

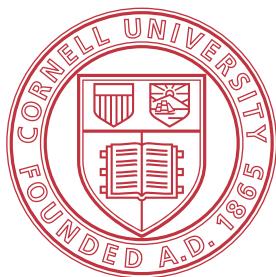
Towards a Personalized, State Aware Model of Trauma

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Introduction

Trauma is the leading cause of death and disability, surpassing all other causes combined, for persons 36 years old and younger [1]. Hemorrhage accounts for 40% of all trauma deaths, where the control of bleeding is especially challenging in the presence of blood coagulation disorders, collectively known as coagulopathy [2]. However, adverse outcomes associated with coagulopathy are not limited to death from acute blood loss. Organ dysfunction, multiple organ failure and increased susceptibility to sepsis [3] are all potential consequences of prolonged shock resulting from coagulopathy [4]. Following a wound, the immediate response of the body is to activate the coagulation cascade, which in turn generates a clot through the fibrinolysis network. Varnerlab has worked for 10 years on understanding the coagulation cascade, using both mechanistic models e.g., [5, 6], and more recently reduced order modeling approaches [7]. These models have also been used by the medical community [8, 9], and licensed by biopharma simulation companies such as Certara, to better understand this important cascade. However, recent studies have demonstrated that coagulation is only one component of the body's response to injury. Coagulation is integrated with inflammation, the autonomic nervous system and adaptive immunity through many factors including complement, a subsystem of innate immunity [10]. Thus, if we are to fully understand trauma, we must model these biochemical subsystems in addition to the physics of bleeding. Toward this challenge, we will develop a collection of reduced order models of blood biochemistry, as well as models which control heart rate and vessel dilation, and integrate these models with whole-body simulation approaches such as Physiologically Based Pharmacokinetic Models (PBPKs). My thesis work will be divided into three specific aims:

Aim 1: Develop a state-aware mathematical model of the human body. [Fill me in 1-2 sentences.]

Aim 2: Develop personalized models of trauma. [Fill me in 1-2 sentences.]

Aim 3: Predict the patient-specific efficacy of trauma interventions. [Fill me in 1-2 sentences.]

Significant previous work

Mathematical modeling of coagulation and fibrinolysis. The coagulation cascade, activated following a wound, is mediated by proteases in the circulation, called factors and a key group of blood cells, called platelets (Fig. 1). The central process in coagulation is the conversion of prothrombin (fII), an inactive coagulation factor, to the master protease thrombin (fIIa). Thrombin generation involves three phases, initiation, amplification and termination [11, 12]. Initiation requires a trigger event, for example vessel injury, which leads to the activation of factor VII (fVIIa). Two converging pathways, the extrinsic and intrinsic cascades, then process and amplify this initial coagulation signal. The extrinsic cascade is generally believed to be the main mechanism of thrombinogenesis in the blood [13–15]. Initially, thrombin is produced upon cleavage of prothrombin by fluid phase activated factor X (FXa), which itself has been activated by TF/fVIIa [16]. Picomolar amounts of thrombin then activate the cofactors factors V and VIII (fV and fVIII) and platelets, leading to the formation of the tenase and prothrombinase complexes on activated platelets. These complexes amplify the early coagulation signal by further activating FXa, and directly converting prothrombin to thrombin.

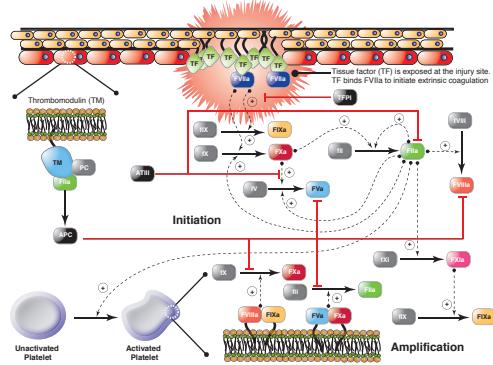


Fig. 1: Schematic of the extrinsic and intrinsic coagulation cascade. Upstream coagulation factors are activated by materials exposed following vessel injury which initiates thrombin production (fIIa) from prothrombin (fII). fIIa catalyzes its own activation (amplification), platelet activation, and its own inhibition by activated protein C (APC). APC and tissue factor pathway inhibitor (TFPI) inhibit initiation and amplification, while antithrombin III (ATIII) directly inhibits thrombin.

inhibitor (TAFI), which inhibits the activity of plasmin, an important enzyme present in blood that degrades many blood plasma proteins, including fibrin clots. Counter balancing the role of activated thrombin is tissue plasminogen activator (tPA), which activates plasmin, thereby promoting the break down of blood clots. Thus, a delicate balance exists between activated thrombin and tPA that controls the rate of clot formation. Too much activated thrombin leads to hypo-fibrinolysis (excessive clot formation resulting in stroke or heart attack risk), while too little or excess tPA leads to hyper-fibrinolysis (inadequate clot formation resulting in increased, sometimes catastrophic bleeding). See [17, 18] for reviews of coagulation and fibrinolysis.

There are several control points that inhibit thrombin formation. Tissue Factor Pathway Inhibitor (TFPI) inhibits FXa formation catalyzed by TF/fVIIa, while antithrombin III (ATIII) neutralizes several of the proteases generated during coagulation, including thrombin. Thrombin itself also inadvertently plays a role in its own inhibition; thrombin, through interaction with thrombomodulin, protein C and endothelial cell protein C receptor (EPCR), converts protein C to activated protein C (APC) which attenuates the coagulation response by proteolytic cleavage of fV/FVa and fVIII/FVIIIa. Termination occurs after either prothrombin is consumed, or thrombin formation is neutralized by inhibitors such as APC or ATIII. Activated thrombin plays a dual role in blood clot formation. First, it catalyzes the cleavage of fibrinogen to fibrin, a key component of a blood clot, thereby promoting clot formation. Activated thrombin also activates thrombin-activatable fibrinolysis inhibitor (TAFI), which inhibits the activity of plasmin, an important enzyme present in blood that degrades many blood plasma proteins, including fibrin clots. Counter balancing the role of activated thrombin is tissue plasminogen activator (tPA), which activates plasmin, thereby promoting the break down of blood clots. Thus, a delicate balance exists between activated thrombin and tPA that controls the rate of clot formation. Too much activated thrombin leads to hypo-fibrinolysis (excessive clot formation resulting in stroke or heart attack risk), while too little or excess tPA leads to hyper-fibrinolysis (inadequate clot formation resulting in increased, sometimes catastrophic bleeding). See [17, 18] for reviews of coagulation and fibrinolysis.

Previous coagulation models have typically been formulated as systems of nonlinear ordinary differential equations, using mass action or more complex kinetics, to describe the rates of biochemical conversions [19–23]. Mechanistic ODE coagulation models from our laboratory [5, 6] were built upon the earlier studies of Jones and Mann [24], Hockin *et al.* [25], and later Butenas *et al.*, [26] who developed and then subsequently refined highly mechanistic coagulation models. Other laboratories have also explored the intrinsic pathway, the role of stochastic fluctuations in coagulation [27], and the dynamics of thrombin mediated clot formation [28] and fibrinolysis [29]. Other aspects of coagulation have also been modeled, such as platelet biochemistry [30], multi-scale models of clot formation [31, 32], and transport inside clots [33]. However, these previous studies were largely based upon extensive mechanistic knowledge. Recently, Papadopoulos and co-workers constructed a phenomenological mathematical model for thrombin generation [34]. Using only four ordinary differential equations and six parameters, the reduced order model showed good agreement with experimental data. However, the Papadopoulos model neglected physiological inhibitors such as ATIII or the protein C pathway.

The central challenge of modeling coagulation or other biochemical cascades important in trauma is estimation of model parameters, and dealing with unknown biochemical mechanisms. Toward this challenge, Varnerlab has developed a reduced order modeling approach that integrates logical rules, which describe interactions that may not be well understood, with traditional ordinary differential equation modeling. In this approach, the abundance of species i (x_i) is governed by:

$$\frac{1}{\tau_i} \frac{dx_i}{dt} = \sum_{j=1}^{\mathcal{R}} \sigma_{ij} r_j(\mathbf{x}, \mathbf{k}) - k_{d,i} x_i \quad i = 1, \dots, \mathcal{M} \quad (1)$$

where \mathcal{R} and \mathcal{M} denotes the number of reactions and species in the model, and $k_{d,i}$ denotes a degradation constant for species i . The quantity σ_{ij} denotes the stoichiometric coefficient for species i in reaction j , $r_j(\mathbf{x}, \epsilon, \mathbf{k})$ denotes the rate of reaction j , and \mathbf{k} ($\mathcal{K} \times 1$) denotes the unknown kinetic parameter vector. If $\sigma_{ij} > 0$, species i is produced by reaction j , if $\sigma_{ij} < 0$, species i is consumed by reaction j , while $\sigma_{ij} = 0$ indicates species i is not connected with reaction j . Species balances were subject to the initial conditions $\mathbf{x}(t_0) = \mathbf{x}_0$. The reaction rate was written as the product of a kinetic term (\bar{r}_j) and a control term (v_j), $r_j(\mathbf{x}, \mathbf{k}) = \bar{r}_j v_j$. In this study, we used either zero- or first-order kinetics. The control term $0 \leq v_j \leq 1$ depended upon the combination of factors which influenced rate process j . For each rate, we used a rule-based approach to select from competing control factors. If rate j was influenced by $1, \dots, m$ factors, we modeled this relationship as $v_j = \mathcal{I}_j(f_{1j}(\cdot), \dots, f_{mj}(\cdot))$ where $0 \leq f_{ij}(\cdot) \leq 1$ denotes a regulatory transfer function quantifying the influence of factor i on rate j . The function $\mathcal{I}_j(\cdot)$ is an integration rule which maps the output of regulatory transfer functions into a control variable.

Physiologically Based Pharmacokinetic Models (PBPK). PBPK models are standard tools to model the physical disposition of blood constituents in the body. PBPK models are composed of collection of well-mixed organ compartment models (of variable complexity) interconnected by a circulatory system (Fig. 2). Classically, PBPK models were developed to predict the tissue distribution of therapeutic agents or toxins [35]. PBPK models readily allow the assimilation of potentially important clinical information such as the demographic

and physical characteristics of patients, and naturally incorporate key clinical information such as cardiac output, dissolved oxygen levels, blood flow rates to well- and poorly-perfused organs and pulmonary function. Thus, PBPK models are ideal candidates to simulate clinically important physical characteristics of patients. However, PBPK models are typically not used in combination with detailed models of blood biochemistry e.g., coagulation or immune system models, or nervous systems models that modulate heart or inhalation rates.

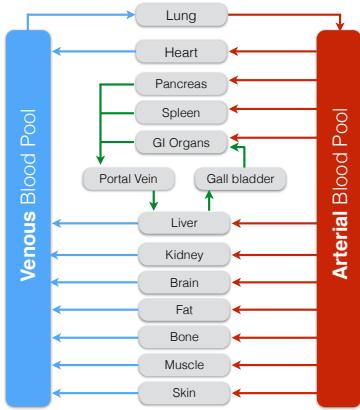


Fig. 2: Fill me in.

Current trauma models either simplify the body or the coagulation process. For example, Ho et al developed a model that considered blood to be two distinct parts, either hematocrit or plasma, and assumed the rate of fluid loss was the same as the rate as fluid replacement. They did not model the coagulation process; instead, they lumped all of the coagulation factors into one variable, which they correlated with prothrombin time [36]. Their model provides guidance on the ratio of fresh frozen plasma (FFP) to packed red blood cells (PRBC) that patients with excessive blood loss should receive. A slightly more complex model by Hirshberg et al modeled blood as having three compartments: red cells, plasma, and water, and allowed flow between these compartments based on systolic blood pressure. [37] They used prothrombin time to quantify if a patient was at risk for dilutional coagulopathy, and did not consider the biological mechanisms behind coagulation.

A model without compartments, but containing more physiological functions was developed by Simpson et al. [38]. Simpson's model allows for both blood pressure and bleed out rate to change over time, with the bleed out rate decreasing as blood pressure decreases. This model predicts hematocrit levels over time, but not the levels of specific proteins. Reisner et al re-purposed their model of the cardiovascular system (which was originally created to predict how the cardiovascular system responds to orthostatic stress) to study hemodynamic responses to haemorrhage [39]. Their model includes the heart and pulmonary circulation as well as four peripheral tissue compartments representing the upper body, legs, viscera and kidneys, each of which received the same fraction of the cardiac output. They modeled blood by separating it into two components: red blood cells and plasma. This model includes transcapillary fluid exchange and lymphatic flow, but groups all proteins together.

Modeling of heart rate. A key parameter in the PBPK model is cardiac output (CO). Cardiac output is the product of the stroke volume (amount of blood pumped per beat) and the heart rate, which can be modulated by a variety of stimuli. Olufsen and Ottesen have developed several models of heart rate regulation [REFs]. Heart rate is determined, in part, by the sympathetic and parasympathetic nervous system. Sympathetic nervous system stimulation releases the hormones epinephrine and norepinephrine into the circulation, which increase heart rate. On the other hand, the parasympathetic system releases acetylcholine which decreases heart rate. These two systems, often described as an accelerator and a brake, are not independent. Rather, they interact through second messengers cAMP and cGMP [40] [DEFINE cAMP and cGMP]. Heart rate is also influenced by

the baroreflex system, which consists of baroreceptors, tension sensitive nerve endings found in the circulatory system [41]. When baroreceptors sense a pressure change, they modulate in the frequency of nerve activity. When pressure (and stretch) rapidly increase, so does the baroreceptor firing rate [42]. The effect of this signal is not instantaneous, rather, there is a time delay on the order of seconds before the sympathetic and parasympathetic nervous systems respond [41]. These models use the baroreflex system and the concentrations of acetylcholine and norepinephrine to predict heart rate. They assume changes in arterial wall stretch are proportionate to filtered blood pressure, $\dot{\bar{p}} = \alpha(-\bar{p} + p)$ where α is a gain, \bar{p} is the filtered blood pressure, and p is the patient's measured blood pressure. From \bar{p} , we can predict the nervous system firing rate, $n = \sum_i n_i + N$, $i = 1, 2$ where N is a baseline firing rate and n_i correspond to the firing rates of nerve fibers of type i . The firing rates for nerves of type i are computed as:

$$\frac{dn_i}{dt} = \kappa_i \frac{d\bar{p}}{dt} \frac{n(M - n)}{(M/2)^2} - \frac{n_i}{\tau_i}, i = 1, 2 \quad (2)$$

where M is the maximum firing rate, and τ_i is the time scale for nerves of type i . The firing rate information is compiled by the central nervous system, which then determines the sympathetic and parasympathetic outputs, f_{sym} and f_{par} , respectively. The parasympathetic output is given by $f_{par} = n/M$, while sympathetic output takes the form:

$$f_{sym} = \frac{1 - n(t - \tau_d)/M}{1 + \beta f_{par}} \quad (3)$$

With these outputs, we can determine the dimensionless concentrations of acetylcholine, c_{ach} and noradrenaline, c_{nor} :

$$\frac{dc_j}{dt} = \frac{f_i - c_j}{\tau_j} \quad (4)$$

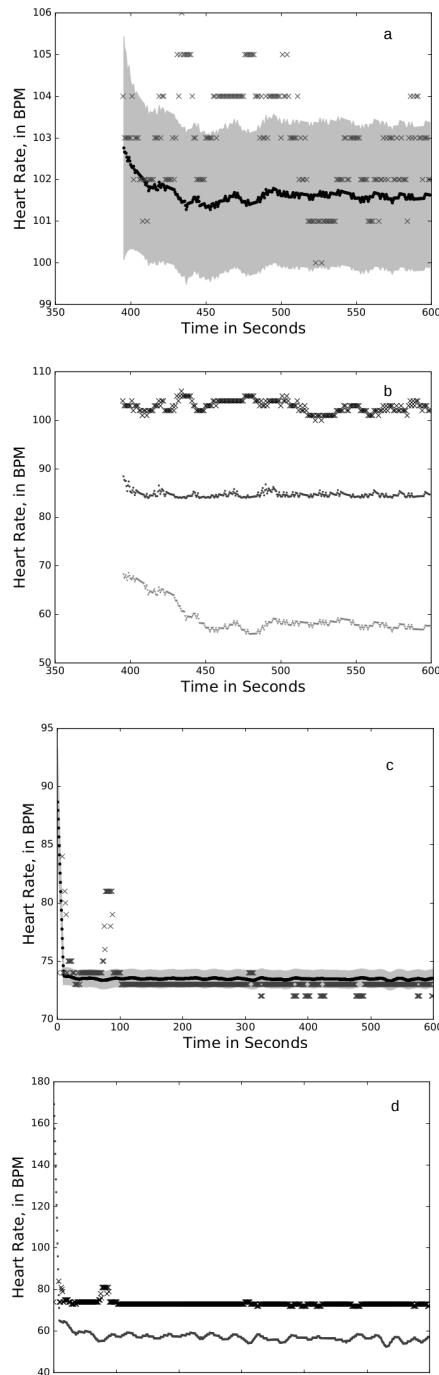
where each τ_j represents a time scale. Finally, the heart rate is calculated from h_0 , the intrinsic heart rate, and m_{nor} and m_{ach} , which are weights for the contributions of acetylcholine and norepinephrine to heart rate $h = h_0(1 + m_{nor}c_{nor} - m_{ach}c_{ach})$.

Research plan

Aim 1: Develop a state-aware mathematical model of the human body. The objective of this aim is to [Fill me in.]. Toward this aim, we have [quickly describe the preliminary studies.]

Reduced order modeling of biochemical trauma cascade. [Describe how does this fit the overall thesis?] A reduced order model of fibrinolysis is currently under development by the Varner group. This model expands on the reduced order coagulation model by including 18 species. We will validate the reduced order fibrinolysis model using ROTEM, TEG, and FIIa time series measurements taken at the University of Vermont. Incorporating it into PBPK will allow us to study both coagulopathy and DIC. The larger reduced order model allows us to track more species, providing us with more points of comparison between the model and experimental data, for better estimation of kinetic parameters. The

development of a reduced order model of complement is also underway. In its current form, this small model predicted the overall trends of C3a and C5a concentration, but the C3 dynamics were too fast. By changing how tickover (the spontaneous hydrolysis of C3) is described in the model, we can better capture the dynamics of C3 formation. We will compare the complement model's prediction to experimental data to insure that it accurately describes the dynamics of the system.[43] Although compliment is a pathway traditionally associated with the immune response, it activated following trauma, and shifts the balances towards coagulation by activating platelets and increasing tissue factor expression. [44] By including a compliment in our trauma model, we will be able to capture the interactions between compliment and coagulation, which may prove important to accurately describe DIC. Furthermore, we will be able model platelet activation, as both C3 and C5b-9 alter platelet behavior.[45].



Identification of a heart rate model for trauma patients. We estimated heart rate model parameters using XX patients from the MIMIC-II/III, a freely-available database comprising deidentified health-related data associated with over forty thousand patients who stayed in critical care units of the Beth Israel Deaconess Medical Center between 2001 and 2012 [46]. When less than 10% of blood volume has been lost, the baroreflex is initiated as pressure has dropped to increase heart rate [47]. After approximately 10% of blood volume has been lost, heart rate begins to decrease, as does blood pressure. When blood loss approaches 30% of blood volume, heart rate increases dramatically [48]. During all three phases, the concentrations of adrenaline, noradrenaline, and vasopressin rise, but then decrease once blood is transfused. Using the blood pressure tracks from MIMIC II, we used Olufsen's and Ottosen's model [49] to predict heart rate within the PBPK model. We used the Nelder Mead algorithm to estimate parameters that better fit the MIMIC II data. Additionally, we used k-means clustering to group the patients into two clusters, based on age, average heart rate, and SAPS (Simplified Acute Physiology) score. We then used the JuPOETS multiobjective optimization package [50] to generate families of parameters for the two clusters (Fig. 3). The estimated parameters reduced the total mean squared error by more than two-thirds. Furthermore, we used the acetylcholine and noradrenaline concentrations predicted by this model to vasodilate and constrict the arterial and venous

blood pools. The aerial and venous blood are constricted if the noradrenaline concentration is above a threshold value, and dilated if the acetylcholine concentration is above a threshold value. The expansion and contraction of the compartments was capped at 125% and 75% of their resting values, respectively. The degree of volume change was based on previous experimental data on volume change as a function of a concentration of acetylcholine and noradrenaline [51, 52]. In general, adding the heart rate change and vassocative factors to the model slightly shifts the curves, but does not alter their overall shape.

Incorporating reduced order biochemical models into the PBPK framework. We constructed an eight

compartment model of the human body, using the Kwatee code generation system [REF]. In this model, blood flowed from a venous pool, to the heart, through the lungs, back through the heart, and into an arterial blood pool. Blood then passed from the arteries through the liver, kidney, or bulk to the veins. All of the proteins flowed freely between compartments, except for thrombomodulin and trigger, which are bound to cell membranes [53]. We simulated a small wound connected to the arterial blood pool, with a volume of 0.5% of the total blood volume. Coagulation dynamics were modeled using the reduced order model constructed by Sagar et al. [54]. In the reduced order model, trigger (which biologically corresponds to the tissue factor-FVIIa complex) activated thrombin. Thrombin then catalyzed its own activation, as well as the activation of Protein C, which inhibited the activation of thrombin. Antithrombin complexed with thrombin and deactivated it. We ran the model in one hundred different patients, each with slightly different organ volumes and initial concentrations, as shown in Figure ???. The patient is bleeding out at a constant rate of 10.5 mL/min, which would lead to complete exsanguination in less than ten hours. As blood volume diminishes, the heart rate of the patient increases. The blood loss comes from the arterial and bulk compartments, leading to a rise in the concentration in thrombomodulin in those compartments, as their volume is decreasing while the amount of thrombomodulin present remains constant.

Proposed studies. [Describe what you are going to do.]

Expected outcomes, potential pitfalls and alternative approaches. We expect Aim 1 will produce [fill me in.] However, if the reduced order models fail to capture the combined dynamics of coagulation, fibrinolysis, and complement, we can consider alternative, more complex ODE or PDE based models of each process. [Is this really going to work? Where does the data come from?]

Aim 2: Develop personalized models of trauma. [Same format as Aim 1]

Aim 3: Predict the patient-specific efficacy of trauma interventions. [Same format as Aim 1]

References

1. Krug EG, Sharma GK, Lozano R (2000) The global burden of injuries. *Am J Public Health* 90: 523-6.
2. Sauaia A, Moore FA, Moore EE, Moser KS, Brennan R, et al. (1995) Epidemiology of trauma deaths: a reassessment. *J Trauma* 38: 185-93.
3. Esmon CT (2005) The interactions between inflammation and coagulation. *Br J Haematol* 131: 417-30.
4. Sauaia A, Moore FA, Moore EE, Haenel JB, Read RA, et al. (1994) Early predictors of postinjury multiple organ failure. *Arch Surg* 129: 39-45.
5. Luan D, Zai M, Varner JD (2007) Computationally derived points of fragility of a human cascade are consistent with current therapeutic strategies. *PLoS Comput Biol* 3: e142.
6. Luan D, Szlam F, Tanaka KA, Barie PS, Varner JD (2010) Ensembles of uncertain mathematical models can identify network response to therapeutic interventions. *Mol Biosyst* 6: 2272-86.
7. Sagar A, Varner JD (2015) Dynamic modeling of the human coagulation cascade using reduced order effective kinetic models. *Processes* 3: 178.
8. Szlam F, Luan D, Bolliger D, Szlam AD, Levy JH, et al. (2010) Anti-factor ixa aptamer reduces propagation of thrombin generation in plasma anticoagulated with warfarin. *Thromb Res* 125: 432-7.
9. Rice NT, Szlam F, Varner JD, Bernstein PS, Szlam AD, et al. (2016) Differential contributions of intrinsic and extrinsic pathways to thrombin generation in adult, maternal and cord plasma samples. *PLoS One* 11: e0154127.
10. Rittirsch D, Flierl MA, Ward PA (2008) Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 8: 776-87.
11. Goldhaber SZ, Colman RW, Clowes AW, editors (2006) Hemostasis and Thrombosis: Basic Principles and Clinical Practice. Lippincott Williams and Wilkins.
12. Brummel KE, Paradis SG, Butenas S, Mann KG (2002) Thrombin functions during tissue factor-induced blood coagulation. *Blood* 100: 148-52.
13. Mann K, Nesheim M, Church W, Haley P, Krishnaswamy S (1990) Surface-dependent reactions of vitamin k-dependent enzyme complexes. *Blood* 76: 1-16.
14. Roberts H, Monroe D, Oliver J, Chang J, Hoffman M (1998) Newer concepts of blood coagulation. *Haemophilia* 4: 331-334.
15. Mann K (1999) Biochemistry and physiology of blood coagulation. *Thromb Haemost* 82: 165-174.
16. Butenas S, Mann KG (2002) Blood coagulation. *Biochemistry (Mosc)* 67: 3-12.
17. Tanaka KA, Key NS, Levy JH (2009) Blood coagulation: hemostasis and thrombin regulation. *Anesth Analg* 108: 1433-46.
18. Chapin JC, Hajjar KA (2015) Fibrinolysis and the control of blood coagulation. *Blood Rev* 29: 17-24.
19. Khanin MA, Semenov VV (1989) A mathematical model of the kinetics of blood coagulation. *J Theor Biol* 136: 127-34.
20. Willems GM, Lindhout T, Hermens WT, Hemker HC (1991) Simulation model for thrombin generation in plasma. *Haemostasis* 21: 197-207.

21. Baldwin SA, Basmadjian D (1994) A mathematical model of thrombin production in blood coagulation, part i: The sparsely covered membrane case. *Ann Biomed Eng* 22: 357-70.
22. Leipold RJ, Bozarth TA, Racanelli AL, Dicker IB (1995) Mathematical model of serine protease inhibition in the tissue factor pathway to thrombin. *J Biol Chem* 270: 25383-7.
23. Kuharsky AL, Fogelson AL (2001) Surface-mediated control of blood coagulation: the role of binding site densities and platelet deposition. *Biophys J* 80: 1050-74.
24. Jones KC, Mann KG (1994) A model for the tissue factor pathway to thrombin. ii. a mathematical simulation. *J Biol Chem* 269: 23367-73.
25. Hockin MF, Jones KC, Everse SJ, Mann KG (2002) A model for the stoichiometric regulation of blood coagulation. *J Biol Chem* 277: 18322-33.
26. Butenas S, Orfeo T, Gissel MT, Brummel KE, Mann KG (2004) The significance of circulating factor ix α in blood. *J Biol Chem* 279: 22875-82.
27. Lo K, Denney WS, Diamond SL (2005) Stochastic modeling of blood coagulation initiation. *Pathophysiol Haemost Thromb* 34: 80-90.
28. Chatterjee MS, Denney WS, Jing H, Diamond SL (2010) Systems biology of coagulation initiation: kinetics of thrombin generation in resting and activated human blood. *PLoS Comput Biol* 6.
29. Mitrophanov AY, Wolberg AS, Reifman J (2014) Kinetic model facilitates analysis of fibrin generation and its modulation by clotting factors: implications for hemostasis-enhancing therapies. *Mol Biosyst* 10: 2347-57.
30. Stalker TJ, Traxler EA, Wu J, Wannemacher KM, Cermignano SL, et al. (2013) Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network. *Blood* 121: 1875-85.
31. Leiderman K, Fogelson A (2014) An overview of mathematical modeling of thrombus formation under flow. *Thromb Res* 133 Suppl 1: S12-4.
32. Bannish BE, Keener JP, Fogelson AL (2014) Modelling fibrinolysis: a 3d stochastic multiscale model. *Math Med Biol* 31: 17-44.
33. Voronov RS, Stalker TJ, Brass LF, Diamond SL (2013) Simulation of intrathrombus fluid and solute transport using in vivo clot structures with single platelet resolution. *Ann Biomed Eng* 41: 1297-307.
34. Papadopoulos K, Gavaises M, Atkin C (2014) A simplified mathematical model for thrombin generation. *Med Eng and Phys* 36: 196-204.
35. Gerlowski LE, Jain RK (1983) Physiologically based pharmacokinetic modeling: principles and applications. *J Pharm Sci* 72: 1103-27.
36. Ho AM, Dion PW, Cheng CA, Karmakar MK, et al. (2005) A mathematical model for fresh frozen plasma transfusion strategies during major trauma resuscitation with ongoing hemorrhage. *Canadian journal of surgery* 48: 470.
37. Hirshberg A, Dugas M, Banez EI, Scott BG, Wall Jr MJ, et al. (2003) Minimizing dilutional coagulopathy in exsanguinating hemorrhage: a computer simulation. *Journal of Trauma and Acute Care Surgery* 54: 454–463.
38. Simpson S, Menezes G, Mardel S, Kelly S, White R, et al. (1996) A computer model of major haemorrhage and resuscitation. *Medical engineering & physics* 18: 339–343.
39. Reisner AT, Heldt T (2013) A computational model of hemorrhage and dehydrat-

- tion suggests a pathophysiological mechanism: Starling-mediated protein trapping. *American Journal of Physiology-Heart and Circulatory Physiology* 304: H620–H631.
- 40. Olshansky B, Sabbah HN, Hauptman PJ, Colucci WS (2008) Parasympathetic nervous system and heart failure pathophysiology and potential implications for therapy. *Circulation* 118: 863–871.
 - 41. Ottesen JT (1997) Modelling of the baroreflex-feedback mechanism with time-delay. *Journal of mathematical biology* 36: 41–63.
 - 42. NEGATIVE ABAA (1999) Reflexes that control cardiovascular function .
 - 43. Morad HO, Belete SC, Read T, Shaw AM (2015) Time-course analysis of c3a and c5a quantifies the coupling between the upper and terminal complement pathways in vitro. *Journal of immunological methods* 427: 13–18.
 - 44. Markiewski MM, Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD (2007) Complement and coagulation: strangers or partners in crime? *Trends in immunology* 28: 184–192.
 - 45. Peerschke EI, Yin W, Ghebrehiwet B (2008) Platelet mediated complement activation. In: *Current Topics in Complement II*, Springer. pp. 77–87.
 - 46. Johnson AEW, Pollard TJ, Shen L, Lehman LWH, Feng M, et al. (2016) Mimic-iii, a freely accessible critical care database. *Sci Data* 3: 160035.
 - 47. Foex B (1999) Systemic responses to trauma. *British medical bulletin* 55: 726–743.
 - 48. Jacobsen J, Søfelt S, Sheikh S, Warberg J, Secher N (1990) Cardiovascular and endocrine responses to haemorrhage in the pig. *Acta physiologica scandinavica* 138: 167–173.
 - 49. Olufsen MS, Ottesen JT (2013) A practical approach to parameter estimation applied to model predicting heart rate regulation. *Journal of mathematical biology* 67: 39–68.
 - 50. Bassen D, Vilkovoy M, Minot M, Butcher JT, Varner JD (2016) Jupoets: A constrained multiobjective optimization approach to estimate biochemical model ensembles in the julia programming language. *bioRxiv* : 056044.
 - 51. Chowienczyk P, Cockcroft J, Ritter J (1994) Blood flow responses to intra-arterial acetylcholine in man: effects of basal flow and conduit vessel length. *Clinical Science* 87: 45–51.
 - 52. Dóra E, Kováč AG (1983) Effect of topically administered epinephrine, norepinephrine, and acetylcholine on cerebrocortical circulation and the nad/nadh redox state. *Journal of Cerebral Blood Flow & Metabolism* 3: 161–169.
 - 53. Esmon CT (1989) The roles of protein c and thrombomodulin in the regulation of blood coagulation. *J Biol Chem* 264: 4743–4746.
 - 54. Sagar A, Varner JD (2015) Dynamic modeling of the human coagulation cascade using reduced order effective kinetic models. *Processes* 3: 178–203.