In graduate school, I have had the pleasure of exploring a variety of open problems in physiology with world-renowned faculty. To understand and study the dynamics of cell and tissue level processes, I have developed an aptitude in various tools in *dynamical systems, numerical analysis, and sensitivity analysis*. Furthermore, I have extensive experience understanding experimental literature and conversing with researchers from different fields. In this statement, I describe the application of these tools to the study of hemostasis and thrombosis, as well as the direction I will take these projects as a postdoctoral researcher. The NSF Graduate Research Fellowship has funded my research over the last five years.

The biological system of interest is in the formation of blood clots; hemostasis describes the proper process of clot formation, and thrombosis describes the pathological process of clot formation. Flowing blood contains protein and cellular species that continuously monitor blood vessels for injuries. In particular, platelets are small anucleated cells that respond to vessel disruptions by adhering to the wall and cohering to other platelets. The mechanisms surrounding formation of stable aggregates across physical scales have been of interest to researchers in medicine and basic science for many years [4]. Expanding our understanding of hemostasis and thrombosis has broad implications for understanding disease states such as hemophilia, Von Willebrand's Disease, and stroke. My contribution to the study of platelet aggregation has been to examine in detail the biochemical processes of platelet activation and platelet-platelet crosslink formation and breakage [9]. Biological variability presents numerous experimental challenges, and thus, we rely on mathematical modeling to validate experimental hypotheses and provide new insights into this complex cellular process. In particular, my work can be divided into two projects that I outline here.

1. The first major project discussed is the study of integrin activation on platelet surfaces by agonist ADP and the governing protein regulatory pathways. This project is in collaboration with Dr. Wolfgang Bergmeier at the University of North Carolina at Chapel Hill (UNC-CH) and my PhD advisor, Dr. Aaron Fogelson. ADP binds to two separate receptors that initiate their own signaling pathways and lead to the transient activation of integrin. We analyze a detailed dynamical systems model of the activation pathways and compute the sensitivity of integrin activation to changes in protein expression. Our inital model and results are currently under review at Biophysical Journal [10]. I aim to determine the individual contribution of each ADP receptor to an integrin response.
2. The second major project discussed is the study of polymeric protein Von Willebrand Factor (vWF) and its role in stabilizing platelet aggregates at high shear rates. This project is in collaboration with Dr. Jian Du at Florida Institute of Technology and Dr. Aaron Fogelson. I plan to study vWF using my developed dynamical systems model and model extensions to a 1D spatial PDE model. I aim to determine the biochemical properties of vWF that increase the likelihood of aggregate formation and vessel occlusion under high shear rate environments.

Determination of individual receptor contribution to platelet activation via sensitivity analysis: My first project objective is to determine the individual contributions of platelet receptors to the activation of platelet integrins. Through my collaboration with the Bergmeier Lab, I have had the opportunity to develop and analyze a model of integrin activation and collect data using mouse blood samples to inform our model.

Activated platelets release the small nucleotide ADP, which induces the transient activation of other platelets by binding to receptors on their surfaces [6]. Among the induced changes is a conformational change to integrins, which allows it to bind to crosslinking protein fibrinogen [14]. ADP initiates two separate pathways by binding to G protein-coupled receptors (GPCR) $P2Y_1$ and $P2Y_{12}$ that work cooperatively to activate the integrin. Activation by ADP is critical to

preventing platelets from activating in regions of the bloodstream where clotting is not required. Most other agonists induce an irreversible activation of platelet integrins, but ADP is unique in that the integrin response only lasts a few minutes before platelets return to their quiescent state.

It is experimentally known that applying chemical inhibitors to either receptor significantly reduces the strength of integrin activation by ADP [11]. Because activation of both receptors is necessary to see a positive experimental result, it is difficult to show how each receptor individually contributes to the propagation of the activation signal. My goal for this project is to determine the exact contribution of each GPCR to integrin response across ADP concentrations. We will also determine how these results are affected by manipulating the proteins in the pathway, both by variation of expression levels and by manipulating protein action by synthetic agents. We will utilize our *dynamical systems model* that describes integrin activation by ADP.

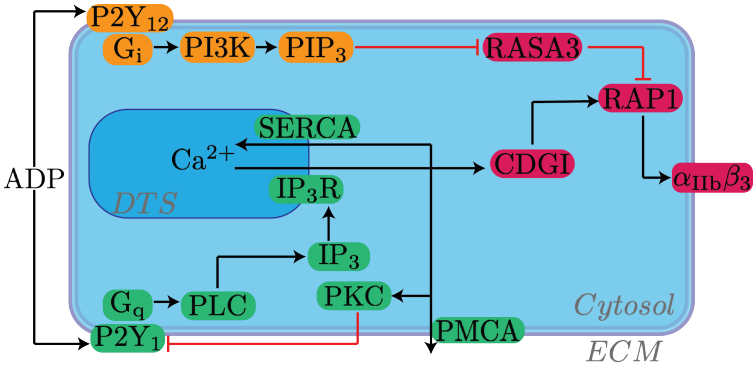

Fig. 1: Diagram GPCR pathways involved in activation of integrin $\alpha_{\mathrm{IIb}}\beta_3$.

The model begins with extracellular ADP binding to receptors and ends with the activation, then inactivation of integrin. Figure 1 shows a schematic of the pathways. Each receptor and second messenger species is explicitly tracked for a total of 13 proteins and 82 differential equations. Each reaction between species is assumed to occur via either mass action or Michalis-Menten kinetics.

We derived our model from experimental literature detailing the pathways [6], past modeling work on relevant subsets of the pathway [12], and protein expression data [16]. For unknown kinetic parameters, we performed a *parameter estimation study* using flow cytometry data collected by Dr. Bergmeier. We obtained data on the release of calcium into the cytosol and the activation of integrin; we scale the data appropriately to determine the concentration of each, then compute the norm of the difference between the model's calcium and integrin curves. We used MATLAB's `fmincon` function to minimize this norm as a function of our unknown parameters.

We then perform hundreds of simulations in parallel using computing clusters available at the University of Utah in which we vary the expression level of a single protein and measure the change in integrin activation. We found that if platelets are presented with a large concentration of ADP, variation in a the expression of $\mathrm{P2Y}_{12}$ affects integrin activation significantly compared to $\mathrm{P2Y}_1$ [10]. Our work points to the $\mathrm{P2Y}_{12}$ pathway as potential therapeutic targets for bleeding disorders.

We plan to utilize our current model to examine in detail the contribution of each protein on integrin response across ADP concentration. We plan to perform an extensive *global sensitivity analysis* using Method of Morris [8] and Sobol' Indices [13]. To match our previous results, we expect that for large concentrations of ADP, the sensitivity in $\mathrm{P2Y}_{12}$ expression should be more significant than $\mathrm{P2Y}_1$. Changes in the sensitivity as platelets are stimulated with lower ADP concentrations would suggest a tradeoff in which signaling pathway impacts the system more. This gives a more realistic insight into platelet activation *in vivo*, where platelets may sense a distribution of ADP concentrations as they flow past an injury site that depends on the shear rate.

Determination of Von Willebrand Factor's effect on vessel occlusion at high shear rate: vWF is a linear polymeric protein that exists constitutively within the bloodstream in a globular conformation; at a high shear rate, the protein stretches to reveal binding sites for platelet GPIb receptors. These receptors bind rapidly to vWF without the need for the platelet to sense aggregation agonists, facilitating aggregation in high shear rate conditions [5]. A critical clinical consequence of vWF is the potential for thrombosis around stenotic arteries — regions in the bloodstream that are pathologically constricted. Thromboses formed here can potentially occlude the stenotic artery or embolize and occlude a downstream artery.

Ongoing work in our group has made progress in developing computational fluid dynamics (CFD) simulations of platelet aggregation [2, 7]. Recent work by others has also detailed the dynamics of vWF folding [15]. However, computational complexity limits the ability of these models to accurately describe the detailed biochemical aspects of aggregation across physiological scales and time. Thus, our methodology is to build a spatially-averaged model that focuses on the biochemical reactions needed to develop and stabilize the aggregate. Each equation is then given by a differential equation in time only. Our model is far more efficient to simulate over several minutes to an hour than 2D or 3D CFD simulations. This allows us to vary both fluid properties, such as shear rate, and chemical properties, such as the concentration of vWF.

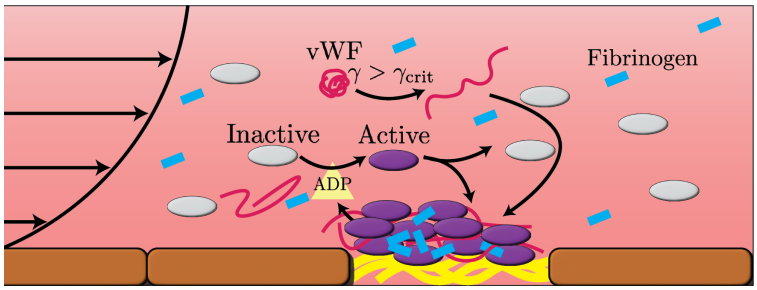

Fig. 2: Platelet aggregation process by vWF. At large shear rate γ, vWF unfolds and facilitates platelet-platelet crosslinking.

My second project objective is to determine the effect that vWF plays in allowing platelets to form stable aggregates in complex geometries. In particular, we plan to extend our work in two meaningful ways: 1) to study the formation of aggregates in so-called "recirculating zones" downstream of a stenosis, and 2) to study the growth of an aggregate up to occlusion.

Our model comprises several equations describing the binding of platelets and crosslinking proteins as they flow in and out of an injury region. Platelet adhesion and cohesion affect the size and density of the aggregate, which then affects the fluid velocity. We assume unidirectional fluid flow in our channel and use theory from porous media to analytically determine a velocity profile of the fluid in and around an aggregate [1]. Our model considers novel additions to spatially averaged platelet aggregation models, in particular, force-dependent platelet detachment as well as the tracking the concentration of crosslink proteins that are bound to only one platelet receptor, and therefore have a free end to form a crosslink. I show within my thesis (under preparation) [9] that platelet aggregate growth nonlinearly depends on shear rate. At low shear rates, platelet aggregates can be formed by fibrinogen; at high shear rates, platelet aggregates can be formed by vWF. At modest shear rates, fluid flow is fast enough to wash away ADP and prevent platelet activation but not fast enough to allow vWF to unfold and cohere platelets.

In our next steps, we seek to understand how geometry contributes to the occlusion of vessels by considering a stenotic artery. My first plan involves applying my current dynamical systems model to track the growth of an aggregate in a recirculation zone. Recirculation zones in steady flow are regions of slow, rotational flow where the fluid does not escape and particles enter/exit primarily through diffusion. These regions can develop immediately downstream of a stenosis, and unfolded vWF polymers that exit the stenotic region can diffuse into the recirculation zone and initiate aggregate formation. A recirculation zone's size, shape, and aggregation propensity heavily depend

on physical quantities like shear rate and size of the stenosis [3]. Moreover, the long timescale over which aggregates can develop makes studying the system experimentally and computationally demanding. Thus, we plan to apply our current dynamical systems model to this physical domain.

We also plan to extend our model to a *1D spatial model* that describes the growth of the aggregate into the blood vessel to occlusion. A current limitation of our spatially averaged model is that as the aggregate grows, a well-mixed assumption fails to capture the experimentally determined structure of an aggregate. Large growing aggregates are known to have a core-shell type structure, where the dense core is relatively stable and does not directly contribute to growth, and the shell, which is less dense but allows for the influx of materials to continue the cohesion process [4]. My plans for a 1D continuum model will consider the same biochemical aspects as in the dynamical systems model. In addition, it will track the growth of the core and shell of the aggregate and allow us to more accurately depict the effect of the growing aggregate on the fluid and the role of vWF in enabling the aggregate to continue growing occlusion.

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