# SUPPLEMENT

**Multiscale prediction of patient-specific clotting function under flow**

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## CONTENTS

<table>
<thead>
<tr>
<th>Supplemental Methods</th>
<th>PAS and NN training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LKMC methods</td>
</tr>
<tr>
<td></td>
<td>LB methods</td>
</tr>
<tr>
<td></td>
<td>FEM methods</td>
</tr>
<tr>
<td></td>
<td>Model coupling</td>
</tr>
</tbody>
</table>

**Supplemental Discussion**

**Supplemental References**

**Supplemental Table S1** Parameters for multiscale modeling of platelet deposition

**Supplemental Fig. S1** Multiscale model domain and grid structure

**Supplemental Fig. S2** Calcium traces and trained NN prediction

**Supplemental Fig. S3** PAC1, anti-P-selectin, Annexin V binding to CVX-activated platelets

**Supplemental Fig. S4** Comparison of microfluidic experiments and multiscale simulations of platelet deposition at an elevated shear rate.

**Supplemental Fig. S5** Sequencing of the TP receptor

**Supplemental Fig. S6** Calcium responses to Convulxin, ADP, or U46619 in Donors

**Supplemental Fig. S7** TP receptor levels in Donors

**Supplemental Fig. S8** Transient expression of TP and TP-V241G in HEK cells

**Supplemental Video S1** Multiscale simulation of platelet deposition

**Supplemental Video S2** Platelet deposition in 8-channel microfluidic device
Supplemental Methods

Pairwise Agonist Scanning (PAS) and Neural Network (NN) Training

Platelet calcium mobilization in response to agonist stimulation was measured in a high throughput assay as reported previously.\(^1\) Briefly, a 384-well “agonist plate” with varying combinations of the three agonists ADP, U46619 and CVX at 0.1,1 and 10x EC50 levels (1\(\mu\)M, 1\(\mu\)M, and 5nM respectively), and the inhibitor iloprost (1 \(\mu\)M) were assembled at 10x final concentration on a Perkin Elmer Janus. Platelet Rich Plasma (PRP) was obtained from each of the three donors tested by centrifuging whole blood at 120 g for 12 min. 2 ml PRP was incubated with each vial of Fluo4-NW dye mixture (Invitrogen) reconstituted into 8 ml buffer for 30 min. A “platelet plate” was assembled on a Perkin Elmer Evolution containing 30 \(\mu\)l dye loaded PRP in every well. Mixtures of agonists or inhibitors were pipetted (20 \(\mu\)l) from individual columns of the “agonist-plate” and dispensed onto the platelet suspension in the “platelet-plate” on a Molecular Devices FlexStation III after a 20 s baseline read in each well. Fluorescence was read at excitation 485nm and emission 535nm for 4 min and relative calcium concentrations were quantified by the ratio of the instantaneous fluorescence \(F(t)\) to the baseline value \(F_0(t)\). All combinatorial conditions were tested in quadruplicate on a single plate. All platelets were pretreated with 28 \(\mu\)M indomethacin to prevent autocatalytic amplification via synthesized thromboxane. Also, all wells contained 2 mM external \(\text{Ca}^{2+}\) (unlike the results reported in\(^1\), where experiments were conducted in the presence of EDTA). Normalized pairwise synergy between agonists (“A” and “B” and their combined use “AB”) was calculated from measurements or NN simulations of integrated calcium curves by:

\[
\text{Pairwise Synergy Score, } S_{AB} = \frac{\int AB - \left( \int A + \int B \right)}{\max \left[ \int AB - \left( \int A + \int B \right) \right]} \tag{S1}
\]

Neural Network training

Neural network training was also carried out as described previously\(^1\) using the Neural Network Toolbox for MATLAB. The training set consisted of concentrations of the 4 molecular inputs (ADP, U46619, CVX and iloprost) to each well, and the outputs consisting of the measured calcium transients at each of the 74 conditions measured. Input concentrations were mapped onto the values -1, -0.333, +0.333 and +1 corresponding to 0, 0.1,1 and 10x EC50 levels of each input. Output concentrations were mapped between 0 (resting calcium levels) and 1 (maximal response). The structure of the NARX (Nonlinear AutoRegressive network with eXogenous inputs) model employed was identical to the one reported previously with 2 processing layers, and a linear transfer function was used in the output layer. Initial states of the feedback to each layer. The hyperbolic tangent transfer function was used in all of the processing layers, and a linear transfer function was used in the output layer. Initial states of the feedback were set to 0 corresponding to the resting platelet. Training was performed using Levenberg-Marquardt backpropagation until the performance of the model (mean squared error) was \(\sim 1 \times 10^5\) and acceptable overall correlation was obtained between training and predicted set. To prevent over-fitting 10% of the experimental data was excluded from the training set, and the weights and biases were adjusted only as long as the error decreased in this “testing-set” in addition to continual decrease in “training-set” error. The output \(y\) at an instant \(t\), for an input vector \(\vec{I}\) of the concentrations of the 4 inputs species can be described by:

\[
y(t) = f\left\{\underbrace{\overbrace{L_3(1x4) \times f\left(\overbrace{H_2(4x8) \times y_{h}(8x1)}^{(4x1)} + L_2(4x4) \times f\left(\overbrace{H_1(8x8) \times y_{h}(8x1)}^{(8x1)} + \overbrace{W(8x4) \times \overbrace{\vec{I}(4x1)}^{(4x1)} + \overbrace{b_1(8x1)}^{(1x1)}}^{(8x1)} + b_2(4x4)}^{(4x4)} + b_3(1x1)})^{(1x1)}}^{(4x1)}\right)\right\} \tag{S2}
\]
where $\mathbf{W}$ is the matrix of input weights, $L^2$ and $L^3$ are the weights that operate on the “inputs” coming from the first and second processing layers respectively. $H^1$ and $H^2$ are matrices that contain history coefficients that weigh the history vector $y^h$ (containing the output of the system 1, 2, 4, 8, 16, 32, 64 and 128 s prior to the current instant). $b^1, b^2$ and $b^3$ are bias vectors that add constant biases to each weighted input and weighted histories to produce the “net input” to each transfer function. $f$ is the transfer function corresponding to each layer that operates on a vector of “net inputs” to yield the corresponding transformed output. Numbers in parentheses show the sizes of relevant matrices or vectors of weights and biases. The NN output with range 0 to 1 is linearly mapped to platelet intracellular calcium with range 100 nM to 1μM. The time step in the NN is 1s, but to achieve greater time resolution in the multiscale model, the solution of the NN at some time $t_1 < t < t_2$ was interpolated from the previous solution time ($t_1$) of the NN and a predicted solution of the NN at the next time ($t_2$) using the current system state.

**LKMC methods**

The input for LKMC is the rate database for all possible events in the system. The rate of a platelet diffusing one lattice space in any direction is $\Gamma_D = \frac{D_{\text{platelet}}}{h_{\text{LKM}}}$. For a platelet with area-averaged velocity, $v$, the rate of convection along a lattice direction $e_i$ is given by $\Gamma_C = \max(v \cdot e_i, 0)/h$. The combined rate of motion is $\Gamma_{\text{motion}} = \Gamma_C + \Gamma_D$. If a platelet is blocked in the direction of flow, its convective rate is passed in the direction of flow to a platelet that is available to move. This is the Pass Forward Algorithm (PFA) and has been shown to correct for particle blocking on the lattice. The motion rates for any particle that is bonded to the surface or another platelet are set to 0. The rates of binding and unbinding are defined in the main text (Equations 1-6). A binding event is only allowed if there is connectivity to the surface through one of the platelets. This rule prevents free-flowing platelet aggregates, which are not expected to significantly contribute to the surface-adhered platelet deposit over the length and time scales of interest.

With the specified rate database at system time $t$, KMC chooses the time step of the next event as

$$\tau = \frac{-\ln(u)}{\Gamma_{\text{tot}}}$$  \hspace{1cm} (S3)

where $\Gamma_{\text{tot}}$ is the total rate of all events in the system and $u \in (0,1)$. In KMC, the probability that event $i$ with rate $\Gamma_i$ will be the next event is

$$P_i = \frac{\Gamma_i}{\Gamma_{\text{tot}}}$$ \hspace{1cm} (S4)

The Next Reaction Method, which satisfies the time step and probability defined in Equations S3 and S4, uses a sorted event-time queue for greater efficiency. The discretization error in the LKMC method manifests as additional particle diffusion in the direction of flow. The additional diffusivity coefficient scales linearly with the velocity and the lattice spacing.

**LB methods**

The flow of pressure driven fluid can be determined from the Navier-Stokes equation
\[
\rho \left( \frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \nabla \mathbf{v} \right) = -\nabla P + \mu \nabla^2 \mathbf{v} \quad (S5)
\]

and the continuity equation
\[
\nabla \cdot \mathbf{v} = 0, \quad (S6)
\]

where \( \rho \) is the density of the fluid, \( P \) is the pressure, and \( \mu \) is the dynamic viscosity. Lattice Boltzmann indirectly solves Equations \( S5 \) and \( S6 \) by simulating the streaming and collision of fluid particles during one time step on a uniform lattice. In this case for a 2D lattice, each lattice node streams particles to its 8 nearest neighbors and itself (D2Q9 lattice). After streaming, the particle distribution at each lattice site is relaxed to an equilibrium configuration. The macroscopic velocity profile is obtained from moments of the particle distribution at each node. The lattice spacing was defined as the same as LKMC \((h_{\text{LVMC}} = h_{\text{LB}})\) where the grid is offset by a half-lattice space (Fig. S1), and the time step was set at \( \Delta t = 10^{-7} \, \text{s} \). The walls of the chamber and adhered platelets defined the no-slip boundary conditions. The inlet had a prescribed velocity profile (fully developed parabolic flow profile), and the outlet had a constant pressure boundary condition. The boundary conditions were implemented using the nonequilibrium bounceback rule (Zou-He boundary conditions), whereby the unknown particle distributions at the boundary nodes are determined by reflecting the nonequilibrium portions of the known particle distributions.6

FEM methods

The convection-diffusion-reaction equation, as defined in Equation 6, was solved with the Finite Element Method (FEM). In FEM, the system domain is split into discrete elements where the solution is approximated within the elements by interpolation functions. The weak form of the original PDE is obtained by weighting each term with the interpolation functions and integrating over each element, i.e.,
\[
\int_{\Omega} \varphi_i \left( \frac{\partial C}{\partial t} + \mathbf{v} \cdot \nabla C - D \nabla^2 C - R \right) = 0, \quad (S7)
\]

where \( \Omega \) is the domain of a single element and \( \varphi_i \) is a single interpolation function within that element. In this work, the elements were chosen to be square and contain 4 LKMC nodes \((h_{\text{FEM}} = 2h_{\text{LVMC}} = 1 \, \mu\text{m})\) with bilinear interpolation functions. A single platelet overlapped several FEM elements, so the release rate of each platelet was partitioned according to the area within each element. The time derivative was approximated using the Crank-Nicolson scheme with a time step of \( \Delta t = 10^{-2} \, \text{s} \).

Model Coupling

The flow of information between models is given in Fig. 1D. LKMC provides the position of all platelets in the domain and the bonding state of each platelet. LKMC requires the velocity field of the fluid (LB) to calculate convective rates of motion and the activation state of each platelet (NN) to determine the bonding and unbonding rates. The NN provides the activation state of each platelet, and the input into the NN is the local concentration of platelet agonists, which requires both the platelet positions (LKMC) and the concentration field of soluble agonists (FEM). The LB method provides the velocity field and requires the location of all bonded platelets for the location of the no-slip surfaces. FEM provides the concentration field and requires the release rate of platelets, which depends on the location of platelets (LKMC) and the activation states (NN), and the velocity field (LB).
The coupling of the individual models only occurred at specified time intervals: \( \Delta t_{LB} = 5 \times 10^{-3} \, s \), \( \Delta t_{FEM} = 10^{-2} \, s \), and \( \Delta t_{NN} = 10^{-2} \, s \). LKMC always exchanged information during updates, so in this case, LKMC simply had a coupling time of \( \Delta t_{LKMC} = 5 \times 10^{-3} \, s \). At the start of the simulation LKMC, LB, FEM, and the NN were all specified by the initial condition for each method. Each method was stepped forward in time until the first coupling time was reached \( (t = 5 \times 10^{-3} \, s) \), and then the appropriate methods exchanged information. During the first update, LB updated the velocity field in LKMC and received the current configuration of the bound platelets from LKMC. Then, all methods resumed stepping forward in time until the next coupling time \( (t = 1 \times 10^{-2} \, s) \) occurred. During this update all models share information: LKMC updated the positions of all platelets and the bound states of all platelets in FEM, LB, and NN; LB updated the velocity field in LKMC and FEM; FEM updated the concentration field in NN; and NN updated the activation state in FEM and LKMC. This process repeats until the end of the simulation time.

The time scale for velocity field relaxation was generally <<10^{-3} \, s, so the velocity field reached a steady state in LB significantly before the next update time. To gain computational efficiency, LB was only simulated until a steady-state was achieved. At the next coupling time, the boundary condition only changed if a platelet underwent a bonding or unbonding event. If this occurred, LB was again simulated until steady-state. If not, the steady-state solution from the previous step was kept, and no LB simulation was needed. In this way, the velocity field was only updated if a platelet had a bonding or nonbonding event.

The software including FEM, LB, LKMC, and evaluation of the NN for the multiscale model was developed in-house using Fortran and run on TACC, University of Texas for the simulations presented here. The LKMC method for tracking particle motion and aggregation has been described in previous papers.\(^3,4\) The Finite Element code was adapted from a previous model of silicon point-defect dynamics,\(^7\) and the matrix operations are handled by LAPACK routines.\(^8\) The lattice Boltzmann code was developed from Chen and Doolen (1998).\(^9\) The coupling (integration) of the separate methods is described in detail in the Supplemental Methods. Briefly, LKMC is used as the time keeper, and the other methods synchronize data at very small time steps. The code that handles the coupling of the algorithms was included within the LKMC code (Fortran).

**Supplemental Discussion**

Prior studies involving transient expression of the TP receptor in a human cell background have shown that untransfected HEK cells are a suitable background cell for TP receptor studies.\(^10\) The slight signal response with addition of buffer in Fig. 4D-E may be due to drift in the baseline during liquid handling of buffer into the well using the Flexstation reader or a very slight sensitivity of the cells to U46619.
Supplemental References
### Supplemental Table S1  Parameters for multiscale modeling of platelet deposition

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Value</th>
<th>Suppl. Ref./Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{\text{platelet}}$</td>
<td>Platelet count</td>
<td>$1.5 \times 10^5$ platelet/$\mu$L</td>
<td>11</td>
</tr>
<tr>
<td>$\rho_{\text{blood}}$</td>
<td>Density of blood</td>
<td>1 g/cm$^3$</td>
<td>12</td>
</tr>
<tr>
<td>$h_{\text{blood}}$</td>
<td>Viscosity of blood</td>
<td>0.03 g/cm s</td>
<td>13</td>
</tr>
<tr>
<td>$R_{\text{platelet}}$</td>
<td>Radius of platelet</td>
<td>$1.5 \times 10^{-7}$ cm</td>
<td>14</td>
</tr>
<tr>
<td>$D_{\text{platelet}}$</td>
<td>Diffusion (dispersion) Coefficient of platelet</td>
<td>$1.25 \times 10^{-7}$ cm$^2$/s</td>
<td>15</td>
</tr>
<tr>
<td>$D_{\text{ADP}}$</td>
<td>Diffusion (dispersion) Coefficient of ADP</td>
<td>$2.37 \times 10^{-6}$ cm$^2$/s</td>
<td>16</td>
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<tr>
<td>$D_{\text{TXA}_2}$</td>
<td>Diffusion (dispersion) Coefficient of TXA$_2$</td>
<td>$2.14 \times 10^{-6}$ cm$^2$/s</td>
<td>16</td>
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<tr>
<td>$M_{\text{ADP}}$</td>
<td>Total amount of ADP released</td>
<td>$1 \times 10^8$ nmol/platelet</td>
<td>17</td>
</tr>
<tr>
<td>$M_{\text{TXA}_2}$</td>
<td>Total amount of TXA$_2$ generated upon activation</td>
<td>$4 \times 10^{-10}$ nmol/platelet</td>
<td>18</td>
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<td>$\tau_{\text{ADP}}$</td>
<td>Characteristic release time constant for ADP</td>
<td>5 s</td>
<td>19</td>
</tr>
<tr>
<td>$\tau_{\text{TXA}_2}$</td>
<td>Characteristic release time constant for TXA$_2$</td>
<td>100 s</td>
<td>18</td>
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<tr>
<td>$C_{\text{CVX}}$</td>
<td>Effective concentration of collagen (in units of CVX)</td>
<td>0.5 x EC$_{50}$</td>
<td>Soluble vs surface ligand</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Relative potency of TXA$_2$/U46619</td>
<td>15</td>
<td>Relative binding affinity for TP receptor</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Resting level of integrin activation</td>
<td>0.001</td>
<td>~ 1 active integrin per 1000 on a resting platelet.</td>
</tr>
<tr>
<td><strong>Estimated parameters</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$n$</td>
<td>Sharpness of activation function</td>
<td>0.75</td>
<td>Expected range: $0.5 &lt; n &lt; 2$</td>
</tr>
<tr>
<td>$\xi_{50}$</td>
<td>Critical value for 50% activation and platelet release</td>
<td>9 μM-s</td>
<td>Corresponding to strong calcium mobilization (e.g. 1 μM persisting for 9 sec.)</td>
</tr>
<tr>
<td>$k_{\text{collagen att}}$</td>
<td>Attachment rate constant for collagen binding</td>
<td>1000 s$^{-1}$</td>
<td>Includes vWF/GPIb.</td>
</tr>
<tr>
<td>$k_{\text{fibrinogen att}}$</td>
<td>Attachment rate constant for fibrinogen-mediated binding</td>
<td>50 s$^{-1}$</td>
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</tr>
<tr>
<td>$k_{\text{collagen det}}$</td>
<td>Detachment rate constant for collagen binding</td>
<td>$1 \times 10^{-5}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k_{\text{fibrinogen det}}$</td>
<td>Detachment rate constant for fibrinogen-mediated binding</td>
<td>$1 \times 10^{-4}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\gamma_c$</td>
<td>Critical shear rate</td>
<td>200 s$^{-1}$</td>
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</tbody>
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Supplemental Fig. S1 Multiscale model domain and grid structure. (A) The microfluidic channel had a cross section of 250 μm x 60 μm with a 250 μm patch of collagen. The 2D LKMC domain, which included the entire collagen patch, was 500 μm long and 60 μm high. In LKMC, the collagen patch was located 100 μm from the inlet and 150 μm from the outlet. (B) In LKMC, a uniform lattice spacing of 0.5 μm was used, where each platelet radius was spanned by several lattice spaces. In LB, the uniform grid had the same lattice spacing as LKMC (0.5 μm), but was offset by 0.25 μm in each direction such that the LB nodes surround the LKMC voxels. In FEM, the uniform grid was aligned with the LB grid, but had a lattice spacing of 1 μm.
Supplemental Fig. S2  Calcium traces and trained NN prediction. Calcium responses normalized to maximum response are presented for Donor 1 PRP treated with low, medium, and high concentrations of ADP, convulxin, and U46619 in the absence (red) or presence (blue) of iloprost, as well as all pairwise combinations of agonists. NN predictions are shown (dotted line) and are essentially identical to all experimentally measured traces (solid lines).
Supplemental Fig. S3  PAC1, anti-P-selectin, Annexin V binding to CVX-activated platelets. Convulxin induced platelet activation normal in flow cytometry. Suspension 100 μL of 1% PRP in HBS stained with FITC PAC-1, PE anti CD62P (AK-4), and Cy5 annexin V (BD Bioscience). Recorded 30,000 events per sample 10 minutes after activation with 53 nM convulxin (Centerchem).
Supplemental Fig. S4: Comparison of microfluidic experiments and multiscale simulations of platelet deposition at an elevated shear rate. Measured platelet deposition dynamics for Donors 2 and 3 at an inlet wall shear rate of 1000 s\(^{-1}\) (left) and corresponding multiscale simulations at 1000 s\(^{-1}\). Normalized to the 200 s\(^{-1}\) condition, Donor 2 had 50.9% and Donor 3 had 29.1% fluorescence in the experiment. Normalized to the 200 s\(^{-1}\) condition, Donor 2 had 28.8% and Donor 3 had 33.3% platelet density in the simulation. p<0.0001 was relative to the 200 s\(^{-1}\) condition for each donor experiment and simulation.
Supplemental Fig. S5 Sequencing of the TP receptor. DNA from Donor 3 was subjected to PCR amplification (primers in red) of part of exon 2 of the TP gene and sequenced revealing a T→G mutation in a codon (underlined) (A). Direct sequencing of the PCR product revealed a heterozygote mutation (B), while sequencing of individual clones resulted in a determination of G at this position (C), resulting in the V241G mutation (D).
Supplemental Fig. S6  Calcium responses to Convulxin, ADP, or U46619 in Donors. Calcium responses were measured in duplicate for Donors 1-3 in response to increasing doses of ADP, convulxin, or U46619. While the calcium response to ADP and convulxin stimulation was normal for Donor 3, the response to U46619 was severely impaired for Donor 3 in tests conducted on separate days.
Supplemental Fig. S7 TP receptor levels in Donors. Flow cytometry was conducted on diluted whole PRP (1%) treated with anti-TP for Donors 1-3, as well as an additional healthy male donor (Donor 4). All donors displayed essentially identical levels of platelet TP.
Supplemental Fig. S8  Transient expression of TP and TP-V241G in HEK cells.
Supplemental Video S1 Multiscale simulation of patient-specific platelet deposition under flow. Platelet activation (black, unactivated; white, fully activated) and deposition at 500 s (inlet wall shear rate, 200 s⁻¹) for Donor 1 in the presence of released ADP (A) and TXA₂ (B) where local shear rate near the platelet deposit varies markedly from <50 s⁻¹ to greater than 1000 s⁻¹ (C). Flow: left to right (streamlines; black lines); Surface collagen (250 μm long): red bar.
**Supplemental Video S2 Platelet deposition in 8-channel microfluidic device.** Whole blood (100 μM PPACK) was perfused over a type 1 collagen surface under the 4 different pharmacological conditions [control, 66 μM ASA, 14 μM indomethacin, and 2 μM MRS 2179]. The microfluidic device has been designed such that 8 separate blood/collagen interactions can be monitored simultaneously in a 2x field of view. Platelets are labeled with a fluorescently conjugated antibody to CD41.