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From branches to fibers - investigating f-actin networks with biochemistry and mathematical modeling

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FROM BRANCHES TO FIBERS - INVESTIGATING F-ACTIN NETWORKS WITH BIOCHEMISTRY AND MATHEMATICAL MODELING

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From Branches to Fibers - Investigating F-Actin Networks with Biochemistry and Mathematical Modeling

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by Melissa Anne Riddle

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3 Abstract

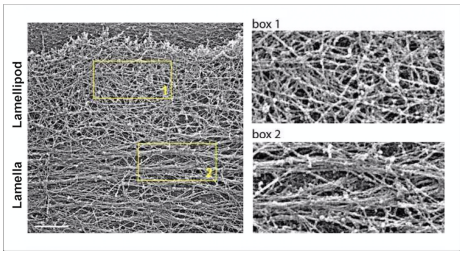
F-actin networks have different structures throughout the cell depending on their location or mechanical role. For example, at the leading edge of a migrating cell, F-actin is organized in a region called the lamellipodia as a branched network responsible for pushing the membrane outwards. Behind the lamellipodia is a lamellar actin network where focal adhesions and stress fibers originate, and then within the cell cortex, actin is arranged in a gel-like network. Stress fibers are an important organization of F-actin and how they arise from either the branched lamellipodia network or the gel-like cortex network is poorly understood. Our approach is to create a computational simulation to model a mechanism of bundling by crosslinking proteins specifically modeling the interaction of two cylindrical rods (individual F-actin filaments). We created a 2D simulation of two cylindrical rods in MATLAB that contact one another. We specified the initial speeds of the rods, and plotted their location after impact based on the principles of linear momentum. Although this model demonstrated how parallel filaments might collide, we also considered how actin-binding proteins work together to form tightly bundled F-actin stress fibers, arising from the branched network of F-actin at the leading edge. Our approach is two-fold: first, we conducted biochemical studies focused on the interactions of actin filaments and the actin binding proteins ARP2/3, α-actinin, dynamin2, and cortactin and second, we developed a computational model to validate experimental hypotheses for actin filament crosslinking to promote filament bundling. Our biochemical studies used quantitative binding analysis and imaged reconstituted networks in real time to show that dynamin2 and cortactin create bundled actin filaments *in vitro*. Additionally, cortactin decreases the association of α-actinin with bundled actin filaments. We hypothesize that the transition from a branched to bundled F-actin network architecture involves balancing the activities of two competing filament crosslinkers: dynamin2 and cortactin vs. α-actinin. We will test this hypothesis by creating an emergent Monte Carlo model in MATLAB that simulates filament crosslinking in the presence of α-actinin, cortactin and dynamin2. Our model lays the foundation for testing hypotheses of the mechanisms by which cortactin and dynamin2 organize F-actin networks.

4 Introduction

Cells and their interactions with other cells are important to understand essential processes such as wound healing and development, in addition to uncontrollable cell growth i.e cancer. Cancer is the second leading cause of death in the United States and was responsible for over 600,000 deaths in 2019 (”Cancer Facts Figures 2019”, 2020). It is important to understand the molecular mechanisms leading to the uncontrollable cell growth. Although there are many structures within the cell that contribute to movement and growth of cells we will focus on filamentous actin, known as F-actin.

The overarching goal of this project is to understand specific architectures of F-actin in the cytoskeleton by simulating F-actin filament interactions and comparing the simulated results with experimental findings. The lamellipodia and the lamellae play a role in these different architectures and their respective F-actin interactions (Figure 1).

[Figure1: Leading Edge of the Cell; Actin Networks]



Specifically we want to answer the question: How do different actin binding proteins give rise to actin networks of different archetectures? We hypothesize that dynamin2 and cortactin do not allow α -actinin to bind to F-actin which results in an actomyosin network based on actin binding protein concentration. We further hypothesize that the competition between actin-binding proteins will allow for bundling rather than branching F-actin structures. We used biochemical experiments as well as computer simulations to test our hypotheses.

Biochemical experiments allow researchers to determine how the cell and components of the cell react

in real time. Dr. Schafer's lab tests the different effects that dynamin2, cortactin and their combination have on filament bundling. We used gel electrophoresis to separate out the proteins within our supernatant and pellet binding experiments. Western blotting allowed us to determine the concentrations of the different proteins within the binding experiments. From these biochemical experiments we were able to quantify the amount of dynamin2, cortactin and actin present in F-actin bundles isolated during low-speed centrifugation.

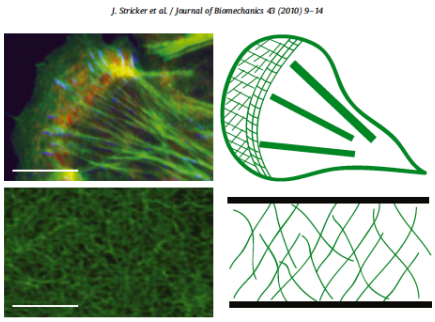
Complement to the experimental work, I worked with Dr. Miller to develop computer s to examine the effects of parameters, such as binding affinity, to the emergence of F-actin bundles. The benefits to using computer simulations include allowing hundreds of simulations to be considered, quickly altering parameter values, validate experimental conclusions and driving new experimental hypotheses.

5 Background

F-actin is an essential part of the cytoskeloton and the cell's ability to notice mechanical changes in its environment. F-actin is the most abundant protein in eukaryotic cells (Stricker, 2009). One organization of F-actin is stress fibers. Stress fibers are uniform, rod shaped networks that are located behind the lamellipodia and lamellae (Figure 2, Stricker 2009). These fibers are unique F-actin structures made of repeated units of myosin-II motor proteins and the actin binding protein α -actinin that are able to sense and respond to mechanical tension within the cell (Stricker, 2009). Within the lamellipodium, on the other hand, F-actin creates branches that sense the external mechanical environment (Stricker, 2009). Actin binding proteins are catalysts for the different roles and organizations of F-actin. My work specifically focuses on the actin binding proteins ARP 2/3, dynamin2, cortactin, and α - actinin.

It is believed that stress fibers and the neighboring lamellae and lamellipodium differ in F-actin formation due to the differing F-actin interactions with actin binding proteins. This will lead to a better understanding of cell growth and understanding how F-actin networks form to facilitate cell movement.

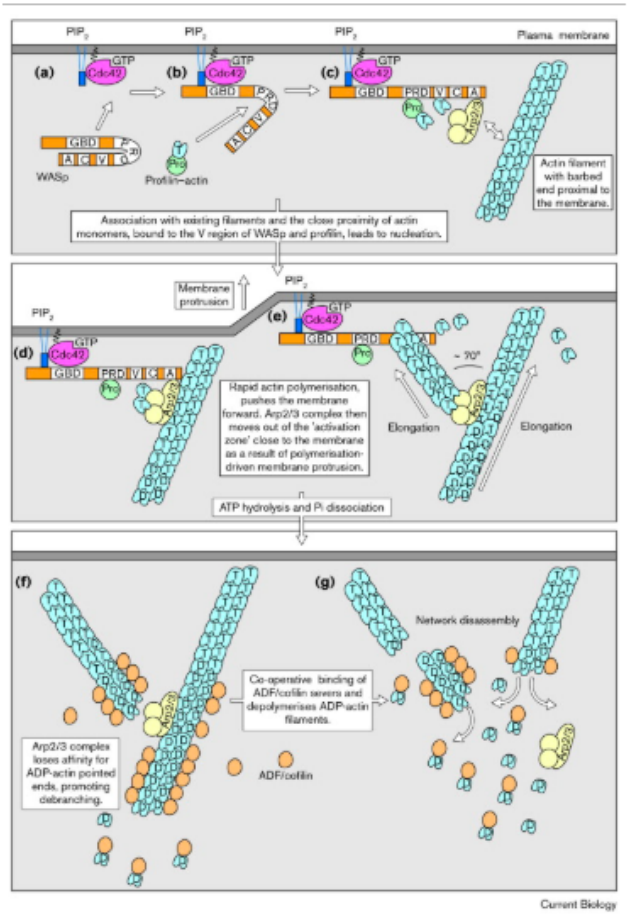
[Figure 2: Leading Edge of the Cell; Actin Networks]



Stress fibers form a uniform rod shape behind the lamellipodia and the lamellae. The lamellipodia is at the forefront of the cell. The lamellipoda forms a diagonal grid like pattern. In contrast, the lamellae sits directly behind the lamellipodia in a straight grid like pattern. This is a zoomed in portion of the cell. (Stricker, 2009)

Interaction between F-actin filaments and ARP 2/3 causes branching. Branching is best described like branching found in trees in nature. There are one or more binding sites with linear protrusions from a central filament (Stricker, 2009). ARP 2/3 binds to an F-actin filament and then a single monomer of F-actin, called G-actin, binds to the single ARP 2/3 to begin creating a new F-actin filament, a process called nucleation. ARP 2/3 is unique in that is creates branches at X_o (Sticker, 2009). These actin branching interactions are essential to the lamellipodia and its maintenance. Previous research at the biology department of The University of Virginia had observed that the presence of dynamin2 inhibited actin nucleation and filament branching.

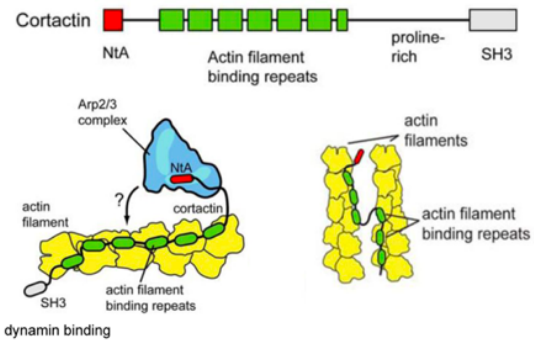
[Figure3]



This image demonstrates branching experienced by F-actin filaments. This is an important part of the role that F-actin plays within the cell. It is the branching, bundling and actin-binding contact that helps define F-actin within the cell.

Bundles are another type of network formed from F-actin interactions with themselves and other actin binding proteins. Bundles are different from branching in their visual appearance and structure. Two actin filaments are aligned beside one another while semi flexible actin binding proteins provide multiple binding sites for the actin filaments to come together. These structures are less likely to dissociate than branching networks because of their numerous binding sites. Numerous binding sites do not allow for easy breakage because if one binding site breaks, there are other sites holding the network together. We examined the relationship between dynamin2 and cortactin for bundling. Both dynamin2 and cortactin are required for filament bundling (Figure 3). We further examined the competition between these two actin binding proteins within our project to see how bundling sites were affected. More specifically, if the inclusion of multiple actin-proteins creates stronger or weaker binding sites.

[Figure 4; Interaction between Actin and Actin Binding Proteins]



Bundling and bundling sites.

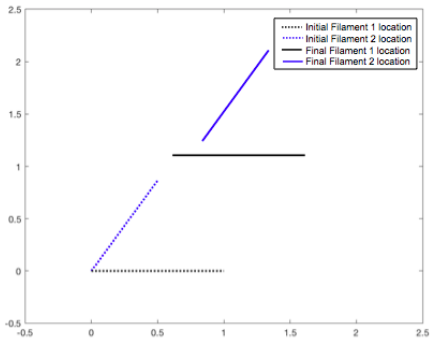
6 Methods

First, to determine how F-actin might create bundles through spontaneous interactions with each other, I created a simulation to model the collision of two F-actin rods.

It was important to start with this simulation in order to see how computer modeling could be implemented on the whole and also to see how actin interacts with itself before adding in other proteins.

I assume that G-actin monomers have bundled together to form F-actin filaments 10nm long. I chose initial random locations for two filament's plus-ends, a term used by biologists to describe where F-actin is most likely to "grow" through the addition of G-actin monomers in a process called polymerization. Additionally, I chose a random angle of orientation, Θ, for each filament. Since I was modeling a collision, I assumed each filament had an initial velocity acting as a vector originating from the filament's plus end. I used the principles of linear momentum and the coefficient of restitution to calculate how fast the filaments would be moving after they collided. My process is described in figure 5, which is an algorithm flow chart.

[Figure 5: Computer Simulation Output]

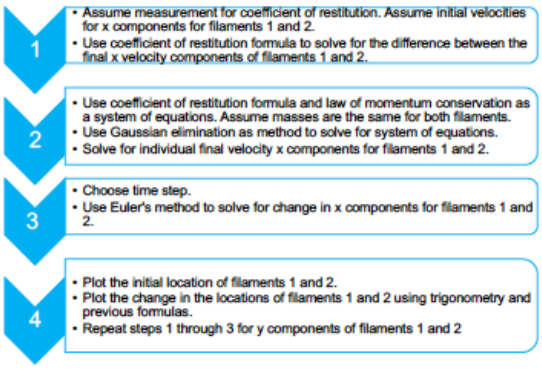


Computer simulation results. This graph simulates F-actin interaction using assumptions and initial velocities listed. The initial positions of the filaments are shown as the bottom left dotted lines while the solid lines in the center and near top right are the movements that the filaments experience after the collision. Assumed: coefficient of restitution is .9; angle between filaments is pi/3 radians; time elapsed

after impact is .5 seconds. Initial velocity in x direction: filament 1 is 1.2, filament 2 is 1.7. Final velocity in x direction (after impact): filament 1 is 1.225, filament 2 is 1.675. Initial velocity in x direction in y direction: filament 1 is 2.2, filament 2 is 2.5. Final velocity in y direction (after impact): filament 1 is 2.215, filament 2 is 2.485. All in micrometers/second.

In order to illustrate this image, two filaments were categorized as filaments 1 and 2 and further separated into individual x and y components (Figure 5).

[Figure 6]



This figure describes the method used through computer simulation 1. Filaments and their respected cartesian coordinates are separated. Gaussian elimination is used to solve for final velocities and graph placement.

The coefficient of restitution (e), is the ratio of the final velocities ($V_b final$ and $V_a final$).

$$e = \frac{V_{2final} - V_{1final}}{V_{1initial} - V_{2initial}} \tag{1}$$

These assumptions allow us to see how manipulation of these assumed and calculated parameters affect the formation of F-actin filament bundles. By separating these filaments into individual cartesian components, it is easier to see the direction of their motion. Using this ratio and assuming initial velocities, we are able to solve for the difference between the x components of final velocities of filaments 1 and 2.

The law of conservation of momentum is an important piece to understanding the collisions of F-actin filaments. This law describes that total momentum before and after a collision is equal (eqn 2). Following solving for the final velocities of filaments 1 and 2, we then created a system of equations using the coefficient of restitution relationship and law of conservation of momentum to solve for the individual final velocity x components for filaments 1 and 2. Gaussian elimination is a method to solve for multiple variables within a system of equations. Using the Gaussian elimination method is a time efficient and systematic way to solve for equations. We assumed equal masses for filaments 1 and 2.

$$M_1 * V_1 + M_2 * V_2 = M_1 * V_1 + M_2 * V_2 \tag{2}$$

Using a previously defined time step, Euler's method was used to solve for the change in the x components for both filaments (Eqn 3). The process is then repeated for the y components for the two filaments. As a visual, the initial location and change in location are then plotted for both x and y components for both filaments.

$$V = \frac{dx}{dt} \tag{3}$$

We hypothesize that filaments with properties similar to that of stress fibers would result in more organized rod-like networks. This simulation resulted in plots that showed the movement and interaction of the actin filaments. We are working to include the interaction of more filaments and different angles of filaments. With these additions, less assumptions will need to be made about the filaments and more information can be gathered from the simulation.

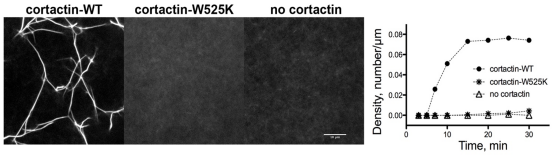
This simulation demonstrated the ability to simulate filament interaction and the influence that different parameter measurements have on these interactions. Future steps in the research will consider validating forward and backward rate constants to match the polymerization and depolymerization rates that are prop-

erties of the inherent polarity of F-actin. Validation based on S-curve of percent of actin monomers in filament structures over time will be examined.By understanding this background information first, I was able to better comprehend interaction between proteins and F-actin.

Following the completion of the first simulation, questions regarding how competition between dynamin2 and cortactin with F-actin arose. Would they work together to create strong bundles? Would they compete and one would overpower the other?

In order to look into this phenomena, biochemistry could to look into what we needed to simulate for our next steps. Image analysis showed that distinct bundles were formed in the presence of cortactin, while in the presence of cortactin-W525K, no bundles were formed. (Figure 7) Cortactin-W525K is a mutant of cortactin.

[Figure 7: Filament Bundling by Dynamin2 and Cortactin]



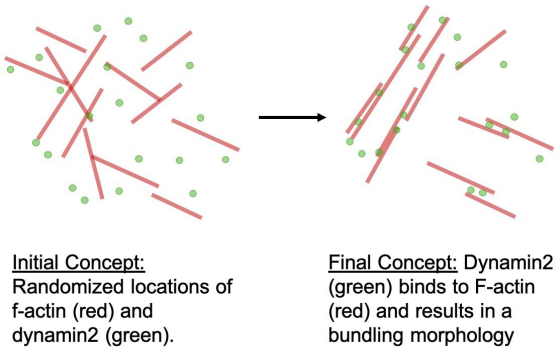
Actin networks were reconstituted from 2.5M actin, 400nM dynamin2, 1.5mM GTP and with either 400nM cortactin, mutant cortactin-W525K (which does not bind dynamin2) or in the absence of cortactin. F-actin is visualized by the presence of 250nM Alexa-647 phalloidin in the reconstitution reaction and formation of the network was recorded over time (20min timepoint is shown). Plotted is the bundle density over time as quantified from the number of maximal intensity peaks of Alexa-647 phalloidin fluorescence per micron along 5 pixel-wide line scans oriented in a grid pattern over the entire image of each frame of the time-lapse movie.

I performed low-speed sedimentation assays to see how dynamin2 and cortactin bundled within the pellet with F-actin. We found that dynamin2 results in F-actin bundles when it is alone as well as with cortactin (Figure 9). In order to understand how dynamin2 and cortactin might work together to form bundles, I began

creating a computer simulation to study the competition that might exist between these proteins.

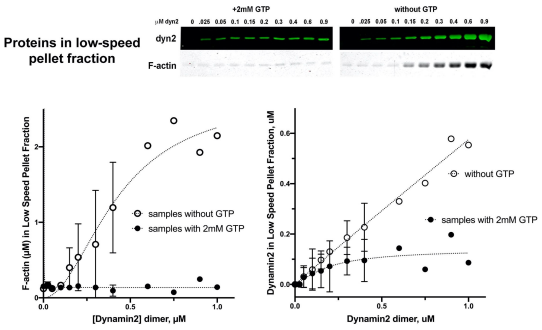
This simulation focuses on the random walking of F-actin filaments and their interaction with actin-binding proteins, specifically dynamin2 and cortactin (Figure 8).

[Figure 8: Initial Concept for Simulation 2]



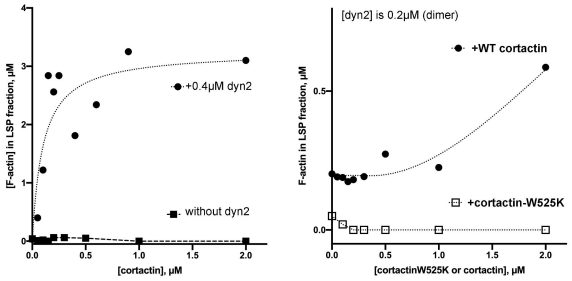
From the biochemistry work we know that dynamin2 and cortactin together leads to bundling of F-actin branched networks. Dynamin2 first binds to cortactin, which then allows cortactin to bind to F-actin within a specific spatial distance and at a given orientation (close to parallel) (Figure 7). Our model had to first simulate the diffusion of cortactin and dynamin2 then determine how they would bind together if they were close together. This would allow us to model the initial interactions of dynamin2 and cortactin within the experimental pellet assay. To model diffusion, we used a random walk method.

[Figure 9: Filament Bundling by Dynamin2 and Cortactin]



Low-speed sedimentation (10,000xg) of actin filaments incubated in the presence of dynamin2 and in the presence or absence of GTP. Plotted is the amount of F-actin (right panel) or dynamin2 (left panel), respectively, in the low-speed pellet fraction with increasing amounts of dynamin2. Pre-assembled F-actin (3M) was incubated for 20 min at room temperature with increasing concentrations of dynamin2 in a buffer containing 20mM imidazole, pH 7, 65mM KCl, 0.1mM EGTA, 2mM MgCl2, 2 mM GTP, 0.2mM ATP, and 0.1mM DTT. Samples were subjected centrifugation at low speed (10,000 x g) for 20 min. Equal volumes of the pellet fraction from each reaction were separated by 11 percent SDS-PAGE; proteins in the upper half of the gel were transferred to nitrocellulose and identified on Western blots probed with anti-dynamin2 (clone C-18; Santa Cruz Biologicals). The lower half of the gel was stained with Coomassie Blue. Gels and blots were analyzed by densitometry on an Odyssey infrared imager (LiCor Biosciences). Scale bar is 10m.

[Figure 10: F-actin Bundling by dyn2 alone occurs only in the absence of GTP]



Low-speed sedimentation (10,000xg) of actin filaments incubated in the presence of dynamin2 and varying concentrations of cortactin (or cortactin-W525K) and in the presence of 2mM GTP. Plotted is the amount of F-actin in the low-speed pellet (LSP) fraction at varying concentrations of WT-cortactin or mutant cortactin-W525K, which does not bind dynamin2. Pre-assembled F-actin (3M) was incubated for 20 min at

room temperature with increasing concentrations of WT-cortactin or cortactin-W525K in a buffer containing 20mM imidazole, pH 7, 65mM KCl, 0.1mM EGTA, 2mM MgCl2, 2 mM GTP, 0.2mM ATP, and 0.1mM DTT. Samples were subjected to centrifugation at low speed (10,000 x g) for 20 min. Equal volumes of the pellet fraction from each reaction were separated by 11 percent SDS-PAGE; proteins in the upper half of the gel were transferred to nitrocellulose and identified on Western blots probed with anti-cortactin (clone 4F11; Invitrogen). The lower half of the gel was stained with Coomassie Blue. Gels and blots were analyzed by densitometry on an Odyssey infrared imager (LiCor Biosciences).

Future steps include the addition of ARP 2/3 into this simulation to see how that allows branches of F-actin to form and influence the dynamin2 and cortactin bundling.

Ultimately, our simulations will provide researchers with an understanding of the interactions that F-actin filaments undergo within the cell in a way that is a visually accessible and manipulative comparison to biochemical experiments.

7 Looking Back: Reflection

I began my research with Dr. Miller my sophomore year as simply an exploration for intellectual stimulation. This thesis and research ended up being a large part of my college career. Being a mathematics and statistics major, I thought at first I was at a disadvantage but quickly found out that my skills would be useful for the work at hand and a fresh set of eyes would be beneficial as well. I was hooked on this idea that we were looking into something that no one else had before. I believe there was a bit of a learning curve because of my lack of biology background but found that when I worked hard and kept up with literature that I was able to contribute just as much as other students.

The hardest adjustment for me was that I was able to ask questions along the way and that research was a team effort. I was taught that finished products get handed in throughout my education but quickly realized that many heads are better than one. I enjoyed learning that questions are valuable and that progress and collaboration were the keys for success. These new found adjustments I have been able to translate to a lot of other parts of my life including career applications.

I spent my summer going into junior year at the University of Virginia's biochemistry department working under Dr. Dorothy Schafer. Every day I was challenged and found myself working harder than ever to learn and improve myself as an academic. This was an unforgettable experience that allowed me to explore biochemistry in a lab setting in a new town. I had never had lab experience before and seeing the proteins that I had been reading about and studying made it all come together. I will emphasize the challenge that came with this study, however. The constant reassurance and help from both Dr. Schafer and Dr. Miller were priceless.

I did find times to be frustrating when my brain would not understand the concepts that were explained to me countless times and when other people already understood the science behind what I was studying. I think overall however, this has made me into a better student and a more intelligent researcher.

I recently went to the Biophysical Society's 64th annual conference. It was here that I realized how much of the world that we do not know about and how many efforts are being made to answer these questions. I enjoyed my conversations with scientists from around the world and their encouragement on my intellectual journey. One of the best aspects that has come from this research are the opportunities that I had to interact with other people from other departments and ways of thinking.

Making my first poster for the VAS conference in 2017 was challenging. I at first did not understand that a story had to be told on the paper just as much as when you're explaining it verbally. It challenged me to decide what was important and what wasn't in my research and how to organize the information in a way that anyone who looked at it would understand. I struggled with this first poster more than I thought that I would and Dr. Miller held my hand during the whole thing. Her guidance helped me immensely.

8 Deliverables

Following the completion of these simulations and collection of wet lab data, I attended the 64th annual Biophysical Society conference in February. This gave me the chance to demonstrate my knowledge and understanding of the material that I have synthesized. I travelled to San Diego, California through James Madison University's Engineering Department and The University of Virginia's Biochemistry Department. Dr. Dorothy Schafer accompanied me to the conference. Funding from the 4VA grant was used throughout my process and travels.

In order to attend I submitted an abstract in addition to a poster that summarized my work.

The work done with Dr. Dorothy Schafer in summer 2018 will be published.

To begin, I worked with Dr. Callie Miller and her research team in the Engineering Department at James Madison University to gain knowledge on the subject of F-actin networks and the way that they worked in the world of cellular biology and bioengineering. I started to explore F-actin networks and MATLAB simulations.

Following this academic year, I worked with Dr. Dorothy Schafer in the Biochemistry Department at The University of Virginia to see how different proteins interact within the cell in the wet lab setting. We collected data on the relationship between dynamin2 and cortactin and their inability to allow α -actinin to bind to F-actin.

During my time at The University of Virginia, I attended The Virginia Academy of Science Conference at Longwood University where I presented my first simulation and conclusions.

I then returned to James Madison University's Engineering department to continue my research and MATLAB simulations.

9 Appendix

9.1 MATLAB Code for Simulation 1: Focusing on filaments before and after collision

```
%--------------------------------------------------------------------------
% Program   : Ploting and Simulating Before and After collision
% Author    : Melissa Riddle and Callie Miller
% Date  : 4/8/18
% Purpose   : This program will plot the filaments before and after
 the collision.
% Mass and length are assumed to be the same for both
% filaments. This means that inertia is also the same. This is to test
% the plotting and see how the interaction would look. All 2D. F-
 Actin
% filaments.
%--------------------------------------------------------------------------

%Variables (all double)
%Vax, Vay = initial velocity A (assumed)
%Vbx, Vby = initial velocity B (assumed)
%Vaxf, Vayf = final velocity A
%Vbxf Vbyf = final velocity B
%e = coefficient of restitution (assumed)
%dt = change in time aka time step
%dxa, dxb = change in x direction for filaments 1 and 2
%dya, dyb = change in y direction for filaments 1 and 2
%xa1 = initial x starting coordinate for filament 1
%xb1 = initial x starting coordinate for filament 2
%ya1 = initial y starting coordinate for filament 1

%using system of equations, solve for Vbxf and Vaxf (respectively).
%Gaussian elimination
%set initial velocity for A and B
%set coefficient of restitution based on research
format long;
Vax = input('what is your initial ax velocity?(in micromillimeters/
second)  ');
Vbx = input ('what is your initial bx velocity?(in micromillimeters/
second)  ');
e = input('what is your coefficient of restitution?  ');
A=[1, -1, (Vax-Vbx)*e ; 1, 1 , Vax+Vbx];
[m, n]=size(A);
for i=1:m %The process of Gaussian elimination progresses row by row
    %pivoting
    if A(i,i)~=0
        i=i; %Do nothing
    else
        for k=i+1:m
            if A(k,i)~=0 %find a nonzero entry under the zero that you
 want to get rid of
                %Swap rows k and i
                Z=A(i,:);
                A(i,:)=A(k,:);
                A(k,:)=Z;
                break;
            end
```

```
        end
    end
    %Finished with pivtoing other than making the pivot equal to 1
    %Make sure the (i,i) entry is equalt to 1
    A(i,:)=A(i,:)/A(i,i);
    %Make sure the two entries below the pivot are equal to zero
    for j=i+1:m
        A(j,:)=A(i,:)*(-A(j,i))+A(j,:);
    end
end
A

%backward substitution
w=zeros(m,1); %Solution vector that we want to fill
for i=m:-1:1
    w(i)=A(i,n)-A(i,1:n-1)*w;
end
soln=w

%final velocities
Vbxf = w(1);
Vaxf = w(2);

%input angle between the 2 filaments, will be the same as OG angle;
angle = input('what is the angle between the filaments?(in radians)
 ');

%input time step
dt = input('how much time has elapsed?(in seconds)  ');

%these are the initial x coordinates change later to inputs when
 combine
%code. weird labeling.
xa1 = 0;
xb1 = 0;

%this is change in x coordinates for filaments
dxa = xa1 + dt*(Vaxf);
dxb = xb1 + dt*(Vbxf);
fprintf('the change in x coordinates for fil1 and fil2 are %d and %d
\n',dxa,dxb);

%using system of equations, solve for Vbyf and Vayf (respectively).
%Gaussian elimination
%set initial velocity for A and B
%set coefficient of restitution based on research
format long;
Vay = input('what is your initial ay velocity?(in micromillimeters/
second)  ');
Vby = input ('what is your initial by velocity?(in micromillimeters/
second)  ');
C=[1, -1, (Vay-Vby)*e ; 1, 1 , Vay+Vby];
[p, h]=size(C);
for i=1:p %The process of Gaussian elimination progresses row by row
```

```
    %pivoting
    if C(i,i)~=0
        i=i; %Do nothing
    else
        for k=i+1:p
            if C(k,i)~=0 %find a nonzero entry under the zero that you
 want to get rid of
                %Swap rows k and i
                Z=C(i,:);
                C(i,:)=C(k,:);
                C(k,:)=Z;
                break;
            end
        end
    end
    %Finished with pivtoing other than making the pivot equal to 1
    %Make sure the (i,i) entry is equalt to 1
    C(i,:)=C(i,:)/C(i,i);
    %Make sure the two entries below the pivot are equal to zero
    for j=i+1:p
        C(j,:)=C(i,:)*(-C(j,i))+C(j,:);
    end
end
C

%backward substitution
b=zeros(p,1); %Solution vector that we want to fill
for i=p:-1:1
    b(i)=C(i,n)-C(i,1:n-1)*b;
end
soln=b

%final velocities
Vbyf = b(1);
Vayf = b(2);

%these are the initial y coordinates change later to inputs when
 combine
%code.
ya1 = 0;
yb1 = 0;

%this is change in x coordinates for filaments
dya = ya1 + dt*(Vayf);
dyb = yb1 + dt*(Vbyf);
fprintf('the change in y coordinates for fil1 and fil2 are %d and
 %d',dya,dyb);



%plot
figure()
%to plot filament a's initial location, consider the initial end point
 of
```

```
%xa1 and ya1, the filament's length, L (defined below as a variable
 for the
%code), and the angle of the filament. I *believe* for how you've
%defined it, filament a has the angle but filament b is at an angle of
 0.
% So you need a line from xa1, ya1 to the end point of the filament
 based
% on trig (using filament length and angle).
L=1;
plot([xa1,xa1+L*cos(angle)],[ya1,ya1+L*sin(angle)],'b:');
hold on
% now add to the plot, the new location of filament a as a solid blue
 line
% instead of a dotted blue line
plot([dxa,dxa+L*cos(angle)],[dya,dya+L*sin(angle)],'b');
% now plot filament b initial
plot([xb1,xb1+L*cos(0)],[yb1,yb1+L*sin(0)],'k:');
% not plot filament b final
plot([dxb,dxb+L*cos(0)],[dyb,dyb+L*sin(0)],'k');


%title and label the plot
title('Interaction between F-Actin Filaments (t=0sec & t=.5sec)');
xlabel('x-axis');
ylabel('y-axis');
```

```
Error using input
Cannot call INPUT from EVALC.

Error in milleristhebomb (line 30)
Vax = input('what is your initial ax velocity?(in micromillimeters/
second)  ');
```

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9.2 MATLAB Code for Simulation 2: Focusing on interaction between filaments and proteins

```
%-------------------------------------------------------------------------
% Program   : Bundling Competition
% Author    : Melissa Riddle and Callie Miller
% Date      : 11/19
% Purpose   : This program will present multiple actin filaments and
%             multiple populations of actin binding proteins.
 Competitions
%             will occur. Bundling/Binding.
%-------------------------------------------------------------------------

%Variables
%C1 C2 CN = Cortactin
%D1 D2 DN = Dyn2
%P3 = Cortactin + Dyn2
%P4 = Alpha-Actinin
%R1 = Rate Cortactin
%R2 = Rate Dyn2
%R3 = Rate Cortactin + Dyn2
%R4 = Rate Alpa-Actinin
%G1 = Gamma1 - Off rate Cortactin
%G2 = Gamma2 - Off rate Dyn2
%G3 = Gamma3 - Off rate Cortactin + Dyn2
%G4 = Gamma4 - Off rate Alpha-Actinin
%xs, ys = starting coordinates
%xe, ye = ending coordinates
%L = length of filament
%theta = angle of filament


%this creates protein populations

%cortactin
tic
% CN = input('What is your population rate for cortactin proteins? ');
% C1 = rand(CN*100,1) *10;
% C2 = rand(CN*100,1) *10;
%dynamin
DN = input('What is your population rate for dynamin2 proteins? ');
D1 = rand(DN*100,1) *10;
D2 = rand(DN*100,1) *10;
%plot protein populations
scatter(D1,D2,'g');
hold on
% scatter(C1,C2, 'k');
% hold on
title('F - Actin Filaments & Actin Binding Proteins');
% axis([0,10 0, 10]);
xlabel('x-axis');
ylabel('y-axis');
toc %measures time elapsed from beginning
```

```
%plot filaments
xs = rand(100,1)*10;
ys = rand(100,1)*10;
theta = rand(100,1)* 2*pi;
L = rand(100,1);
xe = xs + L.*cos(theta);
ye = ys + L.*sin(theta);
for i=1:max(length(xs))
    plot([xs(i),xe(i)],[ys(i),ye(i)],'b');
    hold on;
end
% axis([floor(min(xe)),ceil(max(xe)), floor(min(ye)), ceil(max(ye))]);
xlim = [0,10];
ylim = [0,10];
limits = [-1, 11, -1, 11];

%random walk

angle = rand(max(length(D1)),1)*2*pi;
ds = input('What is your step size? ');
d = rand(max(length(D1)),1)*ds;


 %-------------------------------------------------------------------------
   % Code from Dr. Miller for saving positional data in txt files for
then
   % plotting in ImageJ to create a time-lapse movie.
   % 12/04/19
   % NOTES: I have created two separate txt files because the number
of
   % dynamin and the number of filaments may be different, so if each
line of
   % text code is one of the proteins, they will end up being
different sizes
   % and more difficult to plot in ImageJ, for example.

 %-------------------------------------------------------------------------
   % create a text file to 'write'
   fil_length=fopen('length.txt','w');
   for i=1:max(length(xs))
       fprintf(fil_length,'%f\n',L(i));
   end
   fclose(fil_length);
   clear fil_length;

   fil=fopen('fil0.txt','w');
   for i=1:max(length(xs)) %need to write positional information for
each filament
       fprintf(fil, '%f  %f  %f  %f\n', [xs(i), ys(i), xe(i),
ye(i)]); %saved as floating point numbers where each line is a
different filament's data
   end
   fclose (fil);
   clear fil %just to clear up data space
```

```
    dyn=fopen('dyn0.txt', 'w');
    for i=1:max(length(D1))
        fprintf(dyn, '%f  %f\n', [D1(i), D2(i)]);
    end
    fclose(dyn);
    clear dyn

%-------------------------------------------
% BEGIN TIME LOOP FOR DYN MOVEMENTS
%
%_____________________________________________

%Create a matrix of filament data
Z=[xs, ys, xe, ye];
r=0.1; %search radius for Dyn2 to look for nearby filaments

n=input('How many times do you want the proteins to move? ');
for b=1:n
    %First, check to see if there are any filaments near by for Dyn to
 bind
    %to
    for j=1:max(size(D1)) %loop through every dyn2 protein
        [T9]= bundle_dyn2(Z, D1(j), D2(j), r); %calls the function
 bundle_dyn2 and returns a matrix of 1's and 0's
        %Second, update the location of dynamin2
        %********
        % If there is a filament nearby, update dyn2 position to be on
 the
        % filament
        %********

        %********
        % If there is not a filament nearby, then update dyn2 position
 via
        % a random walk
        %********
        angle = rand()*2*pi;
        d = rand()*ds;
        xdyn_change = D1(j) + d * cos(angle);
        ydyn_change = D2(j) + d * sin(angle);

        %update variables
        D1(j)=xdyn_change;
        D2(j)=ydyn_change;
    end

    %save positional data in text files
    fil=fopen(sprintf('fil%d.txt',b),'w'); %allows the file name to be
 updated based on the iteration variable b
    for i=1:max(length(xs))
        fprintf(fil, '%f  %f  %f  %f\n', [xs(i), ys(i), xe(i),
 ye(i)]);
    end
```

```
    fclose(fil);
    clear fil;

    dyn=fopen(sprintf('dyn%d.txt',b), 'w');
    for i=1:max(length(D1))
        fprintf(dyn, '%f  %f\n', [D1(i), D2(i)]);
    end
    fclose(dyn);
    clear dyn


end

tok
```

```
Error using input
Cannot call INPUT from EVALC.

Error in Bundle_Bind (line 37)
DN = input('What is your population rate for dynamin2 proteins? ');
```

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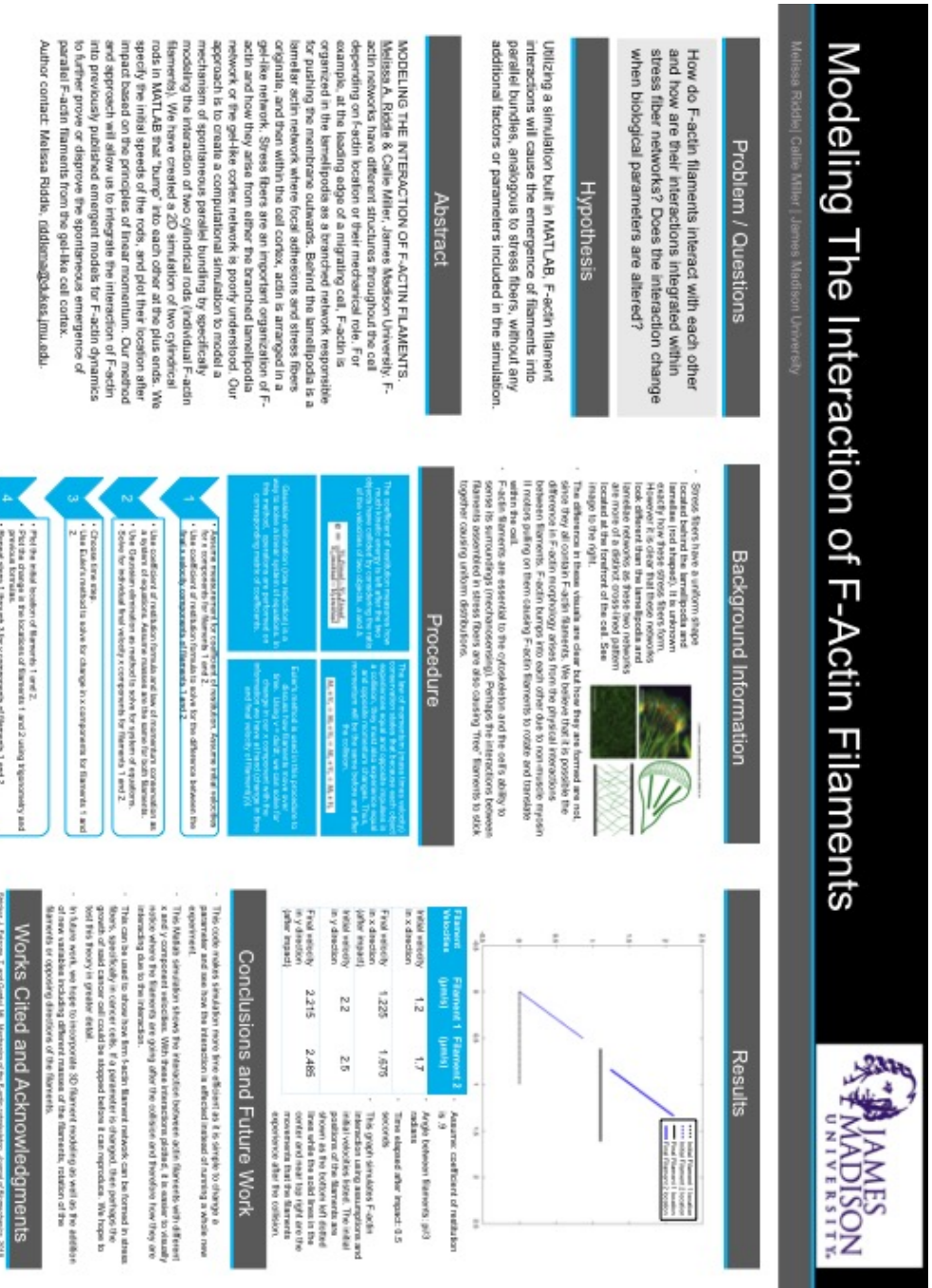
9.3 Poster Presentation Annual Biophysical Society, San Diego CA, February 2020

9.4 Poster Presentation Virginia Academy of Science, Longwood University VA, June 2018



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