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ILLUSTRATED REVIEW

New insight into the traditional model of the coagulation cascade and its regulation: illustrated review of a three-dimensional view

Romualdo Troisi[1,2] | Nicole Balasco[3] | Ida Autiero[2] | Filomena Sica[1] | Luigi Vitagliano[2]

[1]Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Naples, Italy

[2]Institute of Biostructures and Bioimaging, CNR, Naples, Italy

[3]Institute of Molecular Biology and Pathology, CNR c/o Department of Chemistry, University of Rome Sapienza, Rome, Italy

Correspondence
Filomena Sica, Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cintia, 80126 Naples, Italy.
Email: filomena.sica@unina.it

Luigi Vitagliano, Institute of Biostructures and Bioimaging, CNR, Via Pietro Castellino 111, 80131 Naples, Italy.
Email: luigi.vitagliano@cnr.it

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Abstract

The coagulation process relies on an intricate network of three-dimensional structural interactions and subtle biological regulations. In the present review, we illustrate the state of the art of the structural biology of the coagulation cascade by surveying the Protein Data Bank and the EBI AlphaFold databases. Investigations performed in the last decade have provided structural information on essentially all players involved in the process. Indeed, the initial characterization of specific and rather canonical domains has been progressively extended to complicated multidomain proteins. Recently, the application of cryogenic electron microscopy techniques has unraveled the structural features of highly complex coagulation factors, which has led to enhanced understanding. This review initially focuses on the structure of the individual factors as a function of their involvement in intrinsic, extrinsic, and common pathways. A specific emphasis is given to what is known or unknown on the structural basis of each step of the cascade. Available data providing clues on the structural recognition of the factors involved in the functional partnerships of the pathways are illustrated. Recent structures of important complexes formed by these proteins with regulators are described, focusing on the drugs used as anticoagulants and on their reversal agents. Finally, we highlight the different roles that innovative biomolecules such as aptamers may have in the regulation of the cascade.

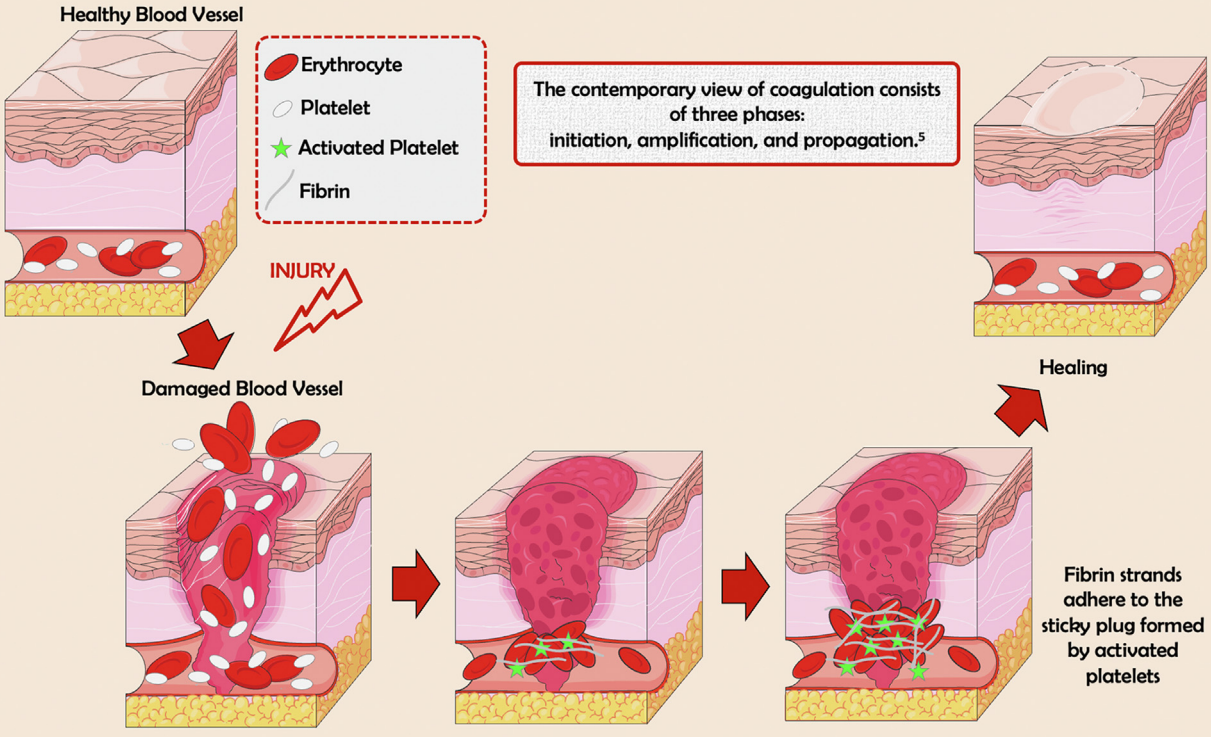
KEYWORDS
anticoagulants, blood coagulation factors, drug interactions, protein conformation, thrombin

Essentials

- Experimental and computational studies have led to an atomic-level understanding of coagulation.
- Key information on the structural aspects of many hemostatic interactions has been elucidated.
- Available data explain the detailed mechanism of action of drugs and reversal agents.
- Structure-based approaches are key to drug optimization.

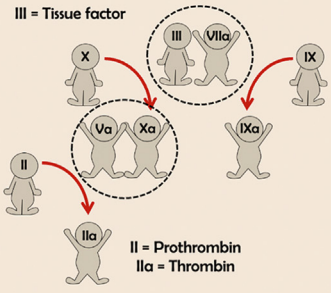
The contemporary view of blood coagulation

Coagulation is a complex biological process that involves a controlled sequence of proteolytic activations of a series of zymogens to accomplish appropriate and timely hemostasis of a damaged vessel through the formation of the final fibrin product.[1-7] All the proteins involved in this process are named coagulation factors.



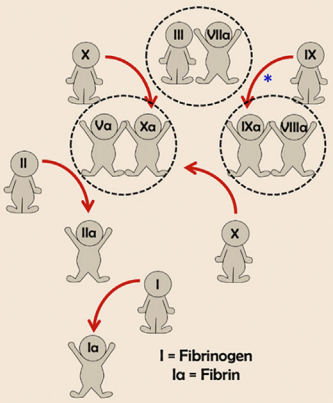
1. Initiation

Exposure of tissue factor to coagulation factors



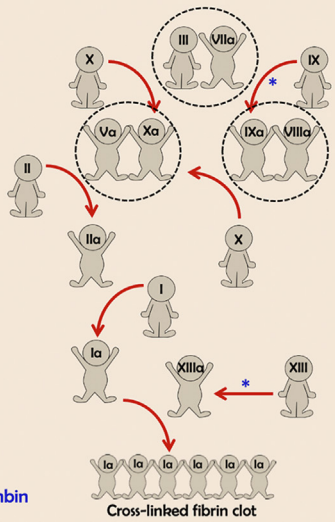
2. Amplification

Conversion from extrinsic to intrinsic thrombin generation



3. Propagation

Thrombin generation with cross-linked fibrin deposition



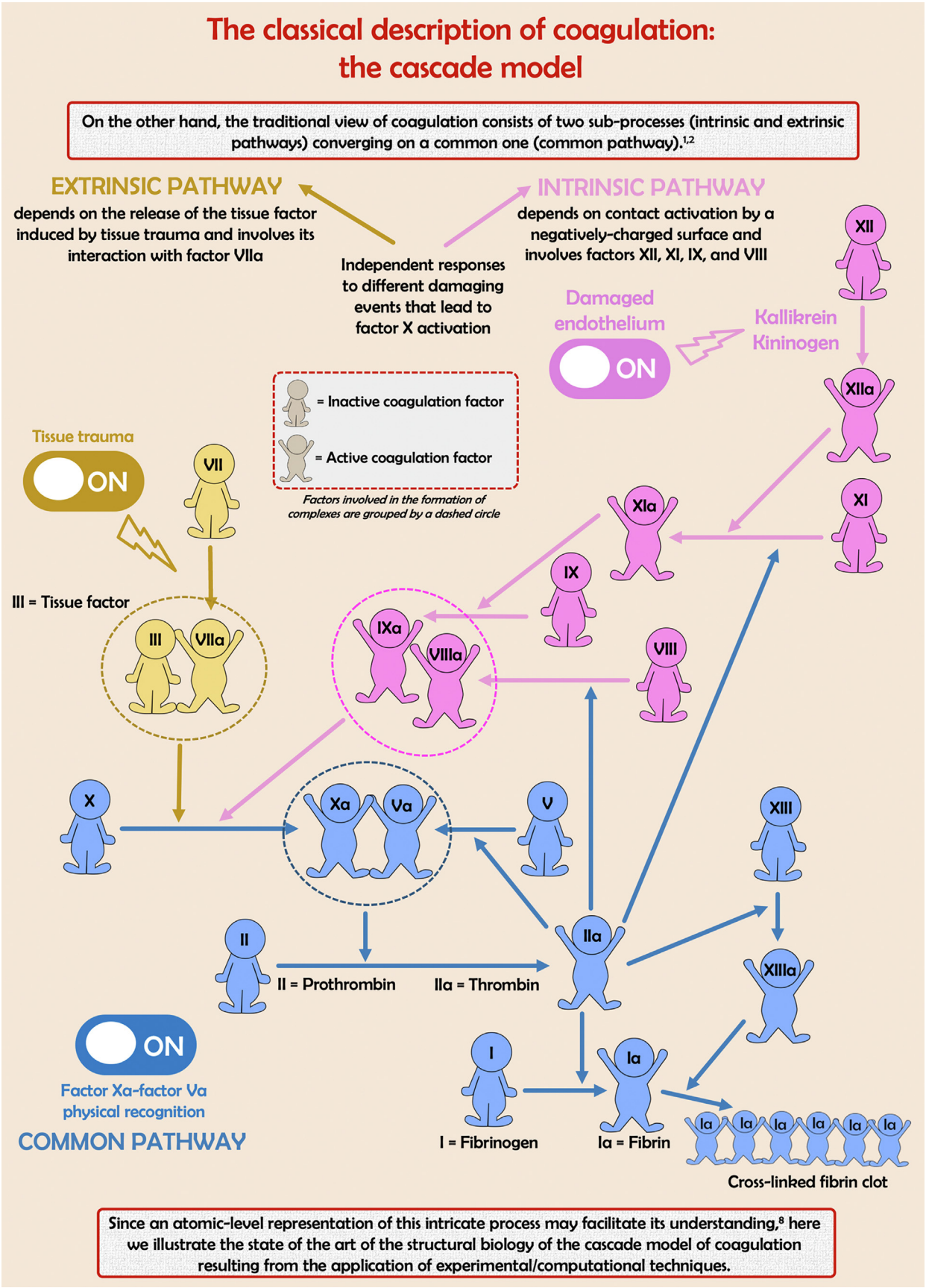
= Inactive coagulation factor

= Active coagulation factor

Factors involved in the formation of complexes are grouped by a dashed circle

* These reactions are activated also by thrombin

The inactive coagulation factors are labelled with roman numerals. The letter "a" follows the roman numerals in the active forms.


The classical description of coagulation: the cascade model
On the other hand, the traditional view of coagulation consists of two sub-processes (intrinsic and extrinsic pathways) converging on a common one (common pathway).[1,2]
EXTRINSIC PATHWAY
depends on the release of the tissue factor induced by tissue trauma and involves its interaction with factor VIIa
INTRINSIC PATHWAY
depends on contact activation by a negatively-charged surface and involves factors XII, XI, IX, and VIII
Independent responses to different damaging events that lead to factor X activation
Damaged endothelium
ON
Kallikrein
Kininogen
= Inactive coagulation factor
= Active coagulation factor
Factors involved in the formation of complexes are grouped by a dashed circle
Tissue trauma
ON
III = Tissue factor
XII
XIIa
XI
XIa
IX
IXa
VIIIa
VIII
VII
III
VIIa
X
Xa
Va
V
XIII
XIIIa
II
IIa
II = Prothrombin
IIa = Thrombin
ON
Factor Xa-factor Va physical recognition
COMMON PATHWAY
I
Ia
I = Fibrinogen
Ia = Fibrin
Cross-linked fibrin clot
Since an atomic-level representation of this intricate process may facilitate its understanding,[8] here we illustrate the state of the art of the structural biology of the cascade model of coagulation resulting from the application of experimental/computational techniques.

Structural biology techniques

The characterization at the atomic level of the biomolecules is an essential prerequisite for a full understanding of biological processes.

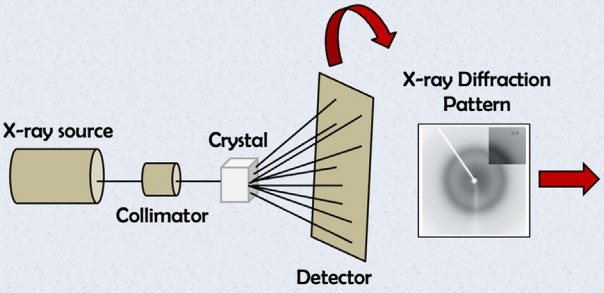
In the last decades, the description of complex biological processes has increasingly benefited from important technological advances that have made structural studies more effective.

A repertoire of different experimental and theoretical techniques, each endowed with specific application properties, is available.

Experimental techniques

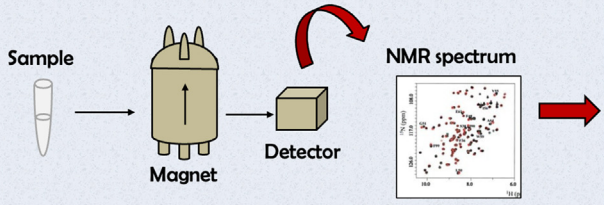
X-ray Crystallography

For many years, most of the available data have been derived by applying the powerful technique based on the X-ray diffraction of ordered crystals composed of biomolecules.[9] The successful application of this technique provides accurate three-dimensional models for biomolecules of different sizes from small molecules to large complexes. However, the application of this technique relies on ordered crystals that are sometimes difficult to be obtained.



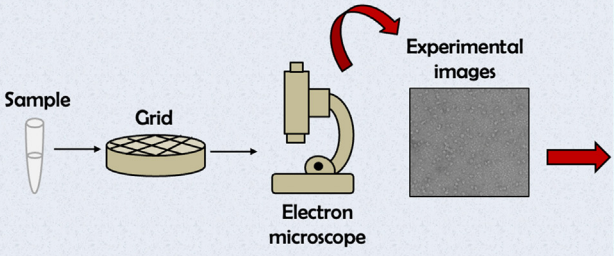
Nuclear Magnetic Resonance - NMR

Complementary information can be collected using the NMR methodology.[10] This technique, in addition to static structural data, can also provide information on the dynamics of biomolecules. The most important limitation of this technique is the limited size (generally up to ~ 30 kDa) of the biomolecules that can be characterized.

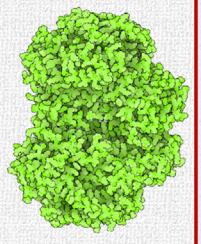


Cryo-electron microscopy - Cryo-EM

In the last decade, the structural biology field has been highly impacted by the enormous technical advancements of cryo-EM[11,12] that have progressively allowed the rapid structural characterization of giant macromolecular machineries.
However, very high-resolution structures are rarely obtained using this technique. Currently, only 0.5% of the cryo-EM structures in the PDB are solved at a resolution better than 2.0 Å.



Atomic level visualization of protein and nucleic acid structures



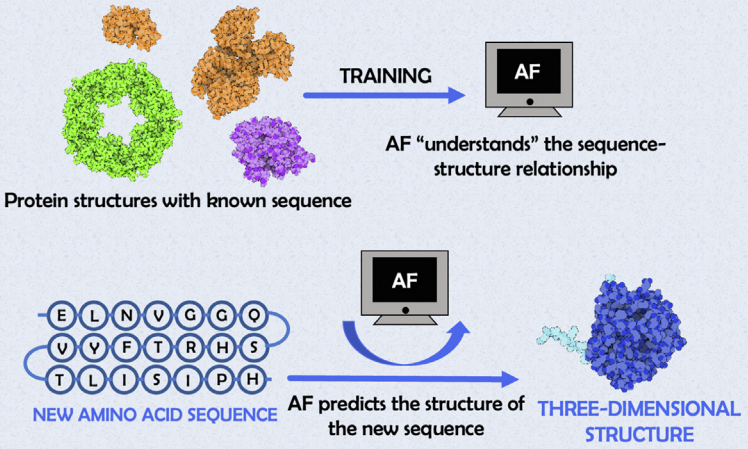
Machine learning approach

In the last couple of years, the field is being further revolutionized by the implementation of innovative algorithms, based on machine learning techniques, that are close to achieving the holy grail of structural biology i.e., a pure computational prediction of the three-dimensional structure of proteins from their amino acid sequence.

AlphaFold - AF

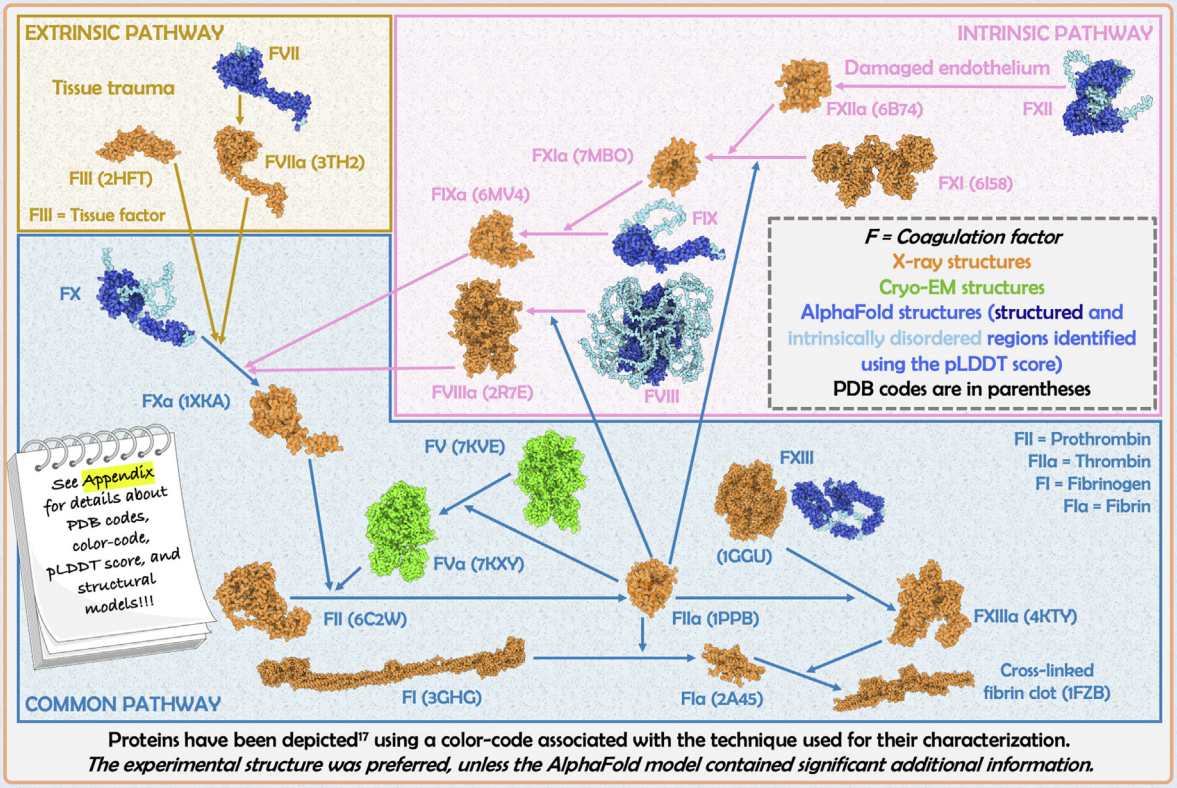
https://alphafold.ebi.ac.uk

This initiative, AlphaFold project developed by DeepMind, has led to the formulation of reliable models for over 200 million proteins, essentially all those with a known sequence.[13-16]



The structural biology of the coagulation cascade

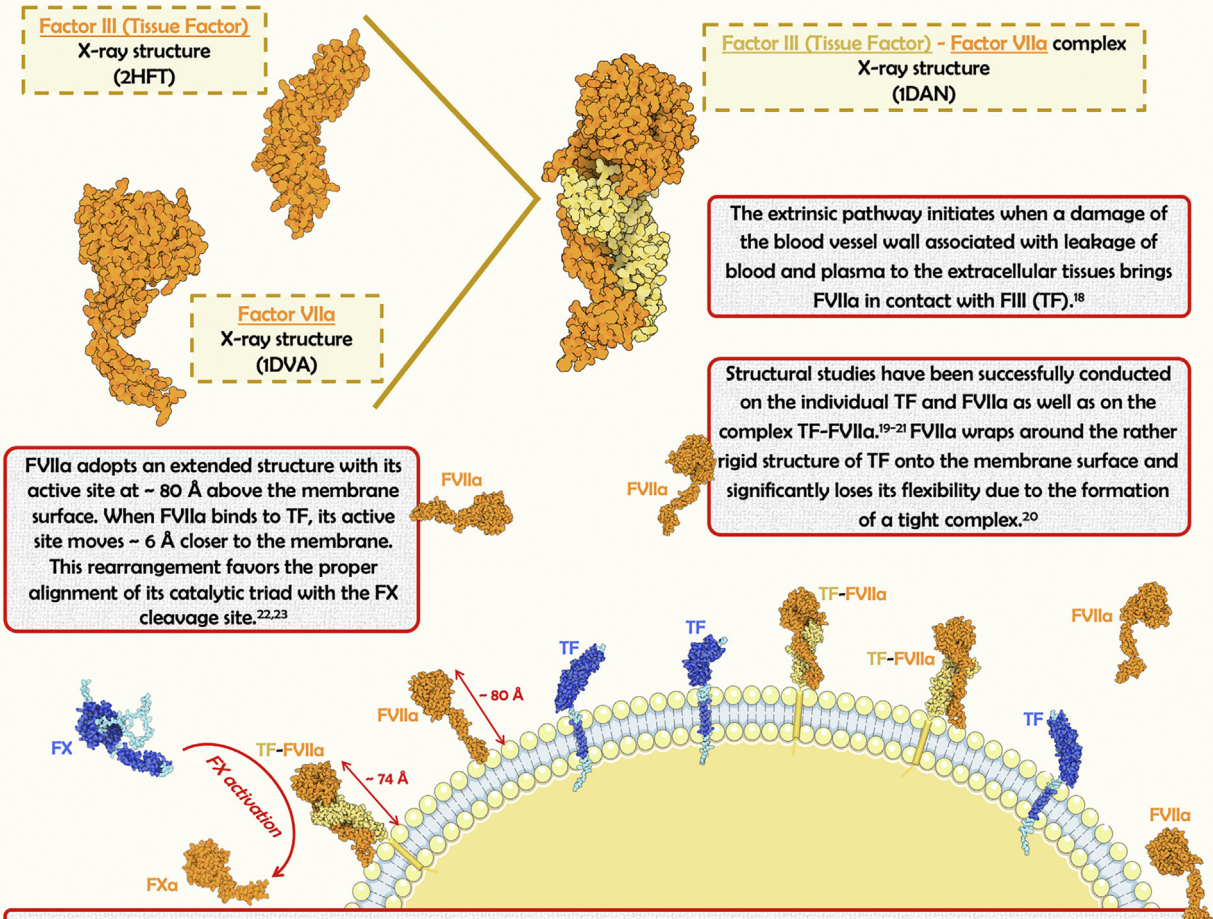
The application of the structural biology methodologies has provided an atomic level representation of all partners involved in the coagulation cascade. Indeed, except for few disordered regions, structural information is essentially available for all coagulation factors, at least as individual protein entities.



Proteins have been depicted[17] using a color-code associated with the technique used for their characterization.
The experimental structure was preferred, unless the AlphaFold model contained significant additional information.

It is worth deepening into the molecular interactions underlying the initiation events of the three pathways.

Molecular interactions that initiate the extrinsic pathway



The extrinsic pathway initiates when a damage of the blood vessel wall associated with leakage of blood and plasma to the extracellular tissues brings FVIIa in contact with FIII (TF).[18]

Structural studies have been successfully conducted on the individual TF and FVIIa as well as on the complex TF-FVIIa.[19–21] FVIIa wraps around the rather rigid structure of TF onto the membrane surface and significantly loses its flexibility due to the formation of a tight complex.[20]

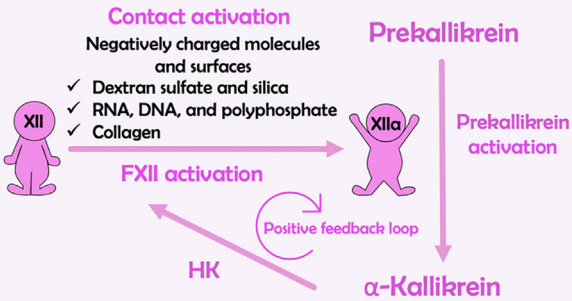
FVIIa adopts an extended structure with its active site at ~ 80 Å above the membrane surface. When FVIIa binds to TF, its active site moves ~ 6 Å closer to the membrane. This rearrangement favors the proper alignment of its catalytic triad with the FX cleavage site.[22,23]

The TF-FVIIa complex leads to the activation of FX. It has been demonstrated that the activity of FVIIa towards FX dramatically increases (nearly one-million-fold) upon TF binding.[23] Unfortunately, no structural data are hitherto available for the TF-FVIIa-FX ternary complex.

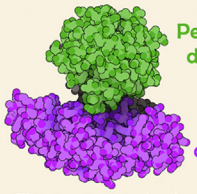
Molecular interactions that initiate the intrinsic pathway

The physiological relevance of the intrinsic pathway has been disputed for long time due to the difficulties in identifying its triggering event. Only in 2009, it has been shown that fibrillar type I collagen provokes a dose-dependent shortening of the clotting time of human plasma *via* activation of FXII followed by the interaction with prekallikrein.[24]

FXII can be activated by exposure to negatively charged molecules and physiological surfaces.[25] No structural information is available on the FXII-collagen interactions.



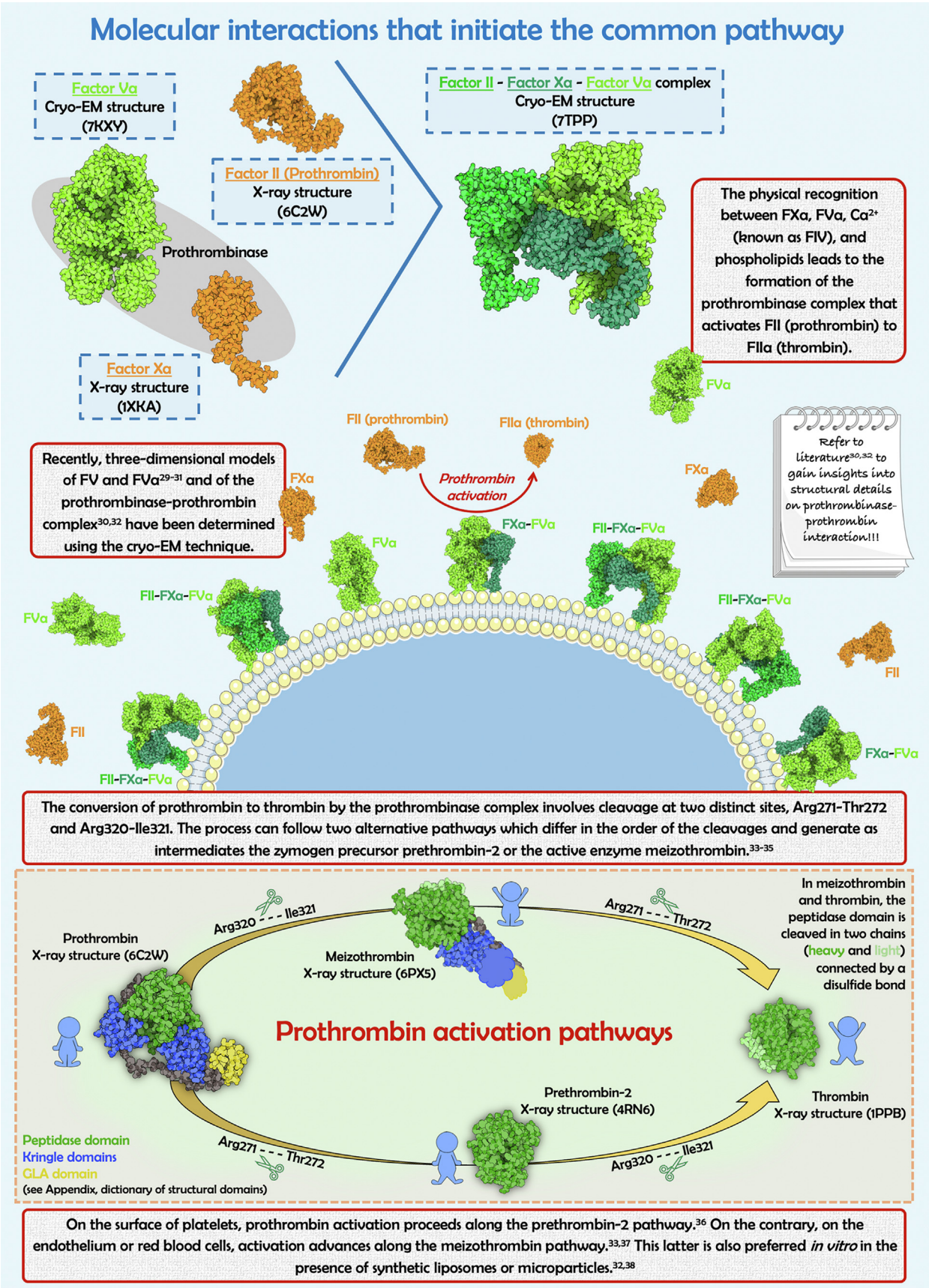
Prekallikrein X-ray structure (6I44)



This structure is closely related to that of FXI[26–28] (see Appendix, dictionary of structural domains)

FXIIa activates prekallikrein forming α-kallikrein that can itself activate FXII. FXII activation mediated by α-kallikrein is nearly 30 times more efficient than FXII autoactivation on a negative surface.[25]

The reciprocal activation of FXII and prekallikrein is further amplified by the activity of the cofactor HK (high molecular weight kininogen).[25]


Molecular interactions that initiate the common pathway
Factor Va
Cryo-EM structure
(7KXY)
Factor II (Prothrombin)
X-ray structure
(6C2W)
Prothrombinase
Factor Xa
X-ray structure
(1XKA)
Factor II - Factor Xa - Factor Va complex
Cryo-EM structure
(7TPP)
The physical recognition between FXa, FVa, Ca2+ (known as FIV), and phospholipids leads to the formation of the prothrombinase complex that activates FII (prothrombin) to FIIa (thrombin).
FVa
FII (prothrombin)
FIIa (thrombin)
Prothrombin activation
FXa
Recently, three-dimensional models of FV and FVa[29-31] and of the prothrombinase-prothrombin complex[30,32] have been determined using the cryo-EM technique.
Refer to literature[30,32] to gain insights into structural details on prothrombinase-prothrombin interaction!!!
FXa-FVa
FII-FXa-FVa
FII
The conversion of prothrombin to thrombin by the prothrombinase complex involves cleavage at two distinct sites, Arg271-Thr272 and Arg320-Ile321. The process can follow two alternative pathways which differ in the order of the cleavages and generate as intermediates the zymogen precursor prethrombin-2 or the active enzyme meizothrombin.[33-35]
Prothrombin
X-ray structure (6C2W)
Arg320 - - - Ile321
Meizothrombin
X-ray structure (6PX5)
Arg271 - - - Thr272
In meizothrombin and thrombin, the peptidase domain is cleaved in two chains (heavy and light) connected by a disulfide bond
Prothrombin activation pathways
Prethrombin-2
X-ray structure (4RN6)
Thrombin
X-ray structure (1PPB)
Arg271 - - - Thr272
Arg320 - - - Ile321
Peptidase domain
Kringle domains
GLA domain
(see Appendix, dictionary of structural domains)
On the surface of platelets, prothrombin activation proceeds along the prethrombin-2 pathway.[36] On the contrary, on the endothelium or red blood cells, activation advances along the meizothrombin pathway.[33,37] This latter is also preferred in vitro in the presence of synthetic liposomes or microparticles.[32,38]

Structural insights into the modulation of the coagulation cascade by drugs

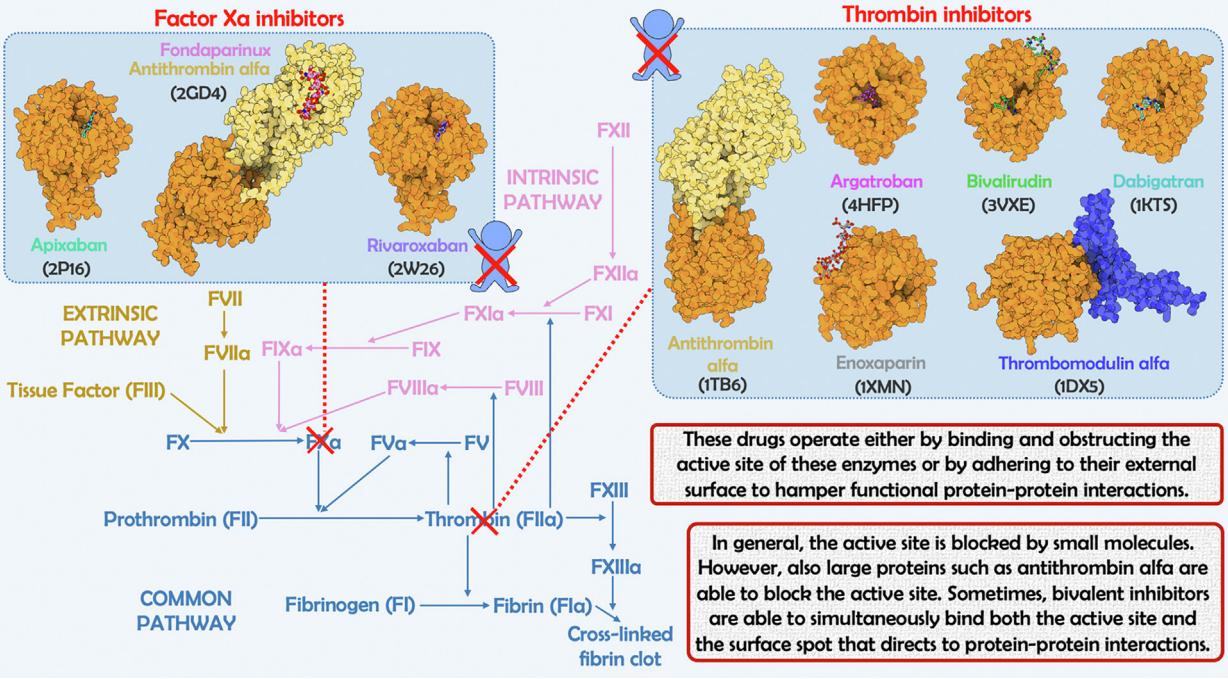
The relevance of the coagulation cascade in the human physio-pathology makes it an attractive target for the drug-developing process.[39] In this context, a full comprehension of the structural details of factor-drug interactions is fundamental.

Table of approved drug targeting factors of the coagulation cascade with anticoagulant activity.

DrugBank* ID	Name	Brand name	Target	PDB code**	Description
DB01109	Heparin	Heparin Leo	FXa, Thrombin	-	Anionic sulfated glycosaminoglycan polymers that, binding to antithrombin III (ATIII), leads to inactivation of FXa and thrombin. Reversal agent: protamine sulfate.
DB01225	Enoxaparin	Lovenox	FXa, Thrombin	1XMN (Thrombin)	Low-molecular-weight heparin, ATIII-mediated inhibitor of FXa and thrombin. Reversal agents: protamine sulfate, andexanet alfa.
DB09258	Bemiparin	-	FXa, Thrombin	-	Ultra-low-molecular-weight heparin, ATIII-mediated inhibitor of FXa. It has lower anti-thrombin activity than classical low-molecular-weight heparins. Reversal agent: protamine sulfate.
DB00569	Fondaparinux	Arixtra	FXa	2GD4	Chemically related low-molecular-weight heparin, ATIII-mediated selective inhibitor of FXa. Reversal agent: andexanet alfa.
DB06228	Rivaroxaban	Xarelto	FXa	2W26, 5VOF	Small molecule, FXa inhibitor. Reversal agent: andexanet alfa.
DB06605	Apixaban	Eliquis	FXa	2P16	Small molecule, FXa inhibitor. Reversal agent: andexanet alfa.
DB12364	Betrixaban	BEVYXXA	FXa	-	Small molecule, FXa inhibitor. Reversal agent: andexanet alfa.
DB09075	Edoxaban	Lixiana, Savaysa	FXa	-	Small molecule, FXa inhibitor. Reversal agent: andexanet alfa.
DB06695	Dabigatran etexilate	Pradaxa	Thrombin	1KTS	Prodrug that is hydrolyzed to the competitive and reversible direct thrombin inhibitor dabigatran. Reversal agent: idarucizumab.
DB00006	Bivalirudin	Angiomax	Thrombin	3VXE, 3VXF	Synthetic peptide, reversible thrombin inhibitor.
DB00278	Argatroban	-	Thrombin	4HFP	Small molecule derived from L-arginine, thrombin inhibitor.
DB11166	Antithrombin alfa	Atryn	FXa, Thrombin	2GD4 (FXa), 1TB6 (Thrombin)	Recombinant ATIII, FXa and thrombin inhibitor.
DB06404	Human C1-esterase inhibitor	Berinert, Cinryze, Haegarda	FXIa, FXIIa, Thrombin	-	Purified human C1-esterase inhibitor isolated from human plasma. It irreversibly inactivates FXIa, FXIIa, and thrombin.
DB09228	Conestat alfa	Ruconest	FXIa, FXIIa, Thrombin	-	Recombinant analogue of human C1-esterase inhibitor, purified from the milk of transgenic rabbits. It irreversibly inactivates FXIa, FXIIa, and thrombin.
DB05099	Ancrod	Viprinex	Fibrinogen alpha chain	-	Anticoagulant protease purified from Malayan pit viper venom. It inactivates circulating plasma fibrinogen.
DB11312	Protein C	Beriplex, Kcentra, Octaplex, Ceprotin	FVa, FVIIIa	-	Protein C (also known as FXIV) is converted by the thrombin/thrombomodulin complex to Activated Protein C (APC). Protein S is a cofactor for APC and together they proteolytically cleave FVa and FVIIIa. Protein S also inhibits FVa and FXa through protein-protein interactions.
DB13149	Protein S	Beriplex, Kcentra, Octaplex	FVa, FVIIIa, FXa	-	
DB05777	Thrombomodulin alfa (ART-123)	-	Thrombin, FVa	1DX5 (Thrombin)	Recombinant and soluble thrombomodulin, a human protein that inhibits thrombin generation by the activation of protein C and the subsequent inactivation of FVa in the presence of protein S.

* DrugBank Online (https://go.drugbank.com/) ** See Appendix, accession-codes

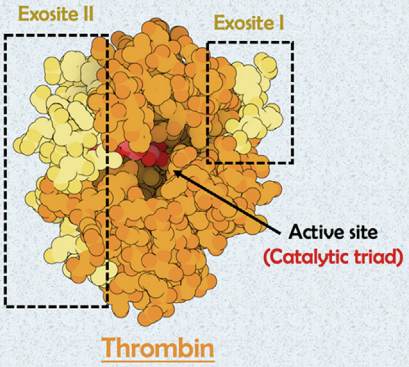
Most of the structurally characterized drugs work by inhibiting the activity of FXa and thrombin.



These drugs operate either by binding and obstructing the active site of these enzymes or by adhering to their external surface to hamper functional protein-protein interactions.

In general, the active site is blocked by small molecules. However, also large proteins such as antithrombin alfa are able to block the active site. Sometimes, bivalent inhibitors are able to simultaneously bind both the active site and the surface spot that directs to protein-protein interactions.

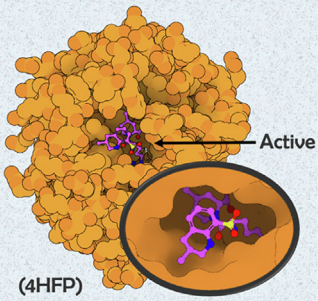
Structural mechanisms of thrombin inhibition

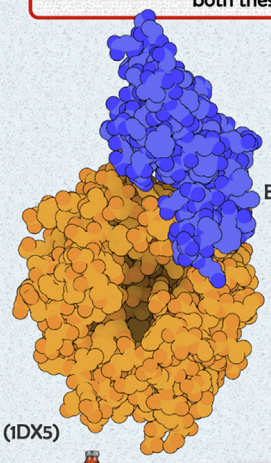


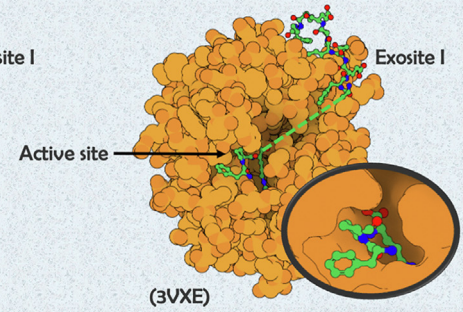
By converting FI (fibrinogen) to the final FIa (fibrin) product, thrombin represents the crucial player of the coagulation cascade. In addition to the active site, thrombin inhibitors have been designed and developed by targeting two protein external regions (exosites I and II).[40]

While argatroban blocks the active site,[41] thrombomodulin alfa anchors exosite I.[42,43] On the other hand, the bivalent molecule bivalirudin interacts with both these hotspots.[44]

Refer to literature[41,42,44] to gain insights into thrombin-inhibitor molecular recognition!!!



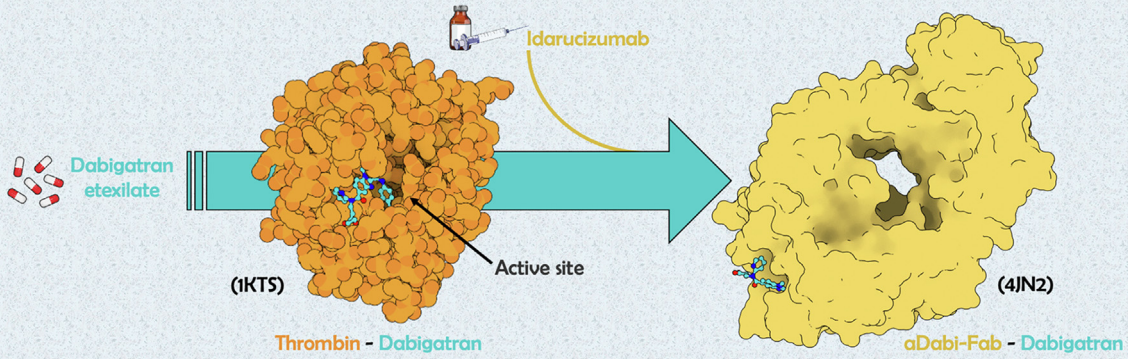




Structural basis of reversal activity

The availability of an antidote that reverses the effect of anticoagulants is fundamental in case of emergencies.[45] The blocking of the anticoagulant action may be achieved by developing molecules that bind the drug with higher affinity and specificity than the coagulation factor.

FDA-approved reversal agents include antibodies (idarucizumab for dabigatran), recombinant proteins (andexanet alfa for anticoagulants targeting FX), and positively charged polypeptides (protamine sulfate for heparin-like drugs).



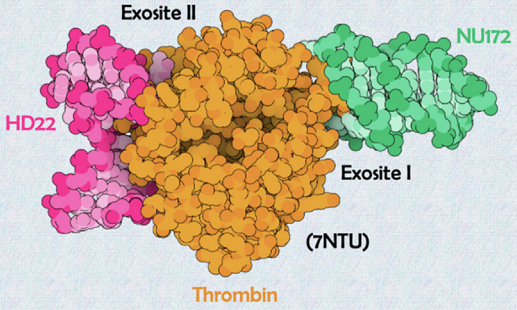
By binding thrombin active site, dabigatran is widely used as an oral anticoagulant for the prevention of stroke in patients with atrial fibrillation.[46] It is inactivated by Idarucizumab, a humanized monoclonal antibody fragment (aDabi-Fab).[47]

The X-ray crystal structure of dabigatran in complex with aDabi-Fab shows many similarities of dabigatran-thrombin recognition. Nevertheless, the antidote does not bind known thrombin substrates and has no activity in coagulation tests or platelet aggregation.[47]

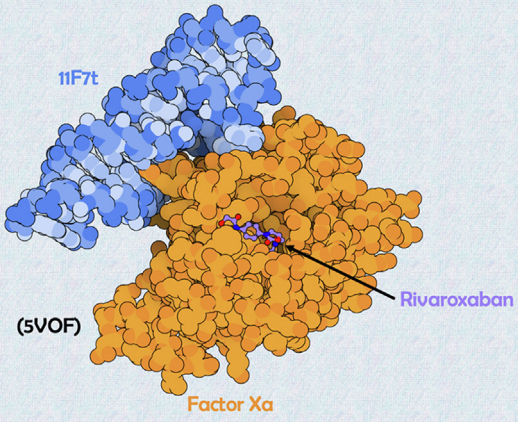
Interestingly, structure-guided approaches have been successfully pursued to generate a potential backup candidate for idarucizumab.[48]

Aptamers as versatile biomolecules for the modulation of the coagulation cascade

Structural studies are fundamental for the design of novel molecules able to modulate the intricate process of coagulation. Particularly interesting is the case of aptamers, DNA/RNA-based molecules specifically selected to tightly bind targets of interest.[49,50]



Many aptamers have been selected against the coagulation factors and some of them are considered in either preclinical studies or clinical trials as anticoagulants.[51,52]
In the case of thrombin, a simultaneous binding of aptamers to the two exosites has been achieved.[53-55]



It has been shown the ability of aptamers to potentiate the activity of known anticoagulants, as in the case of the RNA aptamer 11F7t that enhances the binding of rivaroxaban toward FXa.[56,57]

The interest for these molecules is also due to the possibility of rapidly identifying reversal agents to their actions.[58-60]
In principle, oligonucleotides with a base sequence that is complementary to specific regions of aptamers may hamper their folding and therefore their function.

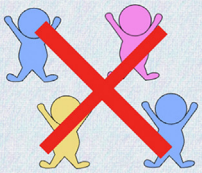
Conclusions & Perspectives

Surveys of literature publications and data mining in structural databases clearly indicate that, over the years, important advances have been made in the structural biology of the coagulation cascade. In particular, the combination of experimental and predictive approaches led to the structural characterization of all factors involved in this biological process.

These studies have also provided insightful information on the mechanism of action of drugs.
Moreover, structure-based approaches have been successfully applied to the optimization of drugs and reversal agents.

Although the structural analysis of the individual coagulation factors has almost reached completeness, much remains to be done to define at atomic level the molecular recognition among the factors involved in the several stages of the coagulation cascade.

A comprehensive analysis of the interfactor interactions will be beneficial for developing strategies aimed at modulating the coagulation cascade and at improving the structural-based design of new drugs and reversal agents. In particular, the design of drugs capable of simultaneously regulating different steps of the coagulation cascade could lead to valuable effects, which potentially include a reduction of therapeutic doses and a decrease of bleeding rates.



Appendix

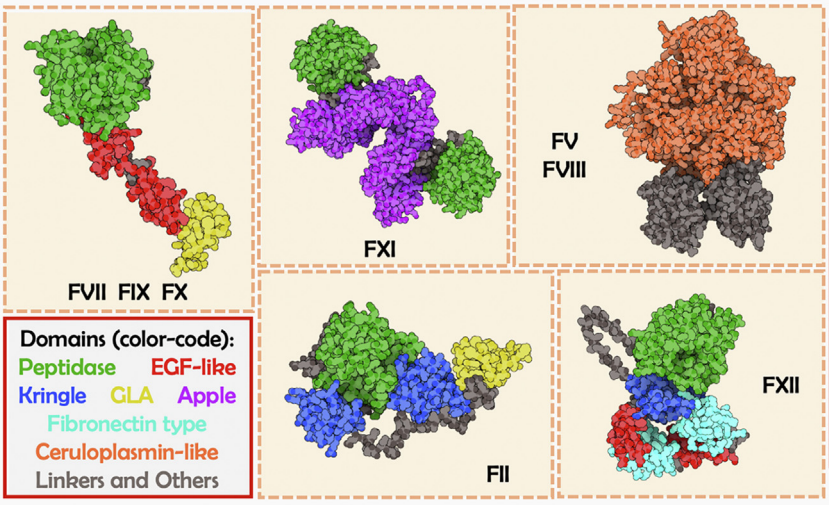
Accession-codes: PDB and UniProtKB

The 4-character codes reported in parentheses close to the structural models represent unique accession with which experimental structures are associated in the Protein Data Bank (PDB - https://www.rcsb.org) database. In the "Model selection" section (see below), the UniProtKB accession number (https://www.uniprot.org/uniprotkb/) has also been provided.

Color-code definition

The structural models are colored in orange and green if solved with X-ray crystallography and cryo-EM, respectively. The AlphaFold predicted models are in different blue tones depending on the pLDDT value, a per-residue estimate of the model confidence on a scale from 0 - 100.[13] Structured (pLDDT ≥ 70) and intrinsically disordered (pLDDT < 70) regions are in dark and light blue, respectively. To discriminate the different factors in a complex (TF-FVIIa, prothrombinase) or specific regions of a factor (thrombin exosite I and II), light and dark tones of the same color have been used. In specific cases, to highlight the multidomain nature of the factors, a different color-code has been used (see the following dictionary of structural domains).

Dictionary of structural domains of coagulation factors



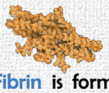
The coagulation factors are composed by different structural domains that sometimes are homologous with each other and with those of other common proteins (epidermal growth factor - EGF, fibronectin, ceruloplasmin). The gamma-carboxyglutamic acid-rich domain (GLA) contains post-translational modifications that enable it to interact with calcium ions (FIV). The latter ones induce the proper folding of the GLA domain, that is necessary for its interaction with the cell surface membranes. In some cases, certain domains are lost upon factors activation ( ).[27]

Model selection

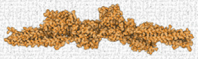
When the experimental structure was available, it was preferred, unless the AlphaFold model contained significant additional information. It must be underlined, that no information about active factors or cross-linked fibrin clot is reported in the AlphaFold database, as only the precursor forms are considered in the predictions.

Below is a detailed description of the factor structures reported in the review. General information on the domain organization and activation cleavage is also reported.[27]

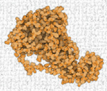
Factor I - Fibrinogen is a heterodimer consisting of three pairs of polypeptide chains termed α (UniProtKB P02671), β (UniprotKB P02675) and γ (UniprotKB P02679). The 3GHG PDB model (X-ray, deposition year 2009) contains structural information on its three chains.



Factor Ia - Fibrin is formed after thrombin cleavage of Arg16-Gly17 and Arg14-Gly15 bonds in the α and β chains of fibrinogen, respectively. Structural information about no-cross-linked human fibrin can be retrieved from the 2A45 PDB model (X-ray, deposition year 2005) of the complex between thrombin and the central region of fibrin.



Cross-linked fibrin clot is formed after FXIIIa-catalyzed cross-linking between γ chains and between α chains of different fibrin monomers. The 1FZB PDB model (X-ray, deposition year 1997) is among the first structures of cross-linked core fragments of human fibrin.



Factor II - Prothrombin (UniProtKB P00734) consists of a GLA domain, two kringle domains, and a proteinase domain. The 6C2W PDB model (X-ray, deposition year 2018) contains structural information on all domains of uncomplexed prothrombin. This structure contains Ser101Cys and Ala470Cys mutations in order to lock the protein in a specific (closed) conformation.

Factor IIa – Thrombin is formed after cleavage (Arg271-Thr272 and Arg320-Ile321) of prothrombin. Thrombin then undergoes intermolecular autolysis to cleave the Arg284-Thr285 bond. It consists of a light chain linked to a heavy chain by a disulfide bridge. The 1PPB PDB model (X-ray, deposition year 1991) contains structural information on both chains.

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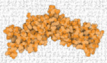
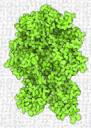
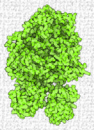


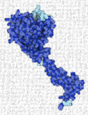
Factor III - Tissue Factor (UniProtKB P13726) consists of two fibronectin type III (FNIII) domains (immunoglobulin-like fold), a transmembrane domain, and a cytoplasmic tail. The 2HFT PDB model (X-ray, deposition year 1995) contains structural information on the TF soluble domain.



Factor V (UniProtKB P12259) consists of three A (ceruloplasmin-like) domains, two C domains, and a connecting B domain between the A2 and A3 domains. The 7KVE PDB model (cryo-EM, deposition year 2020) contains structural information on A and C domains.

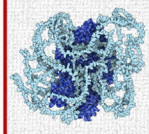


Factor Va is formed after cleavage (Arg709-Ser710, Arg1018-Thr1019, and Arg1545-Ser1546) and release of the B domain of FV. It consists of a heavy chain (A1-A2 domains) and a light chain (A3-C1-C2 domains). The 7KXY PDB model (cryo-EM, deposition year 2020) contains structural information on all chains.

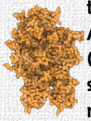


Factor VII (UniProtKB P08709) consists of a GLA domain, two EGF-like domains, and a proteinase domain. The AlphaFold structure is the most complete model of FVII.

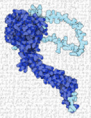
Factor VIIa is formed after cleavage of a peptide bond (Arg152-Ile153) in FVII. It consists of a light chain linked to a heavy chain by a disulfide bridge. The 1DVA PDB model (X-ray, deposition year 2000) contains information about the GLA-domainless factor. The 3TH2 PDB model (X-ray, deposition year 2011) of the complex between FVIIa and the soluble domain of the tissue factor contains information about all domains of FVIIa.



Factor VIII (UniProtKB P00451) consists of three A (ceruloplasmin-like), a B, and two C domains. The nascent form is cleaved (Arg1313-Ala1314 and Arg1648-Glu1649) after secretion and circulates in plasma as heterodimer. It consists of a heavy chain (A1, A2, and a part of B domains) and a light chain (A3, C1, and C2 domains). The AlphaFold structure is the most complete model of nascent FVIII.

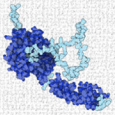


Factor VIIIa is a heterotrimer formed after cleavage at three sites of FVIII: Arg372-Ser373 (between A1 and A2), Arg740-Ser741 (between A2 and B), and Arg1689-Ser1690 (in A3). The B domain and part of A3 are lost. To date, no structures of FVIIIa are available. The 2R7E PDB model (X-ray, deposition year 2007) is an active recombinant form of FVIII consisting of a heterodimer formed by A1-A2 and A3-C1-C2 domains.

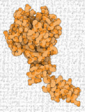


Factor IX (UniProtKB P00740) consists of a GLA domain, two EGF-like domains, and a proteinase domain. The AlphaFold structure is the most complete model of FIX.

Factor IXa is formed after cleavage (Arg145-Ala146 and Arg180-Val181) of FIX. It consists of a light chain linked to a heavy chain by a disulfide bond. The 6MV4 PDB model (X-ray, deposition year 2018) is the most complete model of FIXa. It contains information about the proteinase and one of the EGF-like domains.



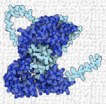
Factor X (UniProtKB P00742) consists of a GLA domain, two EGF-like domains, and a proteinase domain. The AlphaFold structure is the most complete model of FX.



Factor Xa is formed after cleavage (Arg139-Arg140 and Arg142-Ser143, and Arg194-Ile195) of FX. It consists of a light chain linked to a heavy chain by a disulfide bond. The 1XKA PDB model (X-ray, deposition year 1998) is the most complete model of FXa. It only lacks information about the GLA domain.

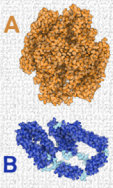
Factor XI (UniProtKB P03951) consists of two disulfide-bound monomers, each containing four apple domains and a proteinase domain. The 6I58 PDB model (X-ray, deposition year 2018) contains information on the whole homodimer.

Factor XIa is formed after cleavage of a peptide bond (Arg369-Ile370) in each monomer of FXI. The FXIa homodimer consists of two heavy and two light chains linked by an intrachain disulfide bond. The 7MBO PDB model (X-ray, deposition year 2021) contains information only on the proteinase domain.

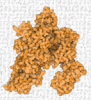


Factor XII (UniProtKB P00748) consists of two fibronectin type domains, two EGF-like domains, a kringle domain, a proline-rich region, and a proteinase domain. The AlphaFold structure is the most complete model of FXII.

Factor XIIa is formed after cleavage of a peptide bond (Arg353-Val354) in FXII. It consists of a light chain linked to a heavy chain by a disulfide bond. The 6B74 PDB model (X-ray, deposition year 2017) contains information on the proteinase domain.



Factor XIII is a heterotetramer consisting of two A (pro-transglutaminase, UniProtKB P00488) subunits and two B subunits (non-catalytic carrier portion, UniProtKB P05160). The 1GGU PDB model (X-ray, deposition year 1998) contains information on the A_2 dimer. The AlphaFold model contains information on the B subunit.



Factor XIIIa is formed when B units are removed from FXIII upon cleavage of a peptide bond (Arg38-Gly39) in the A units. The 4KTY PDB model (X-ray, deposition year 2013) contains information on the A enzymatic subunit of FXIIIa.

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Parts of the figure were drawn using pictures from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 unported license. Molecular graphic images were rendered with Protein Imager.

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AUTHOR CONTRIBUTIONS

R.T., N.B., I.A., F.S., and L.V. conceived the idea and the images, wrote the manuscript, and read and approved the final paper.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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