Modelling fibrinolysis: 1D continuum models

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Fibrinolysis is the enzymatic degradation of the fibrin mesh that stabilizes blood clots. Experiments have shown that coarse clots made of thick fibres sometimes lyse more quickly than fine clots made of thin fibres, despite the fact that individual thick fibres lyse more slowly than individual thin fibres. This paper aims at using a 1D continuum reaction–diffusion model of fibrinolysis to elucidate the mechanism by which coarse clots lyse more quickly than fine clots. Reaction–diffusion models have been the standard tool for investigating fibrinolysis, and have been successful in capturing the wave-like behaviour of lysis seen in experiments. These previous models treat the distribution of fibrin within a clot as homogeneous, and therefore cannot be used directly to study the lysis of fine and coarse clots. In our model, we include a spatially heterogeneous fibrin concentration, as well as a more accurate description of the role of fibrin as a cofactor in the activation of the lytic enzyme. Our model predicts spatio-temporal protein distributions in reasonable quantitative agreement with experimental data. The model also predicts observed behaviour such as a front of lysis moving through the clot with an accumulation of lytic proteins at the front. In spite of the model improvements, however, we find that 1D continuum models are unable to accurately describe the observed differences in lysis behaviour between fine and coarse clots. Features of the problems that lead to the inaccuracy of 1D continuum models are discussed. We conclude that higher-dimensional, multiscale models are required to investigate the effect of clot structure on lysis behaviour.

Keywords: fibrin; enzymatic degradation; lysis front.

FIG. 1. Cartoon of fibrinolysis (not to scale): PLG and tPA diffuse in the plasma and can bind to fibrin. If tPA and PLG bind in close proximity to one another, the tPA can convert the plasminogen to plasmin (PLi). PLi then degrades the fibrin by cutting across the fibre. New binding sites for tPA and PLG are exposed as plasmin cleaves fibrin. Plasma concentration of PLG $\approx 2 \mu\text{M}$, and plasma concentration of tPA $\approx 70 \text{ pM}$.

1. Introduction

Fibrinolysis is the enzymatic degradation of the fibrin mesh that stabilizes blood clots. Plasmin, the main fibrinolytic enzyme, is activated from its inactive precursor plasminogen (PLG) by tissue-type plasminogen activator (tPA). tPA is fibrin-selective and preferentially activates fibrin-bound plasminogen, creating a source of plasmin on the surface of the fibrin fibres. The plasma concentration of plasmin is essentially zero due to strong plasmin inhibitors, so the local creation of plasmin on fibres is a critical step of fibrinolysis. Once plasmin is formed, it degrades the fibrin by cleaving peptide bonds formed by the carboxyl group of lysine. The cleavage exposes new C-terminal lysines to which more tPA and PLG may bind. In this way, plasmin positively feeds back on its own production (Fig. 1).

Plasminogen and tPA are small molecules (88 and 72 kDa, respectively), whose diffusion is not hindered by the presence of fibrin fibres. In fact, non-fibrin-binding proteins an order of magnitude bigger were found to diffuse freely through fibrin clots, without any steric hindrance (Matveyev & Domogatsky, 1992). Therefore, the porosity of a clot does not affect the diffusion of tPA or plasminogen. However, the fibrin-specificity of the molecules results in an effective diffusion rate which is lower than the diffusion rate in the absence of fibrin; when plasminogen and tPA bind to fibres, their diffusional movement is halted until they unbind.

Fibrin clot structure is affected by the environment in which it forms. Clots composed of thin, tightly packed fibres form in conditions of high salinity or high thrombin concentration, while coarser clots composed of thick fibres form in conditions of low salinity or low thrombin concentration. Experiments have shown that coarse clots made of thick fibres sometimes lyse more quickly than fine clots made of thin fibres (Carr & Alving, 1995; Collet *et al.*, 1993), despite the fact that individual thick fibres lyse more slowly than individual thin fibres (Collet *et al.*, 2000, 2003). Proposed mechanisms for this apparent paradox include enhanced movement of tPA through coarse clots (and therefore earlier activation of PLG), and lateral transection of fibres by plasmin (Weisel *et al.*, 1999; Weisel & Litvinov, 2008). If degradation occurs by transverse cutting across fibres (rather than by uniform digestion around fibres) (Blinic *et al.*, 2000; Collet *et al.*, 2000; Veklich *et al.*, 1998), presumably a coarse clot with fewer fibres to transect might be degraded faster than a fine clot with a higher fibre density. While this explanation

seems to be widely accepted, it is difficult to confirm experimentally. This paper aims to use a 1D model of fibrinolysis to elucidate the mechanism by which coarse clots lyse more quickly than fine clots.

The use of continuum models to explore fibrinolysis is the prevailing trend in the literature, with many models consisting of 1D reaction–advection–diffusion equations ([Anand *et al.*, 1995](#); [Diamond & Anand, 1993](#); [Wootton *et al.*, 2002](#); [Zidanšek & Blinc, 1991](#)). All of these models assume that initially fibrin is distributed homogeneously throughout the domain. [Zidanšek & Blinc \(1991\)](#) study the spatial distribution of plasmin inside clots exposed to pressure-driven permeation of urokinase-type plasminogen activator (uPA). Like tPA, uPA converts plasminogen to plasmin, but unlike tPA it does so in plasma. uPA has no fibrin specificity, and therefore the possible positive feedback provided by plasmin exposure of new binding sites is absent in situations of uPA-induced lysis. [Diamond & Anand \(1993\)](#) extend the model of [Zidanšek & Blinc \(1991\)](#) to include more detailed reaction kinetics, but still do not account for the exposure of new binding sites in partially degraded fibrin. Also, [Diamond & Anand \(1993\)](#) incorrectly model lysis as uniform degradation around the fibre diameter, rather than as transverse cutting across fibres. [Wootton *et al.* \(2002\)](#) modify [Diamond & Anand's \(1993\)](#) model to account for the effect of partial fibrin degradation, but by treating fibrin homogeneously, they are unable to study the degradation of single fibres.

We create a 1D continuum model of the fibrinolytic system that extends the preceding models in several ways: we allow for tPA and PLG to compete for newly exposed binding sites; we allow only PLG which is bound to fibrin in close proximity to bound tPA to be converted to plasmin; and perhaps most importantly, we account for the spatially heterogeneous concentration of fibrin within clots. By treating fibrin concentration heterogeneously, we are able to explore for the first time the effects of lysis on single fibrin fibres, as well as on fibrin clots of varying structure.

Patients with blood clots are often treated with a therapeutic bolus of tPA, which has been shown under certain conditions to increase the rate of fibrinolysis when administered near the clot. We model the experiment by [Collet *et al.* \(2000\)](#), which mimics this clinical situation. In the experiment, a bolus of tPA is added to the edge of a native plasma fibrin clot in the absence of fluid flow. This experiment (and therapeutic intervention) does not include uPA, hence, we do not include uPA in the model. First, we test the model with a homogeneous fibrin concentration to verify that it produces results similar to those shown in [Diamond & Anand \(1993\)](#) and [Wootton *et al.* \(2002\)](#). Then spatial inhomogeneity is added to the model (so we can distinguish between fine and coarse clots), and the resulting predictions are compared with published experimental data. We conclude that 1D continuum models are capable of quantitatively predicting the spatial distribution of lytic enzymes, but cannot capture the observed behaviour of lysis in clots of varying structure. 1D continuum models can therefore be used to answer questions about spatiotemporal variation in lytic enzyme concentrations, but more sophisticated models are necessary to answer quantitative questions about structural aspects of lysis.

2. The model

We develop a 1D reaction–diffusion model to study the spatiotemporal dynamics of lytic protein concentrations. In our model, tPA and PLG can each be in one of two states: free in solution (where they diffuse) or bound to fibrin. Because tPA and PLG must be bound to fibrin in close proximity to one another for tPA to convert PLG to plasmin, fibrin is represented by binding doublets. Each binding doublet has two sites to which tPA and/or plasmin(ogen) may bind and from which they may unbind. If tPA occupies one site and PLG occupies the other, then PLG may be converted to plasmin. Such a doubly occupied doublet is analogous to the enzyme–substrate complex in standard enzyme kinetic models. We include binding doublets in an attempt to incorporate small scale spatial effects in the model. Doublets

FIG. 2. Reaction diagram for specific binding doublets: tPA may only bind to the gray sites and PLG and plasmin (PLi) may only bind to the white sites.

provide us with more information about where proteins are located on fibrin; if tPA and PLG are bound to the same doublet, the proteins are close enough together to create plasmin. If we model the concentrations of bound enzymes without the use of doublets, then we know only that tPA and PLG are bound somewhere on fibrin, but not how closely located they are to each other.

In the model, we account for two types of binding doublets: specific and non-specific. On a specific binding doublet, tPA may bind only to the first site, and PLG/plasmin may bind only to the second (Fig. 2). For non-specific doublets, tPA and PLG can each bind to either site. Only specific binding doublets are available initially, but as lysis proceeds, non-specific binding doublets are exposed by plasmin. Multiple doublets may exist at the same location. Our treatment of doublets is biologically motivated. It is believed that initially exposed tPA and plasminogen binding sites on fibrin are specific (Medved & Nieuwenhuizen, 2003). However, the binding sites exposed during degradation are C-terminal lysines to which many types of proteins may bind. Hence, we consider newly exposed binding sites to be nonspecific.

Next, we describe the terms in the model equations before presenting the full model. Two variations of this model are considered, one with a homogeneous concentration of fibrin and the other with a heterogeneous concentration of fibrin.

Model variables: We track the concentrations of free tPA and PLG (denoted a and p , respectively), as well as the concentrations of all possible configurations of binding doublets (e.g. a specific doublet with tPA bound but no PLG). $s^{i,j}$ represents a specific binding doublet with index i describing tPA ($i = 0$ if tPA is not bound, $i = 1$ if tPA is bound), and with index j describing plasmin(ogen) ($j = 0$ if neither PLG nor plasmin are bound, $j = 2$ if PLG is bound, and $j = 3$ if plasmin is bound; Table 1 and Fig. 2). $n^{i,j}$ is the concentration of a non-specific binding doublet with indices $i, j = 0, 1, 2$ or 3 . For example, $n^{2,3}$ refers to non-specific doublets with PLG bound to the first site and plasmin bound to the second. Owing to high concentrations of plasmin inhibitors, the plasma concentration of plasmin is effectively zero. Consequently, we neglect free plasmin. If plasmin unbinds from fibrin, it is assumed to be instantaneously inhibited and is removed from the model. The final variable is n , the concentration of the pool of initially unexposed binding sites that can be exposed by plasmin.

TABLE 1 *Continuum model notation. For a specific binding doublet, i may only be 0 or 1, and j may only be 0, 2, or 3. For a non-specific binding doublet, i and j may take any value 0–3*

s^{ij}	Specific binding doublet concentration
n^{ij}	Non-specific binding doublet concentration
n	Unexposed binding doublet concentration
i or $j = 0$	Nothing bound
i or $j = 1$	tPA bound
i or $j = 2$	PLG bound
i or $j = 3$	PLi bound

Binding and unbinding: Free tPA and PLG can diffuse and interact with fibrin, but once bound to a doublet, the proteins no longer move. Doublet concentrations are changed by protein binding and unbinding. For example,

$$\frac{ds^{1,3}}{dt} = \underbrace{k_{\text{on}}^{a,0,3} a s^{0,3}}_{\text{tPA binds to doublet with PLi already bound}} - \underbrace{k_{\text{off}}^{a,1,3} s^{1,3} - k_{\text{off}}^{i,1,3} s^{1,3}}_{\text{tPA (1st term) or PLi (2nd term) unbind}} + \dots$$

These are basic terms obtained from reaction diagrams like Fig. 2. $k_{\text{on}}^{q,u,v}$ is the forward rate for species q binding to doublet $s^{u,v}$ or $n^{u,v}$, and $k_{\text{off}}^{q,u,v}$ is the reverse rate for species q unbinding from doublet $s^{u,v}$ or $n^{u,v}$, for $q = a, p$, or i (plasmin), and $u, v = 0, 1, 2$ or 3 .

Degradation: The terms for plasmin-mediated degradation of fibrin are derived using a quasi-steady state analysis for two substrates competing for one enzyme. Unexposed non-specific sites, n , and exposed sites, δ , compete for plasmin. The concentration of plasmin is

$$\sigma = s^{0,3} + s^{1,3} + n^{0,3} + n^{1,3} + n^{2,3} + 2n^{3,3},$$

and the total concentration of exposed doublets is

$$\delta = s^{0,0} + s^{0,2} + s^{0,3} + s^{1,0} + s^{1,2} + s^{1,3} + n^{0,0} + n^{1,0} + n^{0,2} + n^{1,1} + n^{1,2} + n^{2,2} + n^{1,3} + n^{2,3} + n^{0,3} + n^{3,3}.$$

Quasi-steady-state analysis gives differential equations for degradation products created from δ (p_d), and for degradation products created from n (p_n):

$$\begin{aligned} \frac{dp_d}{dt} &= \frac{k_{\text{cat}} \sigma \delta}{K_M + \delta + (K_M/K_M^n) n}, \\ \frac{dp_n}{dt} &= \frac{k_{\text{cat}}^n \sigma n}{K_M^n + n + (K_M/K_M) \delta}. \end{aligned}$$

The catalytic rates and Michaelis–Menten constants are k_{cat} and K_M for plasmin degradation of exposed sites, and k_{cat}^n and K_M^n for plasmin ‘degradation’ (i.e. exposure) of unexposed sites, respectively. Assuming that each fibrin species (e.g. $s^{1,3}$) is degraded in proportion to its fraction of the total amount of

exposed binding sites, and that plasmin can degrade any doublet at the same location (not just the doublet to which it is bound), the degradation terms take the form:

$$\begin{aligned}\frac{ds^{1,3}}{dt} &= \left(\frac{s^{1,3}}{\delta}\right) \left(-\frac{dp_d}{dt}\right) + \dots \\ &= -\frac{k_{\text{cat}}\sigma s^{1,3}}{K_M + \delta + (K_M/K_M^n)n} + \dots\end{aligned}$$

The other degradation term, obtained similarly using dp_n/dt , describes the conversion of unexposed sites, n , to exposed sites, $n^{0,0}$:

$$\frac{dn}{dt} = -\frac{k_{\text{cat}}^n\sigma n}{K_M^n + n + (K_M^n/K_M)\delta}.$$

When fibrin is degraded by plasmin, the binding doublets (and any bound plasmin) disappear, and the bound tPA and PLG are released into the solution.

Moving plasmin: On the basis of the observation that plasmin inhibitors have high concentration in plasma, plasmin in solution is ignored. It is believed that plasmin crawls across fibres (Weisel & Litvinov, 2008), so we allow plasmin to move between binding sites at the same spatial location. However, we assume that plasmin does not fully unbind from fibrin, so we set the unbinding rate to 0 (see Table A1, Appendix). Plasmin is released from fibrin only by degradation of the doublet to which it is bound. Consequently, the tPA molecule that created the plasmin is inhibited from producing more plasmin until it unbinds and rebinds elsewhere; it cannot encounter PLG while bound to a doublet occupied by plasmin. Allowing plasmin to move locally (where it is protected from plasmin inhibitors), frees up the binding site for PLG, which can then be converted to plasmin.

Of all the doublets that contain plasmin in the model, $s^{1,3}$ and $n^{1,3}$ are most abundant. For simplicity, we assume that only plasmin from these doublets can move, and that plasmin may move only to the most common doublets in the model, $s^{0,0}$ and $n^{0,0}$. Movement is modelled as a reaction. For example, the reactions

give terms

$$\frac{ds^{1,3}}{dt} = -k_{\text{move}}(s^{1,3}s^{0,0} + 2s^{1,3}n^{0,0}) + \dots$$

The factor of 2 arises because plasmin can move to either of the two available binding sites on non-specific doublet $n^{0,0}$.

Creation of plasmin: Finally, we model the conversion of PLG to plasmin. When tPA and PLG are bound to the same doublet, tPA converts PLG to plasmin at rate $k_{\text{cat}}^{\text{ap}}$. Therefore, tPA-mediated conversion is modelled by $k_{\text{cat}}^{\text{ap}}s^{1,2}$ (for specific doublets) and $k_{\text{cat}}^{\text{ap}}n^{1,2}$ (for non-specific doublets).

Sample equation: We now write out one model equation in its entirety. For the full model, see Appendix. Combining the pieces described above gives the equation:

$$\begin{aligned} \frac{ds^{1,3}}{dt} = & \underbrace{k_{\text{cat}}^{\text{ap}} s^{1,2}}_{\text{tPA converts PLG to PLi}} + \underbrace{k_{\text{on}}^{a,0,3} a s^{0,3}}_{\text{tPA binds to doublet with PLi already bound}} - \underbrace{k_{\text{off}}^{a,1,3} s^{1,3} - k_{\text{off}}^{i,1,3} s^{1,3}}_{\text{tPA (1st term) or PLi (2nd term) unbind}} \\ & - \underbrace{k_{\text{move}}(s^{1,3} s^{0,0} + 2s^{1,3} n^{0,0})}_{\text{PLi from } s^{1,3} \text{ moves onto } s^{0,0} \text{ or } n^{0,0} \text{ at same spatial location}} - \underbrace{\frac{k_{\text{cat}} \sigma s^{1,3}}{K_M + \delta + (K_M/K_M^n)n}}_{\text{PLi degrades fibrin}}. \end{aligned}$$

The other model equations are similar.

Initial and boundary conditions: An indicator function $\chi(x)$ is used to specify where fibrin is found at $t = 0$. For the homogeneous model, the domain is $0 \leq x \leq 300 \mu\text{m}$ ($0 \leq x \leq 1$ non-dimensionally) and $\chi = 1 - 0.5(1 + \tanh((0.1 - x)/0.01))$, which corresponds to fibrin being nearly uniformly distributed over the interval $30 \mu\text{m} < x < 300 \mu\text{m}$. For the heterogenous model, the fibrin concentration varies as indicated in equation (2.3). The fibrin concentration is high at locations occupied by a fibre, and zero between fibres, with transitions smoothed for numerical simulation. The non-zero fibrin concentration is chosen so that the fibrin concentration averaged over the whole domain is $6 \mu\text{M}$. The domain is $100 \mu\text{m}$ long, with the first $10 \mu\text{m}$ free of fibrin (Fig. 3). Using an average fibre diameter from the work of Collet *et al.* (2000) and an average pore size (gap between fibres), we calculate the number of fibres in the clot. For example, in a fine clot (pore size $1 \mu\text{m}$) made of thin fibres (diameter 299 nm), there are $(100 \mu\text{m} - 10 \mu\text{m})/1.299 \mu\text{m} \approx 69$ fibres in the clot domain. Constraints on computational time motivate the use of a variable space step that ranges in non-dimensional size from 2^{-10} to 2^{-14} (Fig. 3).

We assume that bound plasminogen is initially in equilibrium with the plasma plasminogen at concentration $p_0 = 0.5 \mu\text{M}$, and that only specific doublets are exposed. A quasi-steady-state analysis for $s^{0,0}$ and $s^{0,2}$ in the presence of this free plasminogen gives initial conditions

$$s^{0,0}(x, 0) = \frac{k_{\text{off}}^{p,0,2} s_{\text{tot}}}{(k_{\text{on}}^{p,0,0} p_0 + k_{\text{off}}^{p,0,2})} \cdot \chi \quad (2.1)$$

and

$$s^{0,2}(x, 0) = (s_{\text{tot}} - s^{0,0}(x, 0)) \cdot \chi, \quad (2.2)$$

where s_{tot} is the total specific binding site density, taken to be $1 \mu\text{M}$. Because fibrin is concentrated in fibres, the initial conditions involve χ , the initial clot indicator function:

$$\chi = \begin{cases} \frac{H}{5h_f}(x - x_L(i)) & \text{if } x_L(i) \leq x \leq x_L(i) + 5h_f, \\ \frac{H}{5h_f}(x - x_R(i)) & \text{if } x_R(i) - 5h_f \leq x \leq x_R(i), \\ H & \text{if } x_L(i) + 5h_f < x < x_R(i) - 5h_f, \\ 0 & \text{otherwise,} \end{cases} \quad (2.3)$$

for $i = 1, 2, \dots, N_f$ (total fibre number), where H is the height of the trapezoid, h_f the finest numerical space step and $x_L(i)$, $x_R(i)$ are the left-most and right-most points in fibre i , respectively. All other

FIG. 3. Fibrin concentration in a region of the heterogeneous model containing one fibre: Position along the clot, measured in microns, is indicated on the horizontal axis. The total clot length is $100\mu\text{m}$. Fibre width is indicated by the non-zero region of the black curve. x_L denotes the left edge of the fibre, x_R denotes the right edge, and H denotes the fibrin concentration (in μM) within the fibre. Tick marks on the horizontal axis correspond to the numerical grid spacing used in solving the equations. A coarse step, h_c , is used in fibre-free regions of the domain. A fine step, h_f , is used in regions of the domain containing fibres. Four progressively smaller steps are used to transition from h_c to h_f , and those same steps are used in ascending order to transition from h_f to h_c . Inset: A portion of the clot domain before lysis begins. Axes are the same as in main figure. The first fibre is the same one shown in the large figure, and is the first fibre in the clot—there is no fibrin to the left of this fibre. The pattern of fibres continues in this manner to fill the rest of the domain.

specific and non-specific doublets are initially zero. Fibres are aggregates of protofibrils, two-stranded fibrin polymers in which each strand is composed of three chains: α , β and γ (Weisel, 1986). We assume that degradation of a single protofibril requires six cuts (one for each chain), and we take the total fibrin concentration to be $6\mu\text{M}$. This means that the concentration of potential, unexposed binding doublets is five times the concentration of specific doublets, $n(x, 0) = 5 \cdot s_{\text{tot}} \cdot \chi$. At the start of each experiment, a $5 \times 10^{-3}\mu\text{M}$ bolus of tPA is added in the fibrin-free region to the left of the clot (smoothed using a hyperbolic tangent function). The boundary conditions for tPA and PLG at $x = 0$ and 1 (non-dimensional end of the domain) are

$$\begin{aligned} \frac{da(0, t)}{dx} &= 0, \\ p(0, t) &= p_0, \\ \frac{da(1, t)}{dx} &= \frac{dp(1, t)}{dx} = 0. \end{aligned} \tag{2.4}$$

The initial and boundary conditions described above, along with equations (A.1–A.19) (see Appendix A.2), comprise the entire model. These equations hold regardless of clot heterogeneity, which requires only a different definition of the clot function, χ . The full model is comprised of 2

FIG. 4. Dynamics of the homogeneous fibrin model: Total run time is 30 min, with successive traces moving right each separated by 3 min. In the bottom panel, the dotted line indicates the initial clot function. The vertical axes are concentrations measured in μM . ‘Bd’ means ‘bound’.

reaction–diffusion equations and 17 ordinary differential equations. There are 15 distinct parameters, of which 7 have experimentally measured values (see Table A1 in Appendix A.1).

3. Results

The model equations are solved numerically using a fractional step method with a Crank–Nicholson scheme for the diffusion and a second-order Runge–Kutta method for the reactions. For the parameter values in Table A1, the homogeneous model predicts that lysis proceeds as a front (Fig. 4). Free PLG concentrations remain roughly constant and bound concentrations of tPA, PLG and plasmin are all elevated at the lysis front, in qualitative agreement with experiments (Sakharov & Rijken, 1995; Sakharov *et al.*, 1996). Bound PLG concentrations are predicted to be five times higher at the lysis front compared with the initial bound concentration, with elevated concentrations over an average distance of $34.2 \mu\text{m}$, about one-tenth of the domain length.

The homogeneous fibrin model displays important features of lysis, but is unable to predict the degradation of different types of clots. On the basis of the observation that blood clots contain highly concentrated fibrin *fibres* (and not uniform concentrations of fibrin throughout the clot volume), we study a heterogeneous fibrin model. We investigate differences in the lysis speeds of coarse and fine clots by using the same total fibrin concentration, with several different fibre configurations: a fine clot made of 69 299-nm-wide fibres spaced $0.976 \mu\text{m}$ apart; a coarse clot made of 42 476-nm-wide fibres spaced $1.66 \mu\text{m}$ apart; and as an extreme case, a very coarse clot made of 10 1886-nm-wide fibres spaced $7.03 \mu\text{m}$ apart. In each of these cases, the initial fibrin concentration per fibre, H , the value of the non-zero part of the piecewise clot indicator function, is $29.16 \mu\text{M}$.

Since the model is 1D, we must choose fibre widths in a way that reflects the 2D geometry of the fibrin fibres. The amount of fibrin in a cross-section of a thin fibre is the product of fibre cross-sectional

TABLE 2 *Heterogeneous model results: Unless otherwise noted, parameters from Table A1 were used. Each simulation was run for one diffusion time (200 s). After 200 s, the total remaining amount of fibrin was calculated to obtain the percent of clot remaining. ‘Published K_{dis} ’ means $K_{\text{dis}}^a = 0.36 \mu\text{M}$, $K_{\text{dis}}^p = 38 \mu\text{M}$; ‘small K_{dis} ’ means $K_{\text{dis}}^a = 0.0036 \mu\text{M}$, $K_{\text{dis}}^p = 0.38 \mu\text{M}$*

Type of fibres	Fibre width (nm)	Pore size (μm)	Concentration of fibrin per fibre (μM)	Percent remaining (published K_{dis}) (%)	Percent remaining (small K_{dis}) (%)
Thin	299	0.976	29.16	61.3	39.3
Thick	476	1.66	29.16	61.8	36.7
Very thick	1886	7.03	29.16	60.8	26.2
Thick, ‘tall’	376	1.86	37.32	62.2	33.8
Very thick, ‘tall’	751	8.2	75.06	60.6	7.1

FIG. 5. Comparison of lysis front velocities in different clot geometries, for (a) published ($K_{\text{dis}}^a = 0.36 \mu\text{M}$, $K_{\text{dis}}^p = 38.0 \mu\text{M}$) and (b) small ($K_{\text{dis}}^a = 0.0036 \mu\text{M}$ and $K_{\text{dis}}^p = 0.38 \mu\text{M}$) dissociation constants: The first location in the clot where the fibrin concentration remains at least 50% of the maximum concentration is plotted versus time. The lysis front velocities are the slopes of the lines fitted to the above data. The step-like appearance is due to the gaps between fibres. The lysis front must move across the fibrin-free space between the fibres before degradation begins on the next fibre, hence the step-like regions where the location of the front is not moving in time. The clots with thicker fibres have wider pores, so the steps are longer in these cases.

area, $\pi(r_{\text{thin}})^2$, and the concentration of fibrin in a thin fibre, c_{thin} . Likewise, the amount of fibrin in a cross-section of a thick fibre is $c_{\text{thick}}\pi(r_{\text{thick}})^2$. If we assume that the concentration of fibrin in a single fibre is the same regardless of fibre thickness, then $c_{\text{thin}} = c_{\text{thick}}$ and the amount of fibrin in a thick fibre is related to the amount of fibrin in a thin fibre by the factor $(r_{\text{thick}}/r_{\text{thin}})^2$. Taking $W_{\text{thin}}^{\text{1D}}$ (the width of a 1D thin fibre) to be equal to r_{thin} gives the following equation for the 1D width of a thick fibre:

FIG. 6. Average bound PLG and fibrin concentrations (μM) for the heterogeneous model in a fine clot with thin fibres and dissociation constants from the literature ($K_{\text{dis}}^a = 0.36 \mu\text{M}$, $K_{\text{dis}}^p = 38.0 \mu\text{M}$): Averages are plotted at the fibre midpoints. The open symbols denote the initial average concentration, and each successively lighter gray line is 20 s later than the one before it. (a) Average concentration of bound PLG per fibre. (b) Average concentration of fibrin per fibre.

FIG. 7. Average bound PLG and fibrin concentrations (μM) for the heterogeneous model in a fine clot with thin fibres and small dissociation constants ($K_{\text{dis}}^a = 0.0036 \mu\text{M}$, $K_{\text{dis}}^p = 0.38 \mu\text{M}$). Averages are plotted at the fibre midpoints. The open symbols denote the initial average concentration, and each successively lighter gray line is 20 s later than the one before it. (a) Average concentration of bound PLG per fibre. (b) Average concentration of fibrin per fibre.

$$W_{\text{thick}}^{\text{1D}} = \left(\frac{r_{\text{thick}}}{r_{\text{thin}}} \right)^2 \times W_{\text{thin}}^{\text{1D}} = \frac{(r_{\text{thick}})^2}{r_{\text{thin}}}. \quad (3.1)$$

The amount of fibrin in a fibre cross-section does not scale linearly with radius, so rather than simply use the 2D radius as our 1D fibre thickness, we compute the 1D thickness using equation (3.1). Using the radii reported in [Collet *et al.* \(2000\)](#), equation (3.1) gives a 1D thick fibre of width 476 nm.

TABLE 3 *Heterogeneous model single fibre results: Unless otherwise noted, parameters from Table A1 were used. The lysis time is defined as the time at which the average fibrin concentration in the fibre first reaches 10% of its initial concentration*

Type of fibres	Width of fibres (nm)	Lysis time (s)	
		$(K_{\text{dis}}^a = 0.36 \mu\text{M}, K_{\text{dis}}^p = 38.0 \mu\text{M})$	$(K_{\text{dis}}^a = 0.0036 \mu\text{M}, K_{\text{dis}}^p = 0.38 \mu\text{M})$
Thin	299	72.5	6.065
Thick	476	74.0	7.415
Very thick	1886	78.85	20.415

We explore the effect of fibrin concentration per fibre on lysis speed using two simulated clot structures: a coarse clot with 42 376-nm-wide fibres spaced $1.86 \mu\text{m}$ apart and fibrin concentration per fibre $37.32 \mu\text{M}$ (henceforth referred to as a ‘thick, tall’ clot because a higher fibrin concentration results in a taller trapezoid); and a very coarse clot with 10 751-nm-wide fibres spaced $8.2 \mu\text{m}$ apart and fibrin concentration per fibre $75.06 \mu\text{M}$ (henceforth referred to as a ‘very thick, tall’ clot). These concentrations are calculated by keeping the total number of fibres constant and adjusting the fibre widths and pores sizes. For the coarse clot, the fibre diameter is taken from Collet *et al.* (2000), and for the very coarse clot, the fibre diameter is back-calculated using equation (3.1). Once we know the number of fibres and fibre diameter, we calculate the pore size and choose the fibrin concentration per fibre such that the total fibrin concentration in the clot is $6 \mu\text{M}$.

Contrary to experimental evidence (Collet *et al.*, 2000), this model predicts no appreciable difference in the lysis rates for the different clot types (see columns 1–5 of Table 2). With dissociation constants from the literature, the lysis front moves at the same speed regardless of clot structure (Fig. 5a). The model does, however, exhibit a wave-like motion of lysis and higher concentrations of bound enzymes at the lysis front compared with bound enzymes elsewhere in the clot (Fig. 6).

To test whether the model can produce different lysis rates for different clot structures, we increase the binding rates of tPA and PLG by two orders of magnitude while keeping the unbinding rates fixed. This gives dissociation constants that are 100 times smaller than those presented in the literature, and also reflects a stronger binding of proteins to fibrin. Stronger binding reduces the effective diffusion of molecules into the clot, and as a result, bound enzymes localize near the lysis front (Figs 7 and 8), and lysis rates vary for different clot structures (Table 2, column 6 and Fig. 5b). The representation of 2D fibre geometry in the 1D model affects lysis speed; the thick, tall fibres (which have a higher fibrin concentration per fibre than the thin fibres), result in coarse clots lysing ~ 1.2 -times faster than fine. In the very coarse, tall clot, with thicker fibres and wider pores, lysis speeds increase even more (up to 1.6-times the fine speed).

With the smaller dissociation constants ($K_{\text{dis}}^a = 0.0036 \mu\text{M}$ and $K_{\text{dis}}^p = 0.38 \mu\text{M}$), the fibrin-bound concentration of PLG is roughly an order of magnitude higher than the initial bound concentration in a small region at the front of the lysing clot (about $8.35 \mu\text{M}$ for fine clots and $11.4 \mu\text{M}$ for coarse clots, on average). The lysis front is much sharper than when dissociation constants from the literature are used, with almost an entire fibre being degraded before any lysis begins on the next fibre (Fig. 7).

To measure the lysis rate of individual fibres we focus on the first fibre in the domain. Recording the time at which the average fibrin concentration in this fibre first reaches 10% of its initial concentration gives a measure of lysis time (Table 3). Because the first fibre in the different clot types begins at the same position in the domain and we initialize tPA the same way regardless of clot type, we can compare the times it takes the first fibre to lyse for each case. With dissociation constants from the literature, a

FIG. 8. Bound tPA (a), bound PLG plus plasmin (b), free tPA (c) and free PLG (d) concentrations for the heterogeneous model of a fine clot with thin fibres and small dissociation constants ($K_{\text{dis}}^a = 0.0036 \mu\text{M}$ and $K_{\text{dis}}^p = 0.38 \mu\text{M}$). In each plot, the dashed line denotes the initial condition, and each successively lighter shade of gray describes the given spatial distribution 20 s after the previous plot, for a total of 200 s. The peaks are different heights because the times that are plotted do not necessarily correspond to the lysis front speed. All concentrations are in μM .

single thin fibre lyses slightly faster than a thick fibre, which lyses slightly faster than a very thick fibre (72.5, 74.0 and 78.85 s, respectively). With small dissociation constants there is more of an increase in lysis time with increasing diameter, but the predicted times are not physical (6.065, 7.415 and 20.415 s for thin, thick and very thick fibres, respectively).

4. Discussion

The existence of a front of lysis moving through a clot when plasminogen activator is introduced at the clot edge is well supported in the experimental literature (Diamond & Anand, 1993; Sakharov *et al.*, 1996; Sakharov & Rijken, 1995; Wu *et al.*, 1994). With both spatially homogeneous and heterogeneous fibrin concentrations, our model predicts that lysis moves as a front with peaks in the concentrations of lytic proteins at the front. This confirms results from earlier modelling efforts of Diamond & Anand

FIG. 9. Delay in lysis after tPA binds. Average concentrations of tPA (solid black) and plasmin (dashed) bound to a single thin fibre in a fine clot are shown as functions of time. The change in fibrin concentration in the fibre (concentration at time 0 minus concentration at time t) is plotted in gray. There is a delay after tPA binds before plasmin is produced, and another delay after plasmin production before lysis begins, i.e. before the change in fibrin concentration is non-zero. These results were obtained using small dissociation constants ($K_{\text{dis}}^a = 0.0036 \mu\text{M}$ and $K_{\text{dis}}^p = 0.38 \mu\text{M}$).

(1993) and Wootton *et al.* (2002), although consistent with Wootton *et al.* (2002), we find that attaining realistic lysis profiles necessitates using larger binding rates than those proposed by Diamond & Anand (1993). Even with adjusted binding rates, however, neither our homogeneous nor heterogeneous fibrin models, with dissociation constants taken from the literature, can quantitatively reproduce the protein concentration and distribution results from Sakharov *et al.* (1996). There the authors report PLG concentrations in fibrin agglomerates on the lysis front to be 30 times higher than the concentration in the surrounding plasma, PLG concentrations in the $3\text{-}\mu\text{m}$ region comprising the lysis front to be nine times higher than the plasma concentration and PLG concentrations in a $20\text{-}\mu\text{m}$ -wide region adjacent to the front to be at least twice higher than the plasma concentration. The ‘peaks’ of PLG in Figs 4 and 6 are wider (about 34.2 and $51 \mu\text{m}$, respectively), and are barely higher than the free concentration ($0.5 \mu\text{M}$), but nonetheless are qualitatively similar to Sakharov *et al.*’s (1996) results. In reasonable quantitative agreement with Sakharov *et al.* (1996), the heterogeneous model with small dissociation constants results in peaks of PLG occurring in an $8\text{--}12 \mu\text{m}$ region at the lysis front, with a concentration almost 50 times higher than the plasma-phase concentration (Fig. 8). These results are more consistent with Sakharov *et al.*’s (1996) data than any previous models. Wootton *et al.* (2002) show increases in plasma-phase enzyme concentrations at the lysis front, across distances of about $25\text{--}50 \mu\text{m}$, but give no results for bound concentrations. Diamond & Anand (1993), in a model with permeation of proteins, find a high concentration of bound plasmin at the lysis front over a distance of about $2000 \mu\text{m}$, with an increased concentration which is lower than the plasma-phase PLG concentration. Our heterogeneous model accurately predicts the width of the region of accumulating proteins ($8\text{--}12 \mu\text{m}$), and is within a factor of two of the largest measured PLG increase (50 times higher than surrounding plasma concentration in our model vs. 30 times higher in Sakharov *et al.*’s (1996) experiments).

In addition to using our model to investigate concentration profiles, we aim at explaining why coarse clots lyse more quickly than fine clots, even when the total fibrin concentration is constant. When spatial heterogeneity of the clot structure is taken into account and parameter values from the literature are used, fine clots degrade at the same speed as coarse clots (Fig. 5a and Table 2). By lowering the

tPA and PLG dissociation constants from the literature by two orders of magnitude, we observe localization of lytic enzymes at the front and variation in lysis rates for different clot structures (Figs 5b, 7 and 8). Stronger binding results in a decrease in the effective diffusion constant; molecules bind to fibrin and thus are prevented from diffusing farther into the clot. The tPA molecules remain bound until the fibre degrades, and it is only then that they can diffuse to the next fibre and initiate lysis. This localization of tPA results in sequential degradation of fibres, with one fibre degrading before degradation of the next fibre begins. The observed delay after tPA binds before plasmin is produced and lysis begins (Fig. 9), can explain how coarse clots can lyse faster than fine. When lysis is sequential (as when the smaller dissociation constants are used), the number of fibres in the clot is important; with fewer fibres, lysis needs to be started fewer times, and therefore there are fewer delays while plasmin is created. In this situation, a coarse clot with fewer fibres can lyse more quickly than a fine clot.

The large accumulation of lytic proteins appears to be the cause of the variation in lysis rates. Using dissociation constants from the literature, the increase in enzyme concentrations, and therefore lysis, occur over a larger region which contains multiple fibres. With smaller dissociation constants, almost complete lysis of one fibre occurs before degradation of any other fibre begins. This suggests that the number of fibres in a clot (and not their thickness) is the main determinant of lysis rate; with fewer fibres, the lytic process must be started fewer times, which outweighs the longer time required to degrade individual thick fibres. Lysis is slower in the thick and very thick clots compared with their ‘tall’ clot counterparts, because the number of fibres are the same, but fibres are thinner in the ‘tall’ clots, allowing them to lyse more quickly. We cannot explain why it is necessary to have dissociation constants that are 100 times smaller than the measured values in order for the model to predict that coarse and fine clots lyse at different rates. Either the meaning of the dissociation constant is different in the 1D model compared with the experimental quantity or a 1D model is simply incapable of distinguishing between lysis of fine clots and lysis of coarse clots without significant assumptions (like rate constants that are orders of magnitude different than measured values). The challenge of determining whether either of these options explains our results remains to be resolved.

The heterogeneous fibrin model poorly predicts single fibre lysis—not only are the lysis times too fast, but also the relative lysis times between fibres of varying diameter are too small. Experiments show that, on average, a thin fibre takes 3.1 min to degrade, while a thick fibre degrades in 5.4 min (Collet *et al.*, 2000). Therefore, a thin fibre lyses about 1.74 times faster than a thick fibre. With dissociation constants from the literature ($K_{\text{dis}}^a = 0.36 \mu\text{M}$ and $K_{\text{dis}}^p = 38 \mu\text{M}$), our model predicts thin fibres lyse 1.021 times faster than thick fibres (74 s/72.5 s) and only 1.086 times faster than very thick fibres (78.75 s/72.5 s); essentially all fibres lyse in the same amount of time. With smaller dissociation constants ($K_{\text{dis}}^a = 0.0036 \mu\text{M}$ and $K_{\text{dis}}^p = 0.38 \mu\text{M}$) the relative speeds increase, but the individual lysis times are unrealistically fast: thin fibres lyse 1.22 times faster than thick fibres (7.415 s/6.065 s) and 3.37 times faster than very thick fibres (20.415 s/6.065 s).

While model results for single fibres are qualitatively correct and show that thin fibres lyse faster than thick fibres, the results are not quantitatively accurate, perhaps for several reasons. First, our single fibre fibrin concentration may be too small. The fibrin concentration per fibre has been estimated to be about $824 \mu\text{M}$ (Carr & Hermans, 1978; Voter *et al.*, 1986), significantly higher than the $29 \mu\text{M}$ we use. Secondly, the definition of lysis is likely different experimentally than it is in the model. We consider a fibre to be lysed when 90% of its initial fibrin concentration has degraded. Experimentally, lysis of a fibre is simply the time at which the fibre is no longer visible through the microscope. It is unclear how this relates to relative fibrin concentration. Finally, a 1D model cannot account for transverse cutting. Real lysis seems to proceed by transverse cutting across fibres, but with only 1D there is no way to

model plasmin crawling across a fibre. We believe a higher-dimensional model is required to study lysis of individual fibres.

An important feature of blood clots is the *mesh* of fibres. Fine clots contain more fibres and have a higher density of branch points than coarse clots (Collet *et al.*, 2000), which could affect the number of times those enzymes must initiate lysis. A 1D model cannot capture the complicated geometry of a blood clot. This limitation of an 1D model is demonstrated by the inability of the model to produce different fine and coarse front velocities upon inclusion of a more realistic, spatially varying fibrin concentration. Because clot structure plays an important role in the effectiveness of lysis, higher-dimensional models are likely to be required in order to answer questions about the patterns and rates of lysis of different clot configurations.

Using a continuum model to investigate the fibrinolytic system may also be problematic because of the extremely low concentration of tPA. Even in experiments that inject a bolus of tPA at concentration 5 nM (much higher than the plasma concentration of 70 pM), there are only 3 tPA molecules per cubic micrometre in the clot. Assuming a pore size of 1 μm in a fine clot, the volume enclosed by nearest-neighbour fibres is on the order of 1 μm^3 . This observation may partially explain why fibres are cut laterally, rather than uniformly degraded (Veklich *et al.*, 1998). With 3 tPA molecules per cubic micrometre, there is a distinct possibility that a particular fibre will encounter only a single tPA molecule. That tPA molecule will facilitate the local creation of plasmin, but will not contribute to plasmin production outside of this local region. Thus, plasmin activity on the fibrin fibre will be concentrated to a narrow segment of the fibre length.

If there are only three tPA molecules in a characteristic volume of clot, it is not valid to treat tPA as a continuum. This observation, coupled with the conclusion that higher-dimensional models are necessary to study lysis of individual fibres and of varying clot structures, motivates the development of a 3D stochastic model as described in our companion paper (Bannish *et al.*, 2012).

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REFERENCES

- ANAND, S., WU, J. & DIAMOND, S. L. (1995) Enzyme-mediated proteolysis of fibrous biopolymers: dissolution front movement in fibrin or collagen under conditions of diffusive or convective transport. *Biotechnol. Bioeng.*, **48**, 89–107.
- BACHMANN, F. (1994) The plasminogen–plasmin enzyme system. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 3rd edn (R. W. Colman, J. Hirsh, V. J. Marder & E. W. Salzman eds), Chapter 84. Philadelphia: J.B. Lippincott Company, pp. 1592–1622.
- BANNISH, B. E., KEENER, J. P. & FOGELSON, A. F. (2012) Modelling fibrinolysis: a 3-dimensional stochastic multiscale model. *Math. Med. Biol.*, **31**, 17–44.
- BLINC, A., MAGDIC, J., FRIC, J. & MUSEVIC, I. (2000) Atomic force microscopy of fibrin networks and plasma clots during fibrinolysis. *Fibrinolysis Proteolysis*, **14**, 288–299.
- CARR, M. & ALVING, B. (1995) Effect of fibrin structure on plasmin-mediated dissolution of plasma clots. *Blood Coagul. Fibrinolysis*, **6**, 567–573.
- CARR, M. & HERMANS, J. (1978) Size and density of fibrin fibers from turbidity. *Macromolecules*, **11**, 46–50.
- COLLET, J. P., LESTY, C., MONTALESCOT, G. & WEISEL, J. W. (2003) Dynamic changes of fibrin architecture during fibrin formation and intrinsic fibrinolysis of fibrin-rich clots. *J. Biol. Chem.*, **278**, 21331–21335.

- COLLET, J. P., PARK, D., LESTY, C., SORIA, J., SORIA, C., MONTALESCOT, G. & WEISEL, J. W. (2000) Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed : dynamic and structural approaches by confocal microscopy. *Arterioscler Thromb. Vasc. Biol.*, **20**, 1354–1361.
- COLLET, J. P., SORIA, J., MIRSHAHI, M., HIRSCH, M., DAGONNET, F., CAEN, J. & SORIA, C. (1993) Dusart syndrome: a new concept of the relationship between fibrin clot architecture and fibrin clot degradability: hypofibrinolysis related to an abnormal clot structure. *Blood*, **82**, 2462–2469.
- DIAMOND, S. L. & ANAND, S. (1993) Inner clot diffusion and permeation during fibrinolysis. *Biophys. J.*, **65**, 2622–2643.
- HIGGINS, D. L. & VEহার, G. A. (1987) Interaction of one-chain and two-chain tissue plasminogen activator with intact and plasmin-degraded fibrin. *Biochemistry*, **26**, 7786–7791.
- HORREVOETS, A. J. G., SMILDE, A., DE VRIES, C. & PANNEKOEK, H. (1994) The specific role of finger and kringle 2 domains of tissue-type plasminogen activator during *in vitro* fibrinolysis. *J. Biol. Chem.*, **269**, 12639–12644.
- LUCAS, M. A., FRETTO, L. J. & MCKEE, P. A. (1983) The binding of human plasminogen to fibrin and fibrinogen. *J. Biol. Chem.*, **258**, 4249–4256.
- MATVEYEV, M. U. & DOMOGATSKY, S. P. (1992) Penetration of macromolecules into contracted blood clot. *Biophys. J.*, **63**, 862–863.
- MEDVED, L. & NIEUWENHUIZEN, W. (2003) Molecular mechanisms of initiation of fibrinolysis by fibrin. *Thromb. Haemost.*, **89**, 409–419.
- SAKHAROV, D. V., NAGELKERKE, J. F. & RIJKEN, D. C. (1996) Rearrangements of the fibrin network and spatial distribution of fibrinolytic components during plasma clot lysis. *J. Biol. Chem.*, **271**, 2133–2138.
- SAKHAROV, D. V. & RIJKEN, D. C. (1995) Superficial accumulation of plasminogen during plasma clot lysis. *Circulation*, **92**, 1883–1890.
- VEKLICH, Y., FRANCIS, C. W., WHITE, J. & WEISEL, J. W. (1998) Structural studies of fibrinolysis by electron microscopy. *Blood*, **92**, 4721–4729.
- VOTER, W. A., LUCAVECHE, C. & ERICKSON, H. P. (1986) Concentration of protein in fibrin fibers and fibrinogen polymers determined by refractive index matching. *Biopolymers*, **25**, 2375–2384.
- WEISEL, J. W. (1986) Fibrin assembly: lateral aggregation and the role of the two pairs of fibrinopeptides. *Biophys. J.*, **50**, 1079–1093.
- WEISEL, J. W. & LITVINOV, R. I. (2008) The biochemical and physical process of fibrinolysis and effects of clot structure and stability on the lysis rate. *Cardiovasc. Hematol. Agents Med. Chem. (Formerly Current Med. Chem. - Cardiovasc. Hematol. Agents)*, **6**, 161–180.
- WEISEL, J. W., VEKLICH, Y., COLLET, J. P. & FRANCIS, C. W. (1999) Structural studies of fibrinolysis by electron and light microscopy. *Thromb. Haemost.*, **82**, 277–282.
- WOOTTON, D. M., POPEL, A. S. & ALEVRIADOU, B. R. (2002) An experimental and theoretical study on the dissolution of mural fibrin clots by tissue-type plasminogen activator. *Biotechnol. Bioeng.*, **77**, 405–419.
- WU, J.-H., SIDDIQUI, K. & DIAMOND, S. L. (1994) Transport phenomena and clot dissolving therapy: an experimental investigation of diffusion-controlled and permeation-enhanced fibrinolysis. *Thromb. Haemost.*, **72**, 105–112.
- ZIDANŠEK, A. & BLINC, A. (1991) The influence of transport parameters and enzyme kinetics of the fibrinolytic system on thrombosis: Mathematical modelling of two idealised cases. *Thromb. Haemost.*, **65**, 553–559.

TABLE A1 *Parameter values*

Description (units)	Notation	Value
tPA diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)	D_{tPA}	5×10^{-7}
PLG diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)	D_{PLG}	5×10^{-7}
tPA dissociation constant in absence of PLG/PLi (μM)	$K_{\text{dis}}^{\text{a}}$	0.36*
tPA dissociation constant in presence of PLG/PLi (μM)	$K_{\text{dis}}^{\text{a}}$	0.02†
tPA binding rate ($\mu\text{M}^{-1} \text{s}^{-1}$)	k_{on}^{a}	1.0
tPA unbinding rate (s^{-1})	$k_{\text{off}}^{\text{a}}$	$K_{\text{dis}}^{\text{a}} k_{\text{on}}^{\text{a}}$
PLG dissociation constant (μM)	$K_{\text{dis}}^{\text{p}}$	38.0‡
PLG binding rate ($\mu\text{M}^{-1} \text{s}^{-1}$)	k_{on}^{p}	0.1*
PLG unbinding rate (s^{-1})	$k_{\text{off}}^{\text{p}}$	$K_{\text{dis}}^{\text{p}} k_{\text{on}}^{\text{p}}$
PLi unbinding rate (s^{-1})	$k_{\text{off}}^{\text{i}}$	0.0
Rate constant for conversion of PLG to PLi by tPA (s^{-1})	$k_{\text{cat}}^{\text{ap}}$	50
Rate constant for PLi digestion of activated doublets (s^{-1})	k_{cat}	10§
Constant for enzymatic digestion of activated doublets (μM)	k_{M}	0.1
Rate constant for activation of un-activated doublets (s^{-1})	$k_{\text{cat}}^{\text{n}}$	50
Constant for enzymatic digestion of un-activated doublets (μM)	k_{M}^{n}	0.1

*Horrevoets *et al.* (1994), Higgins & Vohar (1987).

†Bachmann (1994).

‡Lucas *et al.* (1983).

*Wootton *et al.* (2002).

§Anand *et al.* (1995).

Appendix

A.1 Parameters

A.2 Model equations

The following dimensional equations comprise the full model. Model variables are described in Table 1 and Section 2, and model parameters are described in Table A1 (Appendix A.1).

$$\begin{aligned}
\frac{\partial a}{\partial t} = & \frac{\partial}{\partial x} \left(D_{\text{tPA}} \frac{\partial a}{\partial x} \right) - k_{\text{on}}^{\text{a},0,0} a(s^{0,0} + 2n^{0,0}) + k_{\text{off}}^{\text{a},1,0} (s^{1,0} + n^{1,0}) - k_{\text{on}}^{\text{a},0,2} a(s^{0,2} + n^{0,2}) \\
& + k_{\text{off}}^{\text{a},1,2} (s^{1,2} + n^{1,2}) - k_{\text{on}}^{\text{a},0,3} a(s^{0,3} + n^{0,3}) + k_{\text{off}}^{\text{a},1,3} (s^{1,3} + n^{1,3}) + 2k_{\text{off}}^{\text{a},1,1} n^{1,1} \\
& - k_{\text{on}}^{\text{a},1,0} a n^{1,0} + \frac{k_{\text{cat}} \sigma s^{1,3}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n} + \frac{k_{\text{cat}} \sigma s^{1,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n} + \frac{k_{\text{cat}} \sigma s^{1,0}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n} \\
& + \frac{k_{\text{cat}} \sigma n^{1,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n} + \frac{k_{\text{cat}} \sigma n^{1,3}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n} + \frac{k_{\text{cat}} \sigma n^{1,0}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n} \\
& + 2 \frac{k_{\text{cat}} \sigma n^{1,1}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^{\text{n}})n},
\end{aligned} \tag{A.1}$$

$$\begin{aligned}
\frac{\partial p}{\partial t} = \frac{\partial}{\partial x} \left(D_{\text{PLG}} \frac{\partial p}{\partial x} \right) &- k_{\text{on}}^{\text{p},0,0} p(s^{0,0} + 2n^{0,0}) + k_{\text{off}}^{\text{p},0,2} (s^{0,2} + n^{0,2}) - k_{\text{on}}^{\text{p},1,0} p(s^{1,0} + n^{1,0}) \\
&+ k_{\text{off}}^{\text{p},1,2} (s^{1,2} + n^{1,2}) + 2k_{\text{off}}^{\text{p},2,2} n^{2,2} - k_{\text{on}}^{\text{p},0,2} p n^{0,2} + k_{\text{off}}^{\text{p},2,3} n^{2,3} - k_{\text{on}}^{\text{p},0,3} p n^{0,3} \\
&+ \frac{k_{\text{cat}} \sigma s^{1,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + \frac{k_{\text{cat}} \sigma s^{0,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + \frac{k_{\text{cat}} \sigma n^{1,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} \\
&+ \frac{k_{\text{cat}} \sigma n^{0,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + 2 \frac{k_{\text{cat}} \sigma n^{2,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + \frac{k_{\text{cat}} \sigma n^{2,3}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n}, \tag{A.2}
\end{aligned}$$

$$\begin{aligned}
\frac{ds^{0,0}}{dt} = &-k_{\text{on}}^{\text{a},0,0} a s^{0,0} + k_{\text{off}}^{\text{a},1,0} s^{1,0} - k_{\text{on}}^{\text{p},0,0} p s^{0,0} + k_{\text{off}}^{\text{p},0,2} s^{0,2} + k_{\text{off}}^{\text{i},0,3} s^{0,3} \\
&- \frac{k_{\text{cat}} \sigma s^{0,0}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} - k_{\text{move}}(s^{1,3} s^{0,0} + n^{1,3} s^{0,0}), \tag{A.3}
\end{aligned}$$

$$\begin{aligned}
\frac{ds^{1,0}}{dt} = &k_{\text{on}}^{\text{a},0,0} a s^{0,0} - k_{\text{off}}^{\text{a},1,0} s^{1,0} - k_{\text{on}}^{\text{p},1,0} p s^{1,0} + k_{\text{off}}^{\text{p},1,2} s^{1,2} + k_{\text{off}}^{\text{i},1,3} s^{1,3} \\
&- \frac{k_{\text{cat}} \sigma s^{1,0}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + k_{\text{move}}(s^{1,3} s^{0,0} + 2s^{1,3} n^{0,0}), \tag{A.4}
\end{aligned}$$

$$\frac{ds^{0,2}}{dt} = -k_{\text{on}}^{\text{a},0,2} a s^{0,2} + k_{\text{off}}^{\text{a},1,2} s^{1,2} + k_{\text{on}}^{\text{p},0,0} p s^{0,0} - k_{\text{off}}^{\text{p},0,2} s^{0,2} - \frac{k_{\text{cat}} \sigma s^{0,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n}, \tag{A.5}$$

$$\begin{aligned}
\frac{ds^{0,3}}{dt} = &-k_{\text{on}}^{\text{a},0,3} a s^{0,3} + k_{\text{off}}^{\text{a},1,3} s^{1,3} - k_{\text{off}}^{\text{i},0,3} s^{0,3} - \frac{k_{\text{cat}} \sigma s^{0,3}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} \\
&+ k_{\text{move}}(s^{1,3} s^{0,0} + n^{1,3} s^{0,0}), \tag{A.6}
\end{aligned}$$

$$\begin{aligned}
\frac{ds^{1,2}}{dt} = &-k_{\text{cat}}^{\text{ap}} s^{1,2} + k_{\text{on}}^{\text{a},0,2} a s^{0,2} - k_{\text{off}}^{\text{a},1,2} s^{1,2} + k_{\text{on}}^{\text{p},1,0} p s^{1,0} - k_{\text{off}}^{\text{p},1,2} s^{1,2} \\
&- \frac{k_{\text{cat}} \sigma s^{1,2}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n}, \tag{A.7}
\end{aligned}$$

$$\begin{aligned}
\frac{ds^{1,3}}{dt} = &k_{\text{cat}}^{\text{ap}} s^{1,2} + k_{\text{on}}^{\text{a},0,3} a s^{0,3} - k_{\text{off}}^{\text{a},1,3} s^{1,3} - k_{\text{off}}^{\text{i},1,3} s^{1,3} - \frac{k_{\text{cat}} \sigma s^{1,3}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} \\
&- k_{\text{move}}(s^{1,3} s^{0,0} + 2s^{1,3} n^{0,0}), \tag{A.8}
\end{aligned}$$

$$\frac{dn}{dt} = -\frac{k_{\text{cat}}^n \sigma n}{K_{\text{M}}^n + n + (K_{\text{M}}^n/K_{\text{M}})\delta}, \tag{A.9}$$

$$\begin{aligned}
\frac{dn^{0,0}}{dt} = &-2k_{\text{on}}^{\text{a},0,0} a n^{0,0} + k_{\text{off}}^{\text{a},1,0} n^{1,0} - 2k_{\text{on}}^{\text{p},0,0} p n^{0,0} + k_{\text{off}}^{\text{p},0,2} n^{0,2} + k_{\text{off}}^{\text{i},0,3} n^{0,3} \\
&- \frac{k_{\text{cat}} \sigma n^{0,0}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + \frac{k_{\text{cat}}^n \sigma n}{K_{\text{M}}^n + n + (K_{\text{M}}^n/K_{\text{M}})\delta} - 2k_{\text{move}}(s^{1,3} n^{0,0} + n^{1,3} n^{0,0}), \tag{A.10}
\end{aligned}$$

$$\begin{aligned}
\frac{dn^{1,0}}{dt} = &2k_{\text{on}}^{\text{a},0,0} a n^{0,0} - k_{\text{off}}^{\text{a},1,0} n^{1,0} - k_{\text{on}}^{\text{p},1,0} p n^{1,0} + k_{\text{off}}^{\text{p},1,2} n^{1,2} + k_{\text{off}}^{\text{i},1,3} n^{1,3} \\
&+ 2k_{\text{off}}^{\text{a},1,1} n^{1,1} - k_{\text{on}}^{\text{a},1,0} a n^{1,0} - \frac{k_{\text{cat}} \sigma n^{1,0}}{K_{\text{M}} + \delta + (K_{\text{M}}/K_{\text{M}}^n)n} + k_{\text{move}}(n^{1,3} s^{0,0} + 2n^{1,3} n^{0,0}), \tag{A.11}
\end{aligned}$$

$$\begin{aligned} \frac{dn^{0,2}}{dt} = & -k_{\text{on}}^{a,0,2} an^{0,2} + k_{\text{off}}^{a,1,2} n^{1,2} + 2k_{\text{on}}^{p,0,0} pn^{0,0} - k_{\text{off}}^{p,0,2} n^{0,2} - k_{\text{on}}^{p,0,2} pn^{0,2} \\ & + 2k_{\text{off}}^{p,2,2} n^{2,2} + k_{\text{off}}^{i,2,3} n^{2,3} - \frac{k_{\text{cat}} \sigma n^{0,2}}{K_M + \delta + (K_M/K_M^n)n}, \end{aligned} \quad (\text{A.12})$$

$$\begin{aligned} \frac{dn^{0,3}}{dt} = & -k_{\text{on}}^{a,0,3} an^{0,3} + k_{\text{off}}^{a,1,3} n^{1,3} - k_{\text{on}}^{p,0,3} pn^{0,3} + k_{\text{off}}^{p,2,3} n^{2,3} - k_{\text{off}}^{i,0,3} n^{0,3} \\ & + 2k_{\text{off}}^{i,3,3} n^{3,3} - \frac{k_{\text{cat}} \sigma n^{0,3}}{K_M + \delta + (K_M/K_M^n)n} + 2k_{\text{move}}(s^{1,3} n^{0,0} + n^{1,3} n^{0,0}), \end{aligned} \quad (\text{A.13})$$

$$\begin{aligned} \frac{dn^{1,2}}{dt} = & -k_{\text{cat}}^{\text{ap}} n^{1,2} + k_{\text{on}}^{a,0,2} an^{0,2} - k_{\text{off}}^{a,1,2} n^{1,2} + k_{\text{on}}^{p,1,0} pn^{1,0} - k_{\text{off}}^{p,1,2} n^{1,2} \\ & - \frac{k_{\text{cat}} \sigma n^{1,2}}{K_M + \delta + (K_M/K_M^n)n}, \end{aligned} \quad (\text{A.14})$$

$$\begin{aligned} \frac{dn^{1,3}}{dt} = & k_{\text{cat}}^{\text{ap}} n^{1,2} + k_{\text{on}}^{a,0,3} an^{0,3} - k_{\text{off}}^{a,1,3} n^{1,3} - k_{\text{off}}^{i,1,3} n^{1,3} - \frac{k_{\text{cat}} \sigma n^{1,3}}{K_M + \delta + (K_M/K_M^n)n} \\ & - k_{\text{move}}(n^{1,3} s^{0,0} + 2n^{1,3} n^{0,0}), \end{aligned} \quad (\text{A.15})$$

$$\frac{dn^{1,1}}{dt} = k_{\text{on}}^{a,1,0} an^{1,0} - 2k_{\text{off}}^{a,1,1} n^{1,1} - \frac{k_{\text{cat}} \sigma n^{1,1}}{K_M + \delta + (K_M/K_M^n)n}, \quad (\text{A.16})$$

$$\frac{dn^{2,2}}{dt} = k_{\text{on}}^{p,0,2} pn^{0,2} - 2k_{\text{off}}^{p,2,2} n^{2,2} - \frac{k_{\text{cat}} \sigma n^{2,2}}{K_M + \delta + (K_M/K_M^n)n}, \quad (\text{A.17})$$

$$\frac{dn^{2,3}}{dt} = k_{\text{on}}^{p,0,3} pn^{0,3} - k_{\text{off}}^{p,2,3} n^{2,3} - k_{\text{off}}^{i,2,3} n^{2,3} - \frac{k_{\text{cat}} \sigma n^{2,3}}{K_M + \delta + (K_M/K_M^n)n}, \quad (\text{A.18})$$

$$\frac{dn^{3,3}}{dt} = -2k_{\text{off}}^{i,3,3} n^{3,3} - \frac{k_{\text{cat}} \sigma n^{3,3}}{K_M + \delta + (K_M/K_M^n)n}. \quad (\text{A.19})$$