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Mathematical models of fibrin polymerization: past, present, and future

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Abstract

Blood clotting is a complex biochemical and biophysical process that leads to the formation of a stabilizing fibrin mesh. Fibrin polymerization is a necessary, multi-stage component of this process and occurs on multiple temporal and spatial scales. These complexities make it difficult to predict how polymerization is affected by perturbations or under varying conditions. Mathematical modeling has been a fruitful approach in generating and testing novel hypotheses about this process. In this review, we focus on the historical context leading to current mathematical models of fibrin polymerization and discuss the contributions of biochemical interactions between thrombin, fibrin(ogen), and factor XIII. We highlight mathematical models that encompass multiple spatial and temporal scales (coarse-grain models, kinetic models, and models incorporating flow and transport effects). We also discuss the unique sets of challenges and benefits of each of these models, and finally, we suggest directions for future focus.

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Introduction

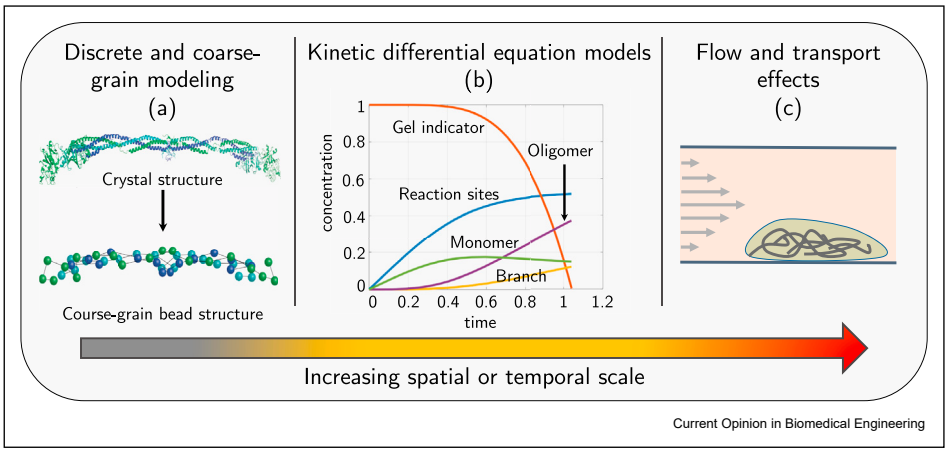
While the formation of fibrin gels can be observed with the naked eye, mathematical models of the fibrin polymerization process enable us to better describe the microscopic biophysical and biochemical events leading up to the point of gelation and beyond. Over the past 40 years, a handful of mathematical models have been developed to study fibrin polymerization. These models span a wide range of spatial and temporal scales (Figure 1). To describe these models and their impact, it is important to highlight three specific proteins, fibrin(ogen), thrombin, coagulation factor XIII (FXIII) and its active form (FXIIIa), which are key contributors to many aspects of the fibrin polymerization process. While thrombin and FXIIIa are critical to the dynamics of fibrin polymerization, their explicit roles have been absent from most mathematical models or have been considered in only simplistic ways. We will discuss gaps that exist in current models of fibrin polymerization and suggest areas that warrant further investigation to address unanswered questions in the field, including the incorporation of FXIIIa into a mathematical model of fibrin polymerization.

Brief biological primer on fibrin polymerization

After vascular injury, the biochemical and biophysical processes of hemostasis work to form a thrombus to prevent blood loss. The first responders are platelets that weakly aggregate and 'plug' the site of vascular injury. Simultaneously, a series of enzymatic reactions that make up the blood coagulation process results in the formation of fibrin, which polymerizes and traps platelets and other blood cells within a dense network of fibers. Together, the aggregated platelets, cells, and fibrin mesh comprise a stable clot.

The most critical enzyme generated during the coagulation process is thrombin, which is necessary for the generation and stabilization of the formed fibrin mesh [1,2]. First, thrombin is the sole enzyme responsible for proteolytically converting fibrinogen into fibrin. Second, it proteolytically activates the zymogen FXIII into its activated form, transglutaminase FXIIIa, which strengthens and stabilizes the fibrin scaffold by covalently cross-linking fibrin fibers [3,4]. Biochemically, thrombin has one active site and two exosites, exosite 1 (E1) and exosite 2 (E2); the exosites serve to localize thrombin by binding it to various substrates and platelet surfaces, which aids in coagulation and platelet activation [5–9]. During fibrin polymerization, thrombin binds both fibrinogen and fibrin and can become trapped within the growing thrombus, where the fibers serve as a sink for its potent enzymatic activity [10,11]. This

Figure 1



Schematic overview of the scope of mathematical fibrin polymerization models that encompass varying spatial or temporal scales. Panel (**a**) illustrates a coarse-grain representation of fibrinogen that is used in molecular dynamic models to better understand intramolecular and intermolecular interactions in polymerization [22,23]. Kinetic, continuum differential equation models, shown in panel (**b**), are often used to determine the temporal dynamics of physical concentrations involved in polymerization [24–26]. Transport effects on the polymerization process are investigated with spatial–temporal models shown in panel (**c**) [27,28].

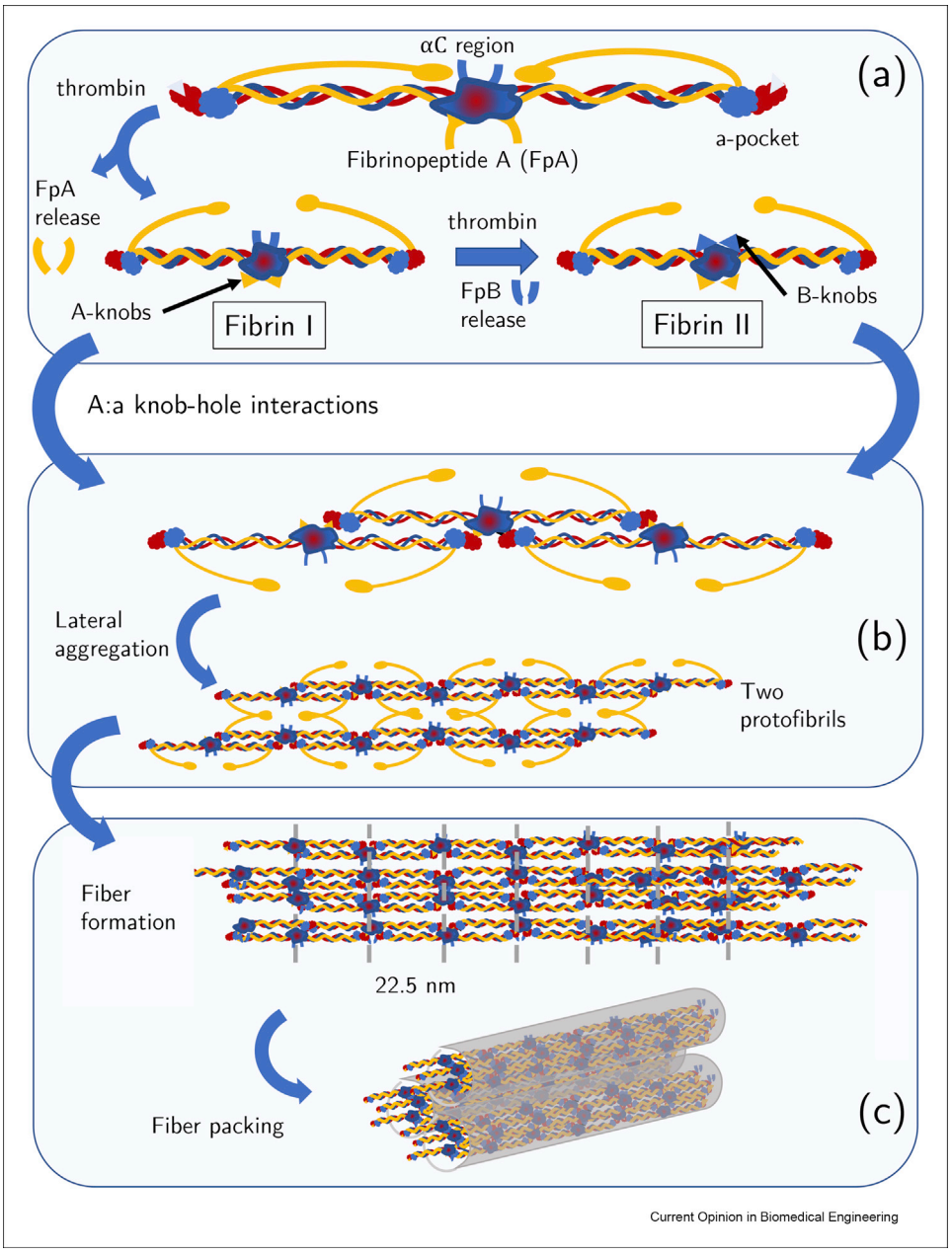
complex coupling of thrombin to the polymerization process is not fully understood.

Fibrin has a dimeric structure with a central E domain flanked by two D-domains. Each D-domain is associated with a γ-chain. Approximately 15% of a fibrinogen's γ-chains are an alternative splice variant denoted γ', which are formed through alternate processing of the γ-mRNA [12]. Details on how the presence of γ'-chains can affect fibrin polymerization are discussed in a later section. Polymerization is initiated when thrombin binds the E domain of fibrinogen and cleaves fibrinopeptide A (FpA), resulting in fibrin I formation. This cleavage event on a single fibrin monomer exposes A-knob binding sites that bind with complementary a-pocket binding sites on the ends of other fibrin monomers to form dimers (Figure 2). Fibrin I monomers can then continue to bind longitudinally, through A:a interactions, to form oligomers. Continual binding of more monomers to oligomers results in half-staggered, two-stranded protofibrils. Thicker fibrin fibers are then formed by lateral aggregation of the protofibrils that have reached a critical length (600–800 nm) [3]. While the exact mechanisms and structures required for lateral aggregation are still not well-defined, Yang et al. [14] proposed a step-wise conceptual model of lateral aggregation, developed from packing interactions in crystal structures of fibrinogen fragments. Their model also suggests that under some conditions, half-staggered packing could enable solvent channels within the fibers through which molecules such as thrombin and FXIIIa could diffuse. A schematic overview of fibrin polymerization is shown in Figure 2, and for more detailed reviews on fibrin polymerization, see the studies by Wolberg [11], Mosesson [12], Weisel and Litvinov [13], Weisel [15,16], and Pieters and Wolberg [17]. In a step sequential to FpA cleavage, thrombin cleaves fibrinopeptide B (FpB), forming fibrin II. Fibrin II has B–knob binding sites that associate with complementary b-pocket binding sites [18,19]. Although this step is not necessary for lateral aggregation and fiber formation, it is thought that B:b interactions allow for thicker fibers because of enhanced lateral aggregation [18,20,21].

Mathematical models of fibrin polymerization

Here, we characterize fibrin polymerization into three broad steps: (1) the enzymatic cleavage of fibrinopeptides from fibrinogen by thrombin to produce fibrin monomers; (2) the binding of fibrin monomers to form half-staggered, two-molecule wide protofibrils; and (3) the lateral aggregation of protofibrils to form fibrin fibers that branch and form a space-filling fibrin gel. These steps occur on multiple spatial and temporal scales and have been modeled with varying degrees of complexity and diverse modeling approaches (Figure 1). This section includes descriptions of these different modeling frameworks/approaches, which are summarized in Table 1, with comments about the appropriateness of these frameworks for addressing different biological questions. Note that while several of these models incorporate many biologically relevant components of fibrin polymerization, all models described in the following do not include the dynamics and contributions of FXIII.

Figure 2



Schematic description of fibrin polymerization. Fibrinogen is a 45 nm-long soluble glycoprotein found in blood and consists of 6 paired, coiled–coiled polypeptide chains (2 Aα- (yellow), 2 Bβ- (blue) and 2γ- (red) chains). The figure shows the following steps of polymerization, (**a**) monomeric fibrin is formed through cleavage of central fibrinopeptides (FpA) of fibrinogen by thrombin, exposing A-knobs and releasing αC regions. (**b**) Fibrin monomers spontaneously polymerize through knob–hole interactions and form half-staggered, two monomer-wide protofibrils. (**c**) Once a critical length is reached (20–25 monomers), protofibrils laterally aggregate through αC interactions to form fibrin fibers that pack to form the fiber network. For more detailed reviews of fibrin polymerization, see studies by Wolberg [11], Mosesson [12], Weisel and Litvinov [13], Weisel [15,16], and Pieters and Wolberg [17].

Early models of fibrin polymerization

Early studies of fibrin polymerization used kinetic models to help explain experimental observations. The first kinetic model of the conversion of fibrinogen to fibrin was developed by Lewis et al. [29]. The model considered thrombin-catalyzed proteolysis of fibrinogen and helped support the data and the idea that there is an ordered, sequential release of FpA and FpB and thus, formation of fibrin I and fibrin II, respectively (Figure 2). Fibrin I in the model could either oligomerize or be further proteolyzed to fibrin II for which oligomerization was not considered. Oligomerized fibrin

Table 1

Mathematical models proposed for fibrin polymerization. Models are organized by modeling framework, and model focus area refers to the three broad steps of polymerization: (1) the enzymatic cleavage of fibrinopeptides on fibrinogen by thrombin to form fibrin monomers, (2) fibrin monomers binding to form half-staggered, two-molecule wide protofibrils, and (3) the lateral aggregation of protofibrils to form fibrin fibers which branch and form a space-filling gel.

Focus Area & Reference	Modeling Methodology	Key Contribution
1, 2, 3 [23]	Coarse-grain molecular dynamic model	Fibrinogen is represented as a coarse-grained molecular chain that is involved in conversion, elongation, and lateral aggregation reactions through reactive dissipative particle dynamics simulations. As the first molecular-based model of fibrin polymerization, the model can recapitulate experimental results, such as the dependence of fiber thickness on thrombin concentration.
1, 2, 3 [22]	Coarse-grain molecular dynamic model	Fibrinogen is represented as a coarse-grained molecular chain and participates in reactions as described in the study by Yesudasan et al. [23]. With a larger time and spatial scale than [23], the model incorporated varying thrombin concentration and showed slower FpB release is essential for realistic fiber development.
1, [29,30]	Kinetic ODE model	The model characterized the kinetics scheme and relevant kinetic constants for thrombin-catalyzed conversion of fibrinogen to fibrin. The model was extended to include thrombin explicitly as well as anti-thrombin inhibition. Experimental data is used to characterize the kinetic rates of fibrin−fibrin binding, as well as fibrinopeptide release rates under various conditions.
1, 2 [36]	Kinetic ODE model	Fibrin-fibrinogen interactions are investigated in a kinetic model of small oligomer formation comprised fibrinogen and fibrin. Fibrinogen-fibrin binding and oligomeric fibrinogen conversion by thrombin were required to reproduce experimental results from size exclusion chromatography.
1, 2, 3 [24]	Kinetic ODE model	The authors present the first model to describe all steps of polymerization, the width and length of fibers (in a number of molecules and the number of protofibrils) that can be extracted. Fiber size was compared with turbidity measurements and validated with SEM, and a minimum protofibril length was required to obtain physically relevant results.
2, Branch formation [26]	Kinetic gelation model	By explicitly incorporating branch formation, three reactions were considered, bimolecular elongation, trimolecular branch formation, and monomer source. The model results can capture experimental thrombin-dependent branch structure [44].
1, 2, 3 [35]	Kinetic gelation model	A kinetic gelation and fragmentation model framework was used, coupled with thrombin activation and inhibition, to determine where in parameter space gelation occurs.
Fibrin-Thrombin Binding [46]	Kinetic ODE model	The model describes the kinetic binding of fibrin and thrombin using ODEs and was created to help understand accompanying experimental results. Parallel plate flow chamber experiments with thrombin bound to fibrin mat showed that active thrombin, protected from some inhibition, remains present for 30+ minutes.
Fibrin-Thrombin Binding [25]	Kinetic ODE/PDE model	Using a PDE diffusion-reaction model, an explicit treatment of bivalent binding kinetics is statistically significant in modeling thrombin-fibrin binding. It was shown that there must be an irreversibly trapped thrombin population, hypothesized to be physically trapped inside fibrin fibers.
1, 2 [28]	Transport effects & flow, kinetic gelation model	As the first fibrin polymerization model to include flow-mediated transport, the model includes a monomer source from thrombin activation/inhibition and a flow-mediated monomer sink. At low shear rates, thrombin availability limits gelation and polymer aggregation is limited by flow removal at high shear rates.
1, 2, 3 [27]	Transport effects & flow, kinetic gelation model with coagulation	A kinetic gelation and fragmentation model coupled with thrombin activation and inhibition is presented and incorporates a 2D spatial model with transport, diffusion, and coagulation reactions.

I could then be converted to fibrin II by fibrin-associated thrombin. Fibrinogen conversion was assumed to obey Michaelis–Menten kinetics, and thus, thrombin-fibrin(ogen) interactions were not explicitly considered. The model was later extended to include antithrombin inhibition of thrombin [30]. These early models of fibrin polymerization provided a foundational framework for the design of future experimental studies and mathematical models. It is now accepted that FpA cleavage allows for oligomerization, and FpB cleavage enhances lateral aggregation of protofibrils [18, 20, 21]. However, the exact mechanism(s) through which fibrin I and fibrin II interact while forming oligomers, protofibrils, and fibers remain unclear.

Coarse-grained molecular dynamic models of fibrin polymerization

Molecular dynamics (MD) simulations have been instrumental to capture structure-to-function relationships within the fibrin polymerization process on a molecular scale. For example, MD simulations have recapitulated the existence of A:a interactions [31], helped to describe characteristics of B:b interactions [32], and uncovered the structural basis for interfacial flexibility of fibrin oligomers [33]. Typically, MD simulations focus on a single protein in water or lipid and track the position and motion of its hundreds of thousands of atoms on microsecond timescales [34].

Yesudasan et al. [22] combined a previously determined crystal structure of fibrinogen together with the efficient computational methodology of MD simulations to examine the fibrin polymerization process. Each fibrin(-ogen) protein was represented as a collection of spring-linked beads. The position, motion, and interactions via reactive attraction bonds were then tracked between thousands of proteins with existing MD software [22]. Their simulations led to realistic phenomena, including the dependence of fiber thickness on thrombin concentration. A follow-up study used many-body dissipative particle dynamics to simulate polymerization on a larger timescale (micro versus nanosecond) and spatial scale (500 nm versus 200 nm) [23]. These models represent the first of their kind on a molecular spatial/temporal scale, and suggested that slower FpB release than that of FpA is crucial for the development of realistic fibrin fibers [23]. While MD and dissipative particle dynamics simulations can provide some insight into molecular interactions on short time scales, computational challenges prevent their application to longer and more biologically relevant time scales (minute, hours, and so on).

Kinetic models of fibrin polymerization

Kinetic models are a computationally efficient approach to model polymerization, which can be based on ordinary [24, 26, 35, 36] and partial differential equations [27,28]. Here, kinetic reaction schemes involved in polymerization are translated into systems of differential equations using the law of mass action, which states that the rate of a reaction is proportional to the product of the concentrations of its reactants. The concentration of each desired species can then be tracked in time and/or space if the transport is included. Species tracked with such models include fibrin monomers, oligomers, protofibrils, and fibers, with reaction schemes that describe elongation and aggregation. The systems of equations tracking these species are solved, often numerically, until some criterion is met. These criteria are dependent on the model framework and biological goal and often based on time or some desired quantity of interest.

A typical quantity of interest is the time at which a fibrin gel forms, called the gel point or clotting time; this is regarded as the time in which soluble polymers transition into an insoluble gel. The transition, also known as gelation, results in a gel which is defined to be a network of polymers in a solution that exhibits solid-like properties. Experimentally, the fibrin gel point is observed by the eye and corresponds to a rapid rise in turbidity due to the formation of fibrin fibers through the lateral aggregation of protofibrils [24,37]. Mathematically, gelation has been defined to occur when the weight-average molecular weight of polymers becomes unbounded [60,61]. In a mathematical model of fibrin polymerization that tracks the formation of protofibrils and fibers, gelation could correspond to the rapid rise in the number of protofibrils per fiber.

Fibrin polymerization has been modeled with a kinetic framework extensively, and Weisel and Nagaswami [24] developed the first kinetic model that included all three steps of the fibrin polymerization process. The enzymatic conversion of fibrinogen to fibrin monomer did not explicitly monitor thrombin-fibrinogen interactions, but rather, conversion was considered to be a constant rate. Small fibrin oligomers, species comprised fibrin monomers, were allowed to form with no fibrinogen interactions. Also considered was protofibril formation through the longitudinal aggregation of fibrin monomers and the lateral assembly of protofibrils to form fibrin fibers. The model considered the dynamics of species over a range of oligomer sizes, defined by the number of monomers, and species type, from fibrinogen to fibrin, and from small oligomers to protofibrils to fibrin fibers. A striking outcome of this study was that to capture the characteristic lag period observed in turbidity (light-scattering) experiments [38], the protofibrils first had to reach a critical length before lateral aggregation could occur.

Wilf and Minton [36] were the first to develop a kinetic model that considered the interactions of fibrinogen with fibrin during polymerization, nicely characterizing the early dynamics of polymerization. The model tracked the temporal evolution of small oligomers comprised both fibrin and fibrinogen. By assuming that

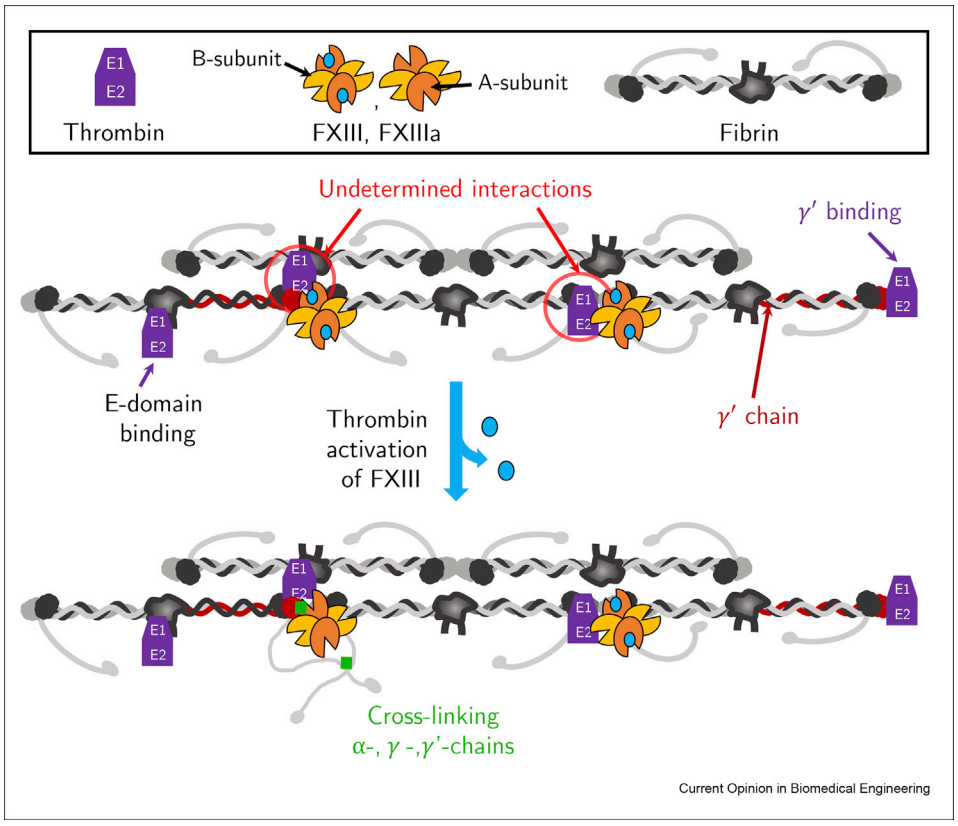
fibrinogen in monomeric and oligomeric form is converted to fibrin and that fibrinogen cannot bind to itself, oligomers form through reactions between either two monomeric species or one monomeric species and one oligomeric species. By tracking oligomers comprised at most five molecules, the model recapitulated experimental data that indicates trimers are favored over dimers throughout the polymerization process and fibrinogen-fibrin binding is essential; this model was not expanded to include the formation of larger oligomers, which lead to gelation. However, oligomers comprised fibrin and fibrinogen, called soluble fibrin complexes, are thought to be important in early polymerization and low thrombin environments [15]. A mathematical approach based on a two-monomer framework, inspired by fibrinogen-fibrin interactions but not yet applied directly to fibrin polymerization, has recently been developed [39] and has the potential to overcome the hurdles limiting the model of Wilf and Minton.

Another modeling approach is to take mean-field approximations of coalescing particles to track the concentration of oligomers, or clusters, comprised of k monomer units, denoted as k-mers. By assuming oligomers do not form loops or cycles (do not bind with self), larger oligomers grow through reactions whose rates, called coagulation kernels in these models, depending on some physical quantity such as particle mass or a number of available reaction sites. The dynamics for all species of k-mers are then defined by an infinite set of ordinary differential equations. Quantities related to the dynamic distribution of k-mers can be analyzed; for example, the total concentration of all species (called the zeroth moment) and the total concentration of monomers (called the first moment). To describe these quantities, moment generating functions are used to simplify the infinite sets of equations to finite sets of differential equations that describe the change in the moments of interest. The second moment is often related to the average oligomer size, and in this context, gelation is defined to be the time at which the second moment goes to infinity. Gelation can only occur in these models if the number of free reaction sites on a monomer unit is greater than two; otherwise, only linear chains would form and these alone cannot form a gel. Kinetic polymerization models with gelation have been studied extensively; a vast body of literature exists and is reviewed in the studies by Galina and Lechowicz [40], Aldous [41], and Wattis [42]. The kinetic polymerization framework with gelation was first analyzed in the study by Ziff and Stell [43] and has only recently been used to investigate fibrin polymerization [26–28,35].

One of the first applications of kinetic gelation models to fibrin polymerization was by Guy et al. [28], which incorporated flow and aspects of coagulation, and will be discussed in further detail in the following. An interesting extension of the gelation framework for fibrin polymerization was to explicitly track branch formation [26]. There, the authors assumed oligomers formed through two types of reactions, bimolecular linear elongation, representing half-staggered protofibrils, and trimolecular branch formation corresponding to branching that occurs either in protofibril formation or lateral assembly [26]. In addition to the moments described previously, they were also able to track the concentration of various types of branches in the gel. Analysis of the model revealed that the time to gelation and the structure of the gel at that time, for example, the mean distance between branch points, depended on the supply rate of the fibrin monomer. This analysis agreed with experimental results indicating branch formation increases proportionally with thrombin concentration, which can be interpreted as a source of fibrin monomer [44]. The authors used their model to understand the mechanism underlying this behavior. In particular, they found that three-monomer reactions dominated early in polymerization and, later, one-monomer and two-monomer reactions occur more rapidly, suggesting that the availability of monomers is critical to the rate at which branches form.

There exist several key differences between kinetic models and kinetic gelation models described previously. In the kinetic gelation framework, concentrations of all possible oligomers are tracked and only form until the gelation singularity occurs. In the context of fibrin polymerization, this framework is used to provide a convenient mathematical indication that a fibrin 'gel' has formed. However, it is notable that models based on this framework do not explicitly track protofibril and fibrin fiber formation and therefore cannot capture the characteristic lag period in polymerization [24]. While fibrin networks continually grow after the gel point [37], the kinetic gelation approach as presented in the study by Fogelson and Keener [26] and Guy et al. [28] is not extendable past gel formation. However, a post-gel framework for Ziff and Stell has recently been established [45] that can be applied to fibrin polymerization. Finally, kinetic models like that of Weisel and Nagaswami [24] assume that the rate at which two monomers react is identical to the rate at which a monomer and an oligomer react; this is not the case for kinetic gelation models where, for example, the reactions could depend on the total mass of the oligomer. While it is not known which of these polymerization mechanisms are more likely physiologically, other modeling frameworks described earlier (such as coarse-grained MD models in section 4.2) could potentially be used to extract this information and predict the most likely mechanism. Therefore, careful consideration must be used when establishing a modeling framework to represent fibrin polymerization.

Figure 3



Schematic description of hypothesized FXIII binding to fibrin. FXIII can be bound to either the γ- or γ′-chain splice variant (dark red) of fibrinogen through the B subunit (yellow) [57]. Once fibrin monomers polymerize, FXIII forms a ternary complex with thrombin and fibrin at the D-E-D junction. Exosite 1 on thrombin is known to bind to fibrin through its E-domain, and exosite 2 can bind to fibrin through only the γ′-chain. Thrombin cleaves peptides from FXIII (light blue), resulting in FXIIIa. However, it remains unclear which thrombin exosites are involved in FXIIIa activation. After thrombin activation, FXIIIa can form crosslinks within and between protofibrils through the α- and γ-chains [3,15]. Questions remain concerning FXIIIa and how it binds to and crosslinks γ′-chains on fibrin [62].

Models of fibrin formation that include flow or transport effects

The first model of fibrin polymerization under flow was developed by Guy et al. [28], where the kinetic gelation framework described previously [43] was extended to include the effects of flow by coupling the model with a one-dimensional partial differential equation. To determine the effects of flow on the height of a growing fibrin gel, the authors combined a minimal coagulation model with flow and polymerization of fibrin monomers. Thrombin converted fibrinogen to fibrin monomer in a prescribed injury zone, and all species were subject to removal by the flow. The authors found that at low shear rates the transport of thrombin from the injury site to the surface of the fibrin gel limits the gel height, while in a high shear regime dilution of fibrin occurs faster than gelation can occur. The transition between these two regimes was dictated by the permeability of the gel.

A two-dimensional spatial–temporal model, based on the same kinetic gelation framework [43], was introduced by Rukhlenko et al. to describe how increased shear stresses associated with plaque (buildup of fat and cholesterol) height, h, affect fibrin gel formation [27]. Procoagulant material was produced along a vessel wall with shear-dependent permeability, and the authors showed that gelation depends on both wall permeability and dilution of procoagulant material due to flow conditions — consistent with the model of Guy et al. Interestingly, the Ruhlenko model also suggests that for large h, gelation occurs for only low Reynolds number flow but intermediate h, a wider range of Reynolds numbers support gel formation.

Models of thrombin–fibrin interactions

Experimental work supports the dogma that clot-bound thrombin retention is due to the dynamic interplay of thrombin binding to both low- and high-affinity binding sites, where the high-affinity binding is associated with γ′ fibrinogen specifically [46,47]. In addition, Haynes et al. developed a kinetic mathematical modeling approach based on this view, where there were two types

of thrombin-fibrin binding considered: high-affinity and low-affinity, using rate constants averaged from measured literature values [46]. Results from their model suggested that, for the amount of thrombin retention they found experimentally, 90% of free thrombin had to be somehow protected from removal by the flow. The mechanism of that protection was undetermined.

To uncover a mechanism of protection from flow, Kelley and Leiderman [25] developed a kinetic binding description based on the idea that thrombin and fibrin bind bivalently, meaning that thrombin is bound by both E1 and E2, to the E domain and γ'-chain, respectively, on two distinct fibrin monomers [48]. This binding is possible because of the half-staggered nature of the protofibrils (see Figure 3), which brings the E domain and γ'-chain in proximity to a single thrombin molecule. Because thrombin binds to γ'-chains, but not γ-chains, bivalent binding can occur with, at most, about 15% of fibrin(ogen). The novel aspect of the mathematical model is not only the bivalent nature of thrombin itself but also the fact that the kinetic scheme captures the physical proximity of binding sites on E domains to binding sites on γ'-chains on adjacent monomers. This was achieved by tracking two types of low-affinity sites on E domains in the model, ones that are within proximity to γ'-chains and ones that are not. The kinetic scheme was embedded in a one-dimensional reaction–diffusion equation to simulate previously published thrombin dissociation experiments [48]. In that study, thrombin bound to fibrin clots made with various mixtures of fibrinogen types, homodimers (γ, γ), and heterodimers (γ, γ') was measured over 24 h periods. The model was able to simulate thrombin binding to fibrinogen within all of these various mixtures because of the explicit consideration of the different binding sites. Using Bayesian parameter inference with a Markov chain Monte Carlo algorithm and subsequent model selection criteria, statistical support was found in favor of the bivalent binding model over a model of only high- and low-affinity binding. An interesting result was that neither model could explain the experimental data without an assumption that some fraction of the initial thrombin became physically trapped with the clot, an observation made in an earlier experimental study [49]. The corollary to this result was that thrombin could be physically trapped inside individual fibrin fibers during polymerization. This hypothesis indicates that thrombin sequestration by fibrin may be a more complicated and nuanced process than simply high-affinity binding driving long-term thrombin retention. An experimental study that can verify this hypothesis and quantify the amount of trapped thrombin as a function of thrombin, fibrinogen, and even flow would help to further this line of inquiry. Extended models to look at polymerization and flow with these thrombin-fibrin interactions will also help to determine the role and potential pathological consequences of trapped and bivalently bound thrombin.

Conclusions and suggestions for future focus

The models described above have been instrumental in providing mechanistic underpinnings of fibrin polymerization and thrombin-fibrin interactions. Molecular dynamic simulations on small temporal and spatial scales, validated by experimental results, illustrate the importance of fibrinopeptide release for fibrin polymerization. Fibrin polymerization models, which cover multiple temporal scales with kinetic differential equations, vary in the number of biochemical species tracked and their targeted quantities of interest. Nonetheless, they have allowed for efficient investigations of the polymerization process and how it is affected by varying thrombin and fibrinogen concentrations and supply rates. Thrombin-fibrin binding and transport effects have only been recently investigated mathematically, but are both found to be important components in understanding the detailed biochemical dynamics of fibrin polymerization. There are still significant gaps in our understanding of the process as a whole as it pertains to the coupling of thrombin, fibrin, and FXIII. Here, we briefly outline where we think models could be focused in the future.

Consideration of γ' fibrin(ogen) and thrombin-fibrin dynamics during fibrin polymerization

It has been reported that γ' decreases the rate of fibrin polymerization, shown by turbidity measurements, but there are conflicting results in the experimental literature regarding the effects of γ' on other aspects of polymerization, including the rates of fibrinopeptide release [50–52] and clot morphology [50,52–54]. These discrepancies could be due to differences across the experiments, including the use of recombinant proteins versus plasma fractionated, the inclusion of calcium or FXIII, the initial concentrations of thrombin and fibrinogen, and whether or not fully hydrated or dehydrated fibrin fibers are measured [55]. No mathematical models of fibrin polymerization currently exist that explicitly consider γ' fibrin(ogen). In addition, most mathematical models of fibrin polymerization do not explicitly track the dynamics of thrombin, neither free nor bound to fibrin(ogen). The coupling of thrombin dynamics with polymerization could be important for understanding how varying ratios of thrombin to fibrin concentration and consideration of γ' fibrin(ogen) affect polymerization. There are conflicting experimental results related to the effects of thrombin binding to γ' on polymerization [53,56]. A model that

considers γ' and explicitly tracks thrombin and the bivalent binding therein would be useful to understand and interpret these disparate experimental results.

FXIII/FXIIIa

FXIII, also called fibrin stabilizing factor, is a circulating protein involved in cross-linking fibrin to protect clots against shear stress when forming underflow. FXIII also slows fibrinolysis, the enzymatic degradation of a fibrin clot by the enzyme plasmin, by crosslinking $\alpha 2$ – PI, the major plasmin inhibitor, to fibrin [3]. FXIII circulates in a state bound to either the γ- or γ'-chain of fibrinogen [4,57]. The conversion of FXIII to FXIIIa by thrombin occurs while FXIII, thrombin, and fibrin are in a ternary complex, where fibrin acts as the cofactor [58], but the details of these interactions and how they might directly affect polymerization are not fully understood. For more details on FXIII/FXIIIa see the following reviews [3,4].

Consider a junction between three fibrin monomers during polymerization where the junction consists of two γ-chains, each one from separate monomers that are end-to-end, and an E domain of a laterally bound monomer (Figure 3). If FXIII is bound to one of the γ-chains within the junction, it is likely well-positioned near thrombin that is bound to the E domain. That thrombin molecule could then proteolytically activate FXIII into FXIIIa (the transglutaminase), which is subsequently available to crosslink the monomers end-to-end via the γ-chains in the junction. FXIIIa could then translocate to form cross-links between α-chains of the end-to-end monomers and/or between an α-chain and other proteins. FXIIIa also protects the clot from degradation during fibrinolysis because the covalent crosslinking irreversibly alters the biophysical and biochemical properties [59].

There remain several unanswered questions relating to the triad of interactions between FXIII(a), thrombin, and fibrin(ogen) that integrated experiments and mathematical modeling could help to address. While fibrin is a cofactor for thrombin in activating FXIII, the biochemical features of the ternary complex remain unclear. Which exosites/binding sites are involved and can bivalently-bound thrombin bind/activate FXIII? Does FXIII or FXIIIa bound to a γ'-chain compete with thrombin binding to that γ'-chain through E2? And finally, it is unknown how FXIIIa affects the distribution of other proteins within fibers and if these distributions vary with the proportion of γ'.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Kattula S, Byrnes JR, Wolberg AS: **Fibrinogen and fibrin in hemostasis and thrombosis**. *Arterioscler Thromb Vasc Biol* March 2017, **37**:e13–e21.
2. Nesheim M: **Thrombin and fibrinolysis**. *Chest* September 2003, **124**:33S–39S.
3. Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona É: **Factor XIII: a coagulation factor with multiple plasmatic and cellular functions**. *Physiol Rev* July 2011, **91**:931–972.
4. Byrnes JR, Wolberg AS: **Newly-recognized roles of factor XIII in thrombosis**. *Semin Thromb Hemost* June 2016, **42**:445–454.
5. Lane DA, Philippou H, Huntington JA: **Directing thrombin**. *Blood* October 2005, **106**:2605–2612.
6. Meh DA, Siebenlist KR, Mosesson MW: **Identification and characterization of the thrombin binding sites on fibrin**. *J Biol Chem* September 1996, **271**:23121–23125.
7. Myles T, Yun TH, Hall SW, Leung LLK: **An extensive interaction interface between thrombin and factor V is required for factor V activation**. *J Biol Chem* July 2001, **276**:25143–25149.
8. Esmon CT, Lollar P: **Involvement of thrombin anion-binding exosites 1 and 2 in the activation of factor V and factor VIII**. *J Biol Chem* June 1996, **271**:13882–13887.
9. Ayala YM, Cantwell AM, Rose T, Bush LA, Arosio D, Di Cera E: **Molecular mapping of thrombin-receptor interactions**. *Proteins: Structure, Function, and Bioinformatics* 2001, **45**:107–116.
10. Mutch NJ, Robbie LA, Booth NA: **Human thrombi contain an abundance of active thrombin**. *Thromb Haemost* 2001, **86**: 1028–1034.
11. Wolberg AS: **Thrombin generation and fibrin clot structure**. *Blood Rev* May 2007, **21**:131–142.
12. Mosesson MW: **Fibrinogen and fibrin structure and functions**. *J Thromb Haemostasis* August 2005, **3**:1894–1904.
13. Weisel JW, Litvinov RI: **Mechanisms of fibrin polymerization and clinical implications**. *Blood* 2013, **121**:1712–1719.
14. Yang Z, Mochalkin I, Doolittle RF: **A model of fibrin formation based on crystal structures of fibrinogen and fibrin fragments complexed with synthetic peptides**. *Proc Natl Acad Sci Unit States Am* 2000, **97**:14156–14161.
15. Weisel JW: *Fibrinogen and fibrin*: *Advances in protein chemistry*, **70**. January: Academic Press; 2005:247–299.
16. Weisel JW: **Structure of fibrin: impact on clot stability**. *J Thromb Haemostasis* July 2007, **5**:116–124.
17. Pieters M, Wolberg AS: **Fibrinogen and fibrin: an illustrated review**. *Res Pract Thromb Haemost* 2019, **3**:161–172.
18. Weisel JW, Litvinov RI: **Fibrin formation, structure and properties**. *Fibrous Protein Struct Mech* 2017:405–456.
19. Litvinov RI, Gorkun OV, Galanakis DK, Yakovlev S, Medved L, Shuman H, Weisel JW: **Polymerization of fibrin: direct observation and quantification of individual B:b knob-hole interactions**. *Blood* 2007, **109**:130–138.
20. Blomback B, Hessel B, Hogg D, Therkildsen L: **A two-step fibrinogen-fibrin transition in blood coagulation**. *Nature* 1978, **275**:501–505.

21. Weisel JW: **Fibrin assembly. Lateral aggregation and the role of the two pairs of fibrinopeptides**. *Biophys J* 1986, **50**: 1079–1093.

22. * Yesudasan S, Wang X, Rodney D: **Averett. Coarse-grained molecular dynamics simulations of fibrin polymerization: effects of thrombin concentration on fibrin clot structure**. *J Mol Model* April 2018, **24**:109.
By defining fibrin(ogen) as a collection of spring-linked beads, this article uses coarse-grain molecular dynamic simulations to illustrate the realistic dependence fiber thickness has on thrombin concentration on very short time scales (nanosecond).

23. Yesudasan S, Wang X, Averett RD: **Fibrin polymerization simulation using a reactive dissipative particle dynamics method**. *Biomech Model Mechanobiol* 2018, **17**:1389–1403.

24. ** Weisel JW, Nagaswami C: **Computer modeling of fibrin polymerization kinetics correlated with electron microscope and turbidity observations: clot structure and assembly are kinetically controlled**. *Biophys J* July 1992, **63**:111–128.
The authors provide the first kinetic model of fibrin polymerization that includes all three steps of fibrin polymerization: conversion, elongation, and lateral aggregation. Their results show that to accurately represent the characteristic lag time and subsequent rise in turbidity, protofibrils cannot aggregate until a critical length is reached.

25. * Kelley M, Leiderman K: **A mathematical model of bivalent binding suggests physical trapping of thrombin within fibrin fibers**. *Biophys J* October 2019, **117**:1442–1455.
The authors propose a new kinetic binding scheme and corresponding reaction-diffusion PDE model of fibrin thrombin binding interactions. They use Bayesian parameter estimation and model selection criteria to validate the new scheme using published data. The authors use the results of the modeling to hypothesize that thrombin can become irreversibly trapped inside fibrin fibers during the polymerization process.

26. Fogelson AL, Keener JP: **Toward an understanding of fibrin branching structure**. *Phys Rev E* May 2010, **81**, 051922.

27. Rukhlenko OS, Dudchenko OA, Zlobina KE, Guria GT: **Mathematical modeling of intravascular blood coagulation under wall shear stress**. *PloS One* July 2015, **10**, e0134028.

28. * Guy RD, Fogelson AL, Keener JP: **Fibrin gel formation in a shear flow**. *Math Med Biol: A Journal of the IMA* March 2007, **24**: 111–130.
The authors present the first mathematical model to quantify the interactions of fibrin gelation, coagulation, and ow and their subsequent inhibition of fibrin gel formation. The results presented indicate that fibrin gel height is limited by both shear rate and thrombin availability.

29. Lewis SD, Shields PP, Shafer JA: **Characterization of the kinetic pathway for liberation of fibrinopeptides during assembly of fibrin**. *J Biol Chem* August 1985, **260**:10192–10199.

30. * Naski MC, Shafer JA: **A kinetic model for the alpha-thrombin-catalyzed conversion of plasma levels of fibrinogen to fibrin in the presence of antithrombin III**. *J Biol Chem* July 1991, **266**: 13003–13010.
The authors extend their previous model of fibrinogen conversion into fibrin, explicitly including thrombin cleavage of fibrinopeptides-A and -B. They perform experiments measuring FpA and FpB release and used those results to validate the model. Their results show that thrombin bound to fibrinogen and fibrin I is competitively inhibited by thrombin binding to fibrin II and AT, resulting in little to no active thrombin released from a fibrin clot.

31. Zhmurov A, Protopopova AD, Litvinov RI, Zhukov P, Mukhitov AR, Weisel JW, Barsegov V: **Structural basis of interfacial flexibility in fibrin oligomers**. *Structure* 2016, **24**: 1907–1917.

32. Kononova O, Litvinov RI, Zhmurov A, Alekseenko A, Cheng CH, Agarwal S, Marx KA, Weisel JW, Barsegov V: **Molecular mechanisms, thermodynamics, and dissociation kinetics of knob-hole interactions in fibrin**. *J Biol Chem* 2013, **288**: 22681–22692.

33. Zhmurov A, Kononova O, Litvinov RI, Dima RI, Barsegov V, Weisel JW: **Mechanical transition from α-helical coiled coils to β-sheets in fibrin(ogen)**. *J Am Chem Soc* 2012, **134**: 20396–20402.

34. Hospital A, Goñi JR, Orozco M, Gelpí JL: **Molecular dynamics simulations: advances and applications**. *Comput Biol Chem Adv Appl* 2015, **8**:37–47.

35. Guria GT, Herrero MA, Zlobina KE: **A mathematical model of blood coagulation induced by activation sources**. *Discrete Continuous Dyn Syst - A* 2009, **25**:175.

36. Wilf J, Minton AP: **Soluble fibrin-fibrinogen complexes as intermediates in fibrin gel formation**. *Biochemistry* 1986, **25**: 3124–3133.

37. Chernysh IN, Weisel JW: **Dynamic imaging of fibrin network formation correlated with other measures of polymerization**. *Blood* 2008, **111**:4854–4861.

38. Hantgan RR, Hermans J: **Assembly of fibrin. A light scattering study**. *J Biol Chem* November 1979, **254**:11272–11281.

39. Nelson AC, Keener JP, Fogelson AL: **Kinetic model of two-monomer polymerization**. *Phys Rev E* 2020, **101**(022501).

40. Galina H, Lechowicz JB: **Mean-field kinetic modeling of polymerization: the Smoluchowski coagulation equation**. *Adv Polym Sci* 1998, **137**:135–172.

41. Aldous DJ: **Deterministic and stochastic models for coalescence (aggregation and coagulation): a review of the mean-field theory for probabilists**. *Bernoulli* 1999, **5**(1):3.

42. Wattis JAD: **An introduction to mathematical models of coagulation-fragmentation processes: a discrete deterministic mean-field approach**. *Phys Nonlinear Phenom* 2006, **222**.

43. Ziff RM, Stell G: **Kinetics of polymer gelation**. *J Chem Phys* 1980, **73**:3492–3499.

44. Ryan EA, Mockros LF, Weisel JW, Lorand L: **Structural origins of fibrin clot rheology**. *Biophys J* 1999, **77**:2813–2826.

45. Fogelson AL, Keener JP: **A framework for exploring the post-gelation behavior of Ziff and Stell's polymerization models**. *Society for Industrial and Applied Mathematics* 2015, **75**: 1346–1368.

46. Haynes LM, Orfeo T, Mann KG, Everse SJ, Brummel-Ziedins KE: **Probing the dynamics of clot-bound thrombin at venous shear rates**. *Biophys J* April 2017, **112**:1634–1644.

47. Zhu S, Chen J, Diamond SL: **Establishing the transient mass balance of thrombosis: from tissue factor to thrombin to fibrin under venous flow**. *Arterioscler Thromb Vasc Biol* 2018, **38**:1528–1536.

48. Fredenburgh JC, Stafford AR, Leslie BA, Weitz JI: **Bivalent binding to γA/γ'-Fibrin engages both exosites of thrombin and protects it from inhibition by the antithrombin-heparin complex**. *J Biol Chem* February 2008, **283**:2470–2477.

49. Bänninger H, Lämmle B, Furlan M: **Binding of α-thrombin to fibrin depends on the quality of the fibrin network**. *Biochem J* February 1994, **298**:157–163.

50. Siebenlist Kevin R, Mosesson Michael W, Hernandez Irene, Bush Leslie A, Di Cera Enrico, Shainoff John R, Di Orio James P, Stojanovic Laurie: **Studies on the basis for the properties of fibrin produced from fibrinogen-containing γ' chains**. *Blood* 2005, **106**:2730–2736.

51. Gersh KC, Nagaswami C, Weisel JW: **Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes**. *Thromb Haemostasis* 2009, **102**:1169.

52. Cooper Amy V, Standeven Kristina F, Ariëns RAS: **Fibrinogen gamma-chain splice variant γ' alters fibrin formation and structure**. *Blood* 2003, **102**:535–540.

53. Allan P, Uitte de Willige S, Abou-Saleh RH, Connell SD, Ariëns RAS: **Evidence that fibrinogen γ' directly interferes with protofibril growth: implications for fibrin structure and clot stiffness**. *J Thromb Haemostasis* 2012, **10**:1072–1080.

54. Collet JP, Nagaswami C, Farrell DH, Montalescot G, Weisel JW: **Influence of γ' fibrinogen splice variant on fibrin physical properties and fibrinolysis rate**. *Arterioscler Thromb Vasc Biol* 2004, **24**:382–386.

55. Domingues Marco M, Macrae Fraser L, Duval Cédric, McPherson Helen R, Bridge Katherine I, Ajjan Ramzi A, Ridger Victoria C, Connell Simon D, Philippou Helen, Ariëns Robert AS: **Thrombin and fibrinogen γ' impact clot structure by marked effects on intrafibrillar structure and protofibril packing**. *Blood* January 2016, **127**:487–495.

56. Lovely RS, Kazmierczak SC, Massaro JM, D'Agostino RB, O'Donnell CJ, Farrell DH: **γ' fibrinogen: evaluation of a new assay for study of associations with cardiovascular disease**. *Clin Chem* May 2010, **56**:781–788.

57. Byrnes JR, Wilson C, Boutelle AM, Brandner CB, Flick MJ, Philippou H, Wolberg AS: **The interaction between fibrinogen and zymogen FXIII-A2B2 is mediated by fibrinogen residues γ390-396 and the FXIII-B subunits**. *Blood* October 2016, **128**:1969–1978.

58. Philippou H, Rance J, Myles T, Hall SW, Ariens RA, Grant PJ, Leung L, Lane DA: **Roles of low specificity and cofactor interaction sites on thrombin during factor XIII activation competetion for cofactor sites on thrombin determines its fate**. *J Biol Chem* August 2003, **278**: 32020–32026.

59. Chernysh IN, Nagaswami C, Prashant KP, Weisel JW: **Fibrin clots are equilibrium polymers that can be remodeled without proteolytic digestion**. *Sci Rep* 2012, **2**.

60. Stockmayer WH: **Theory of molecular size distribution and gel formation in branchedchain polymers**. *J Chem Phy* 1943, **11**: 45–55.

61. Flory PJ: **Molecular size distribution in three dimensional polymers**. *J Am Chem Soc* 1941, **63**:3083–3090.