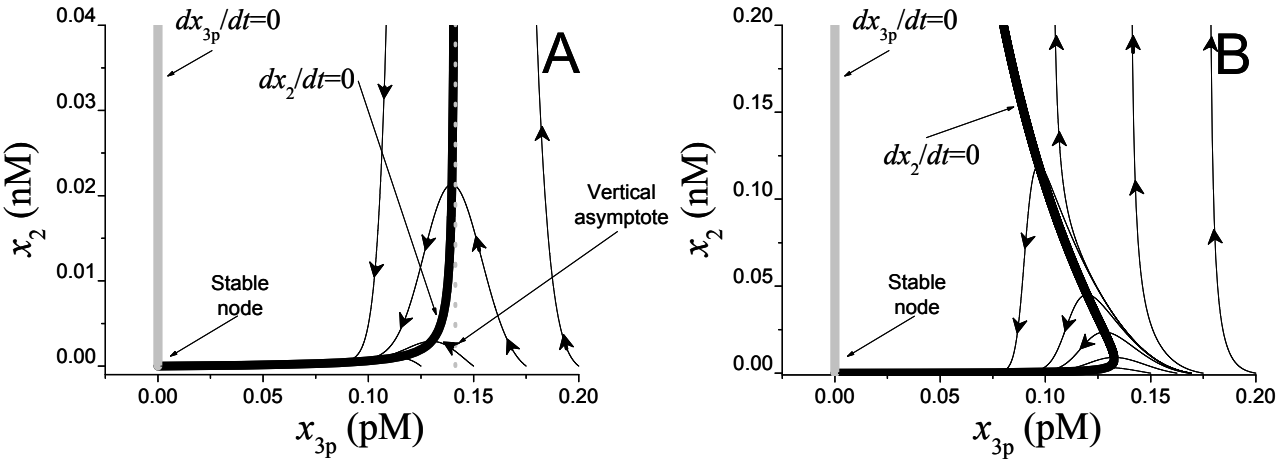
$$\begin{cases} \dfrac{dx_{3p}}{dt} = -b_2 \cdot x_{3p} \\ \dfrac{dx_2}{dt} = x_{3p} \cdot (\alpha_1 + \alpha_2 \cdot x_2) - h \cdot x_2 \\ x_{3p}\big|_{t=0} = x_3\big|_{t=0} \\ x_2\big|_{t=0} = 0 \end{cases} \tag{S113}$$

New parameters α_i and h are combinations of the old ones (a_i) and are used for better readability. They are all positive and non-zero. The null-clines for this set are:

$$\begin{aligned} &\frac{dx_{3p}}{dt} = 0 \quad \Rightarrow \quad x_{3p} = 0 \\ &\frac{dx_2}{dt} = 0 \quad \Rightarrow \quad x_{3p} = \frac{h \cdot x_2}{\alpha_1 + \alpha_2 \cdot x_2} \end{aligned} \tag{S114}$$

A phase portrait for this set is shown in Fig. S3A. The null-clines intersect in the point of origin, which is a stable and robust node. There is no true activation threshold in this system. All phase paths are qualitatively similar: thrombin is generated as a pulse in response to TF addition. However, because of the non-linearity of the null-cline for $dx_2/dt = 0$, which has a vertical asymptote at $x_{3p} = \alpha_3/\alpha_2$, peak thrombin concentration grows nonlinearly as a function of initial TF.

Fig. S3. Phase diagrams of the reduced models. (A) The diagram for the linear model (equations S113). (B) The diagram for the model taking into account the feedback effect of thrombin on factor Xa (equations S115). Parameters used are listed in Supporting Information 4. Null-clines $dx_2/dt = 0$ and $dx_{3p}/dt = 0$ are shown as thick black and gray lines, respectively. Directions of phase trajectories are shown by short arrows. Typical phase trajectories are thin solid lines.



Let us next take into account the effect of factor VIII feedback activation on thrombin generation although we already know that it does not contribute to fibrin generation. Assuming factor VIIIa and factor IXa concentrations to be rapid variables gives an additional thrombin-dependent item in the factor Xa production, i.e. $x_{10} \propto x_{3p} \cdot (1 + \alpha_3 + \alpha_4 \cdot x_2)$. The model can be rewritten as follows:

$$\begin{cases} \dfrac{dx_{3p}}{dt} = -b_2 \cdot x_{3p} \\ \dfrac{dx_2}{dt} = x_{3p} \cdot (\alpha_1 + \alpha_2 \cdot x_2) \cdot (1 + \alpha_3 + \alpha_4 \cdot x_2) - h \cdot x_2 \end{cases} \tag{S115}$$

The resulting quadratic non-linearity in the second equation makes the respective null-cline more complex:

$$\frac{dx_2}{dt} = 0 \quad \Rightarrow \quad x_{3p} = \frac{h \cdot x_2}{(\alpha_1 + \alpha_2 \cdot x_2) \cdot (1 + \alpha_3 + \alpha_4 \cdot x_2)} \tag{S116}$$

The resulting phase diagram is shown in Fig. S3B. This modification makes the response more explosive.

Finally, let us evaluate possible effect of the long-range feedback of factor XI activation adding this pathway back into the reduced model. Assuming factor XIa concentration to be a rapid variable, we obtain:

$$\begin{aligned} x_{11} &\propto x_2 \\ x_9 &\propto x_{3p} + \alpha_5 \cdot x_2 \end{aligned} \tag{S117}$$

Algebraic equation for factor Xa becomes more complex:

$$x_{10} \propto x_{3p} + (\alpha_3 + \alpha_4 \cdot x_2) \cdot (x_{3p} + \alpha_5 \cdot x_2) \tag{S118}$$

And the model becomes:

$$\begin{cases} \dfrac{dx_{3p}}{dt} = -b_2 \cdot x_{3p} \\ \dfrac{dx_2}{dt} = \left(x_{3p} + (\alpha_3 + \alpha_4 \cdot x_2) \cdot (x_{3p} + \alpha_5 \cdot x_2)\right) \cdot (\alpha_1 + \alpha_2 \cdot x_2) - h \cdot x_2 \end{cases} \tag{S119}$$

For the original parameters listed in Table S1, the values of α_i are:

α_1=1.70 min^{-1}

$\alpha_2 = \dfrac{1}{h_{x_5}} \cdot$ 2327.4 min^{-2}nM^{-1}=6649 min^{-1}nM^{-1} for h_{x_5} =0.35 min^{-1}

α_3=0.0022 (S120)

α_4=3.8 nM^{-1}

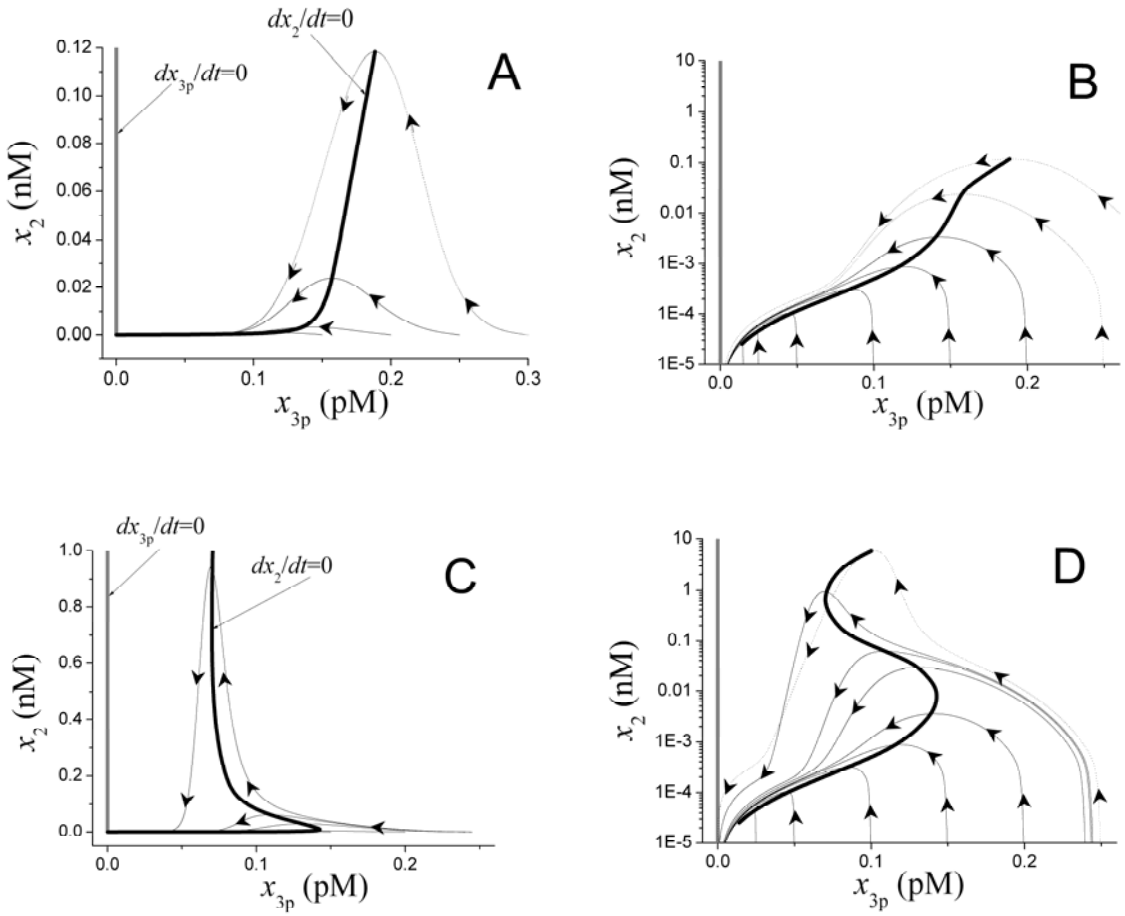
α_5=0.0073

h=0.94 min^{-1}

Analysis of the complete "robusterized" model

In order to test the qualitative predictions obtained in the reduced model, phase diagrams for the complete model of blood coagulation were calculated. To make this model robust, factor Va was assumed to be inhibited at a constant rate identical to that of factor VIIIa. Fig. S4 shows the results obtained for a model without (panels A and B) or with (panels C and D) the positive feedback of factor VIII activation.

Fig. S4. Phase diagrams of the complete "robusterized" model. The panels show projections of phase diagrams on the x_{3p}-x_2 plane. They were calculated for the complete model (equations S1-S35) with a single modification: factor Va is inhibited at a rate of 0.35 min^{-1}. (A, B) Simulation of clotting without factor VIII; (C, D) simulation of clotting in normal plasma. Panels B and D show the same data as panels A and C, respectively, in a semi-logarithmic scale. Null-clines $dx_2/dt = 0$ and $dx_{3p}/dt = 0$ are shown as thick black and gray lines, respectively. Directions of phase trajectories are shown by short arrows. Thin solid lines are typical phase paths. Thin dot lines show phase paths at high TF concentration when limitations on the amount of inactive precursors become important.



The diagrams agree very well with the predictions (Fig. S3), with the only exception that the null-cline $dx_2/dt = 0$ is curved to the right at high initial TF concentrations, because limitations on the amount of inactive precursors become important. Strongly non-linear, explosive dependence of thrombin impulse amplitude on the initial TF concentration is best observed in the semi-logarithmic plots.

Fig. S5. Clot times do not qualitatively depend on factor V. Theoretical (A) and experimental (B) clot times versus TF concentration curves for normal (*solid curves*) and factor V-deficient (*dot curves*) plasmas. The data shown are for the same experiments as those in Fig. 5. The results are means ± S.E.M. for n=3 experiments. In the insets, the clot time axis is reciprocal.

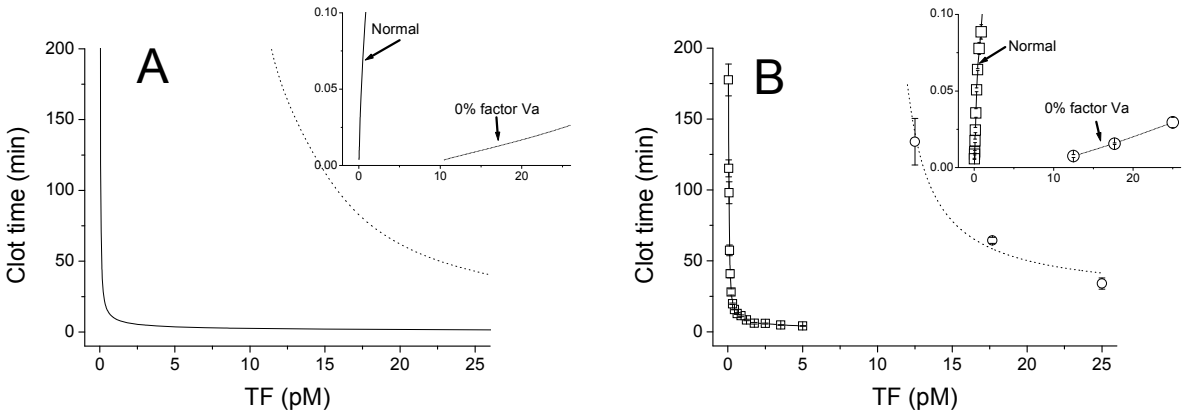
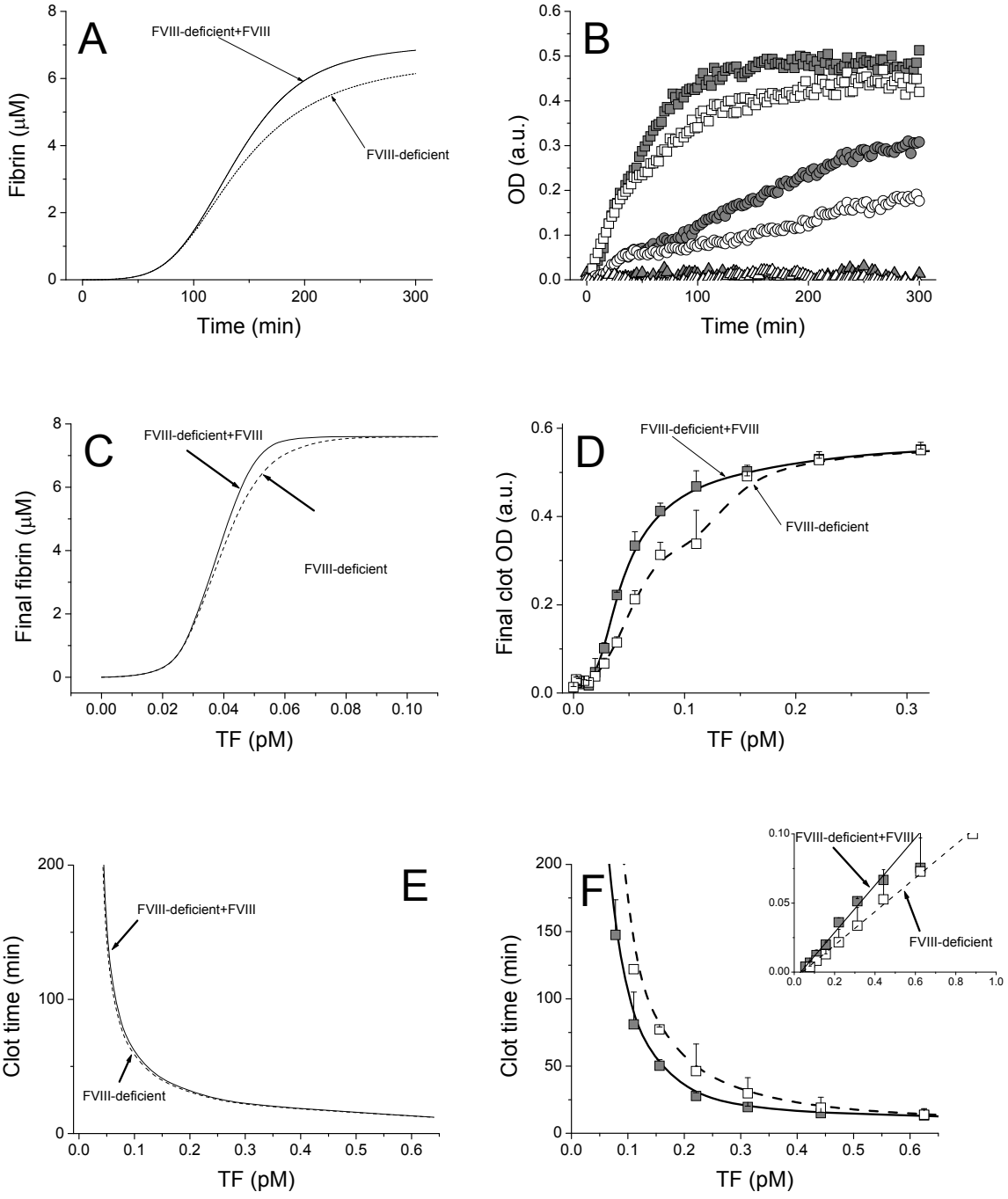


Fig. S6 The role of factor VIII: theory and experiments. Coagulation in factor VIII-deficient plasma supplemented with 0.037 nM factor VIII (filled symbols, solid lines) or without additions (empty symbols, dashed lines); computer simulations (A. C. E) and experiments (B, D, F). Clotting kinetics (A, B), final clot density as a function of TF (C, D), and clot formation time as a function of TF (E, F) are shown. Error bars show S.D. for n=2 experiments. TF concentration in (A) is 0.05 pM; TF concentrations in (B) are 0.156 (squares), 0.055 (circles), and 0.0024 (triangles) pM. Calculations were performed as in the legend to Fig. 1; the experiments were performed as described in the Materials and Methods.



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