Developing Biochemical Probes for a Required Activation Step of Arp2/3 Complex

by

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Brad Nolen

The Arp2/3 complex is a branched actin filament nucleator consisting of seven protein subunits. The Arp2/3 complex plays a crucial role in dynamic actin assembly, which is required for important cellular processes such as cell motility, cell division, chemotaxis, and phagocytosis. Previous studies have shown that Arp2/3 complex requires actin monomers, actin filaments, and nucleation-promoting factors such as WASp for activation. However, it is still unclear how these factors cooperate to activate the Arp2/3 complex due to the lack of high resolution crystal structure of the active Arp2/3 complex. This study seeks to develop biochemical probes to investigate the structural changes that activate the Arp2/3 complex. We hypothesize that there will be an additional conformational change after Arp2 and Arp3 subunits form a pseudo dimer that allows the Arp2/3 complex to bind to the actin filaments and nucleate actin polymerization. We have developed a crosslinking assay with *Saccharomyces cerevisiae* Arp2/3 complex that contains double cysteine mutations at the nucleotide cleft to test for the new proposed conformational change in the Arp2/3 complex. I found that R64C/Q214C mutant successfully crosslinked with bis-maleimidoethane (BMOE), and the presence of ATP significantly decreased the crosslinking reaction rate. However, this study only shows the preliminary data and further studies are needed to investigate the role of actin filaments in the activation of Arp2/3 complex.

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Table of Contents

Introduction	1
Methods	7
Results	13
Future Direction	21
Glossary	22
Bibliography	25

Introduction

Actin is a highly conserved protein found in most eukaryotic organisms. Actin is the monomeric subunit of microfilaments and thin filaments, which are the major components of the cytoskeleton and the contractile apparatus in muscle cells, respectively (8). The actin cytoskeleton is one of the major structural components of cells, and plays crucial roles in a number of dynamic cellular processes including cell migration, cytokinesis, membrane trafficking, and morphogenesis (9). For example, actin creates protrusions that engulf extracellular materials and assists in inward movement of vesicles during endocytosis (9). During muscle contraction, actin and myosin filaments slide past one another to generate movement (7). Neutrophils are the most abundant type of white blood cells in mammals and it responds to chemotactic stimuli by increasing actin polymerization (2). Lamellipodia are broad flat structures supported by orthogonal actin networks and are found at the leading edge of motile cells such as endothelial cells, neurons, immune cells, and epithelial cells (15). Eukaryotic cells depend on proper control of actin polymerization for mobility, phagocytosis, endocytosis, and forming contractile structures (8).

In addition to its role in healthy cells, the actin cytoskeleton plays critical functions in the development and progression of diseased states in humans. For example, a malfunction in actin can lead to Alzheimer's disease and metastasis of cancer cells (3). The microtubule associated protein tau has been known to play a role in the neuropathology of Alzheimer's diseases, and recent studies suggest that tau may be mediating neurotoxicity by disrupting the organization and dynamics of the actin cytoskeleton (3). Metastasis requires extensive remodeling of the cytoskeleton, thus

mutations or abnormal expression of cytoskeletal and cytoskeletal-associated proteins cause the cancer cells to resist chemotherapy and metastasize (3). Therefore, spatial and temporal regulation of actin polymerization is directly related to human diseases and further studies on how actin cytoskeleton is regulated will improve the current understanding of diseased states in humans and how to treat them.

Actin exists in two forms in the cells: monomers and filaments. Actin monomers assemble into filaments in a process called polymerization, which occurs over two phases: nucleation and elongation phase (8). During the nucleation phase, an actin nucleus consisting of three actin monomers is formed. While monomers can spontaneously collide to form dimers, these dimers are highly unstable and usually fall apart before colliding with a third monomer to form a nucleus (8). This large kinetic barrier to nucleation tightly controls the initiation of actin polymerization in cells. However, an actin nucleator shown in red in Figure I can stabilize the actin nucleus to allow the formation of actin filaments (8). In the elongation phase, monomers are rapidly added to the actin nucleus to form filaments, which is often facilitated by elongation factors such as formins (7).

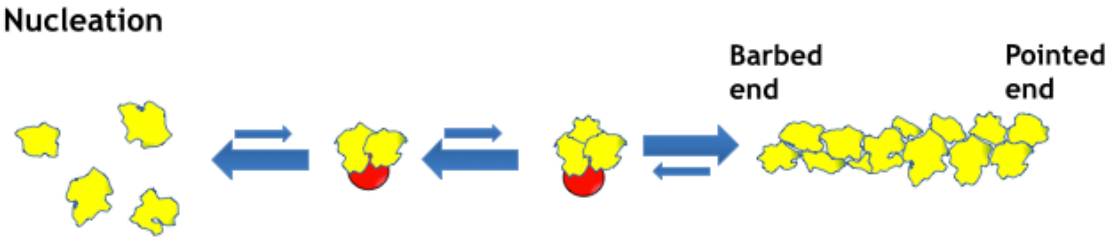


Figure I. A cartoon showing the nucleation step of actin polymerization. This step is energetically unfavorable until a actin nucleator (shown in red) binds to three actin monomers to stabilize the actin nucleus. This energy barrier allows the cells to regulate actin polymerization and actin nucleators are required to nucleate new actin filaments.

While capping proteins can bind at the ends of actin filaments to stop the polymerization, other actin-binding proteins can severe actin filaments and cause disassembly of actin filaments (7). Actin filaments can be either assembled in bundles or branched networks as shown in Figure II, and this is determined by actin-binding proteins that crosslink actin filaments (8). In bundles, actin filaments are crosslinked into closely packed parallel arrays, while in branched actin networks, actin filaments are loosely crosslinked orthogonal arrays that form a three-dimensional meshwork (8). The assembly of actin filaments is highly regulated in cells and actin-binding proteins are key regulators controlling where and when actin monomers will assemble into filaments.

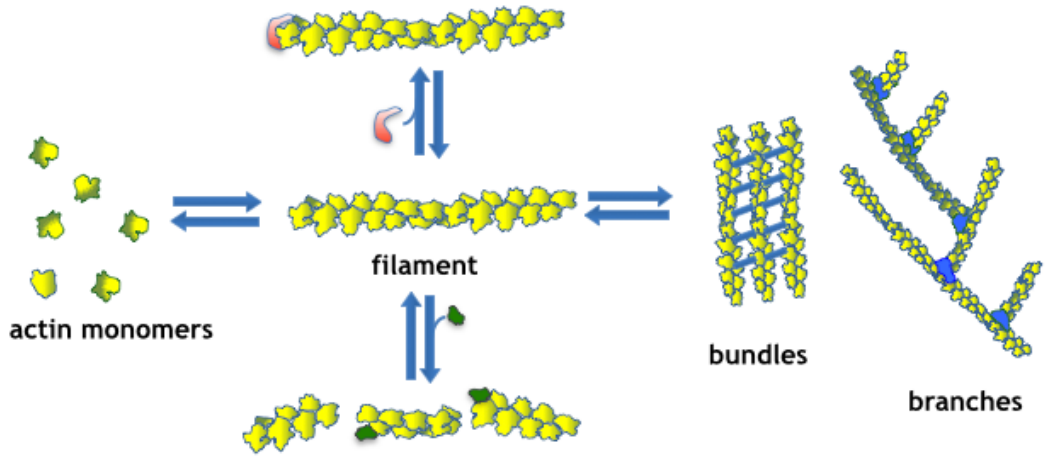


Figure II. A cartoon showing the dynamic nature of the actin cytoskeleton. Actin-binding proteins are responsible for promoting or inhibiting the actin polymerization. Capping protein (in pink), and it binds at the ends of filaments to block polymerization. Other actin binding proteins such as ADF/cofilin (in green) can bind to actin monomers and filaments and sever actin filaments to cause disassembly of actin filaments. Depending on which actin filament nucleator (in blue) binds to actin filaments, actin bundles or branches are formed. In vitro, actin filaments are constantly shrinking or growing in length.

Actin related protein 2/3 complex (Arp2/3 complex) is one of three major classes of actin filament nucleator, and it is conserved in diverse species from yeast to human. The Arp2/3 complex is a seven-subunit protein complex capable of *de novo* formation of actin filaments, and is unique among other actin nucleators since it only creates branched actin filaments under most circumstances (14). The Arp2 and Arp3 subunits are structurally homologous to actin, which allows the Arp2/3 complex to bind to the mother filament and initiate the growth of a new daughter filament to create branched actin networks (5). Branched actin networks are required for cell locomotion, phagocytosis, and intracellular motility of lipid vesicles (13). Previous experiments have shown that Arp2/3 complex is required for viability in many species including

yeast, flies, and worms, and Arp2/3 complex knockouts in most human cells cause cell death (14). Therefore, proper control of the Arp2/3 complex is required for normal cellular functions.

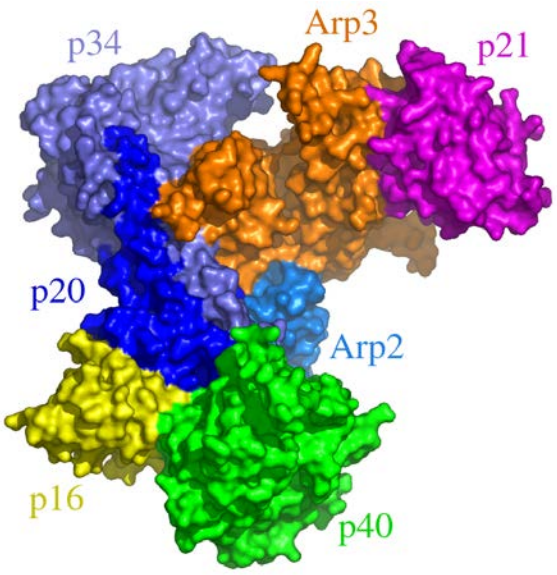
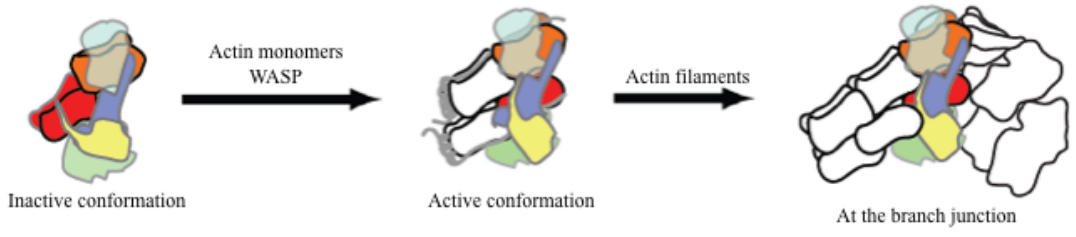


Figure III. A space-filling model of bovine Arp2/3 complex from the x-ray crystal structure. The Arp2/3 complex consists of seven subunits, and Arp2 and Arp3 subunits closely resemble the structure of monomeric actin, which are thought to mimic an actin dimer during nucleation.

The Arp2/3 complex is intrinsically inactive, and the precise mechanism for the activation of the Arp2/3 complex is still unknown. However, it is clear that activation of the Arp2/3 complex requires adenosine triphosphate (ATP), actin monomers, pre-formed actin filaments, and nucleation promoting factors (NPFs) such as WASP (4). High resolution crystal structure of the inactive Arp2/3 complex shows that Arp2 and Arp3 subunits are touching each other in an end-to-end fashion. Upon activation, the Arp2/3 complex undergoes a conformational change where the Arp2 subunit moves

relative to the rest of the complex towards the Arp3 subunit. Previous experiments showed that WASP and actin monomers alone can stimulate the Arp2/3 complex to adopt this active conformation even in the absence of actin filaments (5). However, actin filaments are still required to activate the Arp2/3 complex, which led us to hypothesize that there may be a second set of activating conformational change in the Arp2/3 complex. During the actin polymerization, actin monomers become flattened when incorporated into the filaments, and this flattening causes all the subunits to align into one plane. Therefore, we hypothesize that actin filaments may cause flattening of Arp2 subunit and Arp3 subunit into a conformation that mimics a stable actin dimer, and that this conformational change is required for nucleation. No biochemical assays to test for the flattened conformation currently exist, so the main thrust of this work was to design and produce mutant Arp2/3 complexes that could be used to test our hypothesis. Here we describe that process, and also show our preliminary data using the mutant complexes.

Figure IV. A cartoon model of the proposed conformational pathway to the activation of the Arp2/3 complex. In the inactive conformation, the Arp2 and Arp3 subunits are touching each other in an end-to-end fashion. Upon activation, actin monomers and WASP cause the



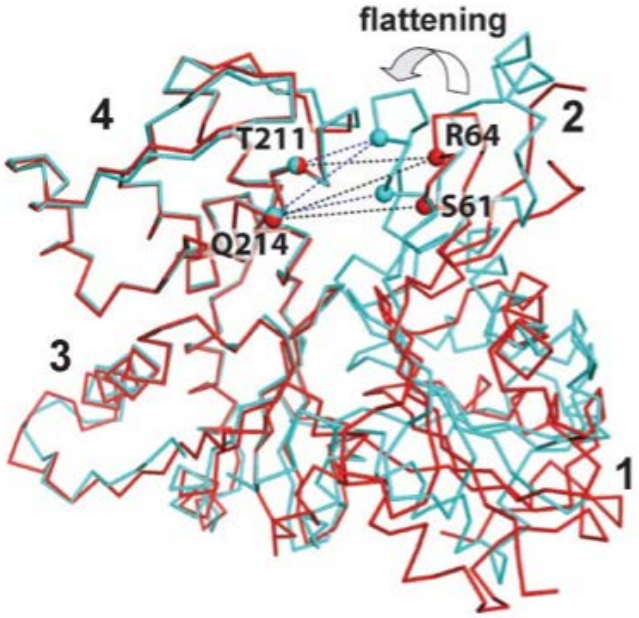
Arp2 subunit to move towards the rest of the complex. The active Arp2/3 complex at the branch junction is thought to structurally mimic an actin dimer and adopt the flattened conformation to template filament formation.

Methods

This study seeks to develop a biochemical assay to probe the flattening of the Arp2/3 complex. We have designed a crosslinking assay with *S. cerevisiae* Arp2/3 complex that contains a double cysteine mutation in the same subunit located at the nucleotide binding cleft (Figure V). Based on the active structure of actin monomers, we have carefully selected and designed five cysteine pairs that are on opposite sides of the nucleotide binding cleft, which are listed in Table I. Bis-maleimidoethane (BMOE) is a short crosslinker that can crosslink two cysteine residues that are approximately 8 Angstroms apart, and it can crosslink these selected cysteine residues only when the Arp2/3 complex is in the flattened conformation. In the inactive conformation, these cysteine residues are located further than 8 Angstroms, and BMOE will not be able to crosslink them (Figure V).

Table I. Mutations of Arp2/3 Complex

Arp2	Arp3
R64C/T211C	V103C/N294C
R64C/Q214C	L102C/K261C
S61C/Q214C	



	twisted dist. (Å)	flattened dist. (Å)
R64/T211	13.1	8.1
R64/Q214	16.4	12.1
S61/Q214	14.1	10.4

Figure V. Mutations in Arp2 subunit of the Arp2/3 Complex and the expected changes in the distance between these residues in the inactive conformation and the active conformation.

Since the Arp2 and Arp3 subunits are required for yeast viability (14), the general strategy was to introduce point mutations into the genes for Arp2 and Arp3 subunits in the context of an expression plasmid. These plasmids were then transformed into the yeast harboring a second copy of that subunit on another plasmid that could later be removed by a plasmid shuffle with 5-fluoroorotic acid (5-FOA) selection (1). After selecting for the strains that contain the mutant Arp2/3 complex, the final Arp2/3 complex protein with double cysteine mutations were harvested from the yeast. The overall strategy involves mutagenesis, transformation, plasmid shuffle, growing and harvesting, and purification of the mutant Arp2/3 complex from *S. cerevisiae* (Figure VI).

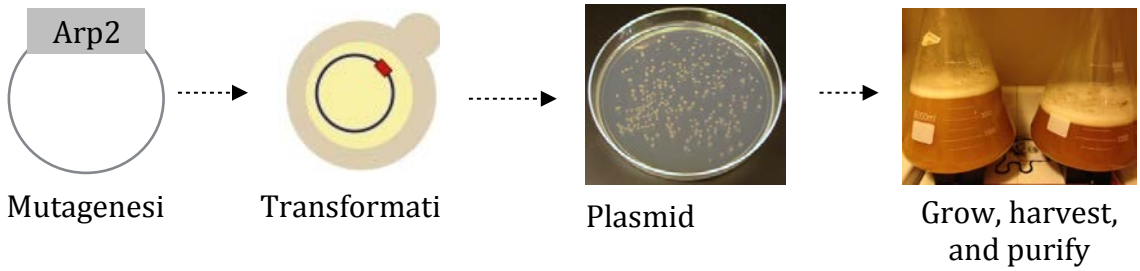


Figure VI. The overall scheme of strategies used in this project. We used site-directed mutagenesis to introduce point mutations into the Arp2 and Arp3 genes. We transformed these plasmids into the yeast strain that has a second copy of the Arp2 and Arp3 genes on another plasmid. Then, we used plasmid shuffle to remove the wild-type Arp2 and Arp3 genes. We then grew and harvested the mutant Arp2/3 complex and purified with gel filtration column.

Site-directed mutagenesis is an in-vitro method to create specific mutations using polymerase chain reaction (PCR) (13). Mutations introduced by PCR can only be incorporated into regions of sequence complementary to the primers. The primers were designed with substitutions that code for cysteine at these specific residues listed in Table I. Denaturation at 95 degrees activates the DNA polymerase and the double stranded DNA melts open to single stranded DNA. Annealing at 55-65 degrees allows the designed primers to bind to the DNA template and extension at 72 degrees allows the new DNA strand to be synthesized. These steps were repeated 28 to 30 times to make multiple copies of DNA with double cysteine mutations. Before transforming these plasmids into the yeast genome, they were sequenced to confirm that point mutations were successfully created during mutagenesis.

Transformation using the Arp2 knockout strain and the Arp3 knockout strain of *S. cerevisiae* allows these mutant plasmids to be introduced into yeast and causes the expression of the mutant Arp2 and Arp3 subunits. These knockout strains have the URA3 marker inserted adjacent to the wild-type Arp2 or Arp3 genes that will be later used for negative selection. These mutant plasmids were first transformed into the yeast genome using lithium acetate to express the mutant Arp2 subunit and the mutant Arp3 subunit in *S. cerevisiae*. Then, plasmid shuffling with 5-fluoroorotic acid (5-FOA) was used to select for cells that were successfully transformed. Although 5-FOA is usually non-toxic to yeast, yeast strains expressing the functional URA3 gene converts 5-FOA to the toxic form, 5-flurouracil, and causes cell death (1). Since the wild-type Arp2/3 gene has the functional URA3 gene, only the mutant Arp2/3 genes will survive and grow on the 5-FOA plates.

After confirming the DNA sequences of the yeast cells that survived on the 5-FOA plates, these mutants were harvested with yeast extract peptone dextrose or YPD media, which is a rich medium for yeast growth. The YPD media contain all the amino acids required for yeast growth, so it is commonly used as the growth medium to grow yeast cultures (13). These mutant Arp2/3 complex were purified using a protocol developed in the Nolen lab which involves both an affinity column and a gel filtration column to separate Arp2/3 complex from other yeast proteins (13). First, yeast pellets were lysed using sonication and the proteins were separated from the mixture by high-speed centrifugation. Then, adding ammonium sulphate and overnight dialysis allowed precipitation of the proteins, and the

affinity column step was performed using the GST-VCA column to only elute the Arp2/3 complex protein. The SDS-page analysis was performed with coomassie staining to visualize the Arp2/3 complex. The Arp2/3 complex was further purified using SOURCE15Q ion exchange chromatography and Superdex 200 gel filtration chromatography. The SDS-page analysis was performed again as the last step to confirm the final product was the pure Arp2/3 complex, and the final concentration was measured with a spectrophotometer, UV lamp, and quartz cuvette.

To ensure these mutations did not disrupt their normal function and activity, the pyrene actin polymerization assay was performed with these Arp2/3 mutants (13). The actin polymerization assay is based on the enhanced fluorescence of pyrene-labeled actin monomers that occurs during polymerization and it monitors and records the fluorescence of pyrene-labeled actin over time. The polymerization is initiated once pyrene-labeled actin monomers are added to the mixture of Arp2/3 complex, WASP, and ATP, and the fluorescence was measured with a fluorescence spectrophotometer. The fluorescence increases as actin monomers are incorporated into the actin filament, and levels off when actin polymerization reaches the steady-state phase. The results will be analyzed by comparing the kinetics of the mutants to the kinetics of the wild-type Arp2/3 complex since our goal is to find mutants that show similar activity as the wild-type Arp2/3 complex.

One of the challenges of studying proteins and their 3-dimensional structures stems from the fact that proteins are not static and subunit interactions

are often transient and occurs only briefly. Therefore, capturing these momentary contacts to study how they interact is one of the main goals of biochemical research, and crosslinking reagents provide the means for capturing these interactions by covalently binding them together as they interact. The crosslinking assay is a powerful tool for probing conformational changes of a protein, and involves mixing the protein of interest with a crosslinker and an activator for one minute and then stopping the crosslinking reaction with a quenching agent. Crosslinking reagents will covalently link together interacting domains by forming chemical bonds between specific amino acid functional groups that are in close proximity. For this project, the Arp2/3 complexes with double cysteine mutations were used with bis-maleimidoethane (BMOE) and WASP and the crosslinking reaction will be quenched with dithiothreitol (DTT). BMOE will crosslink these selected cysteine residues only when the Arp2 subunit and Arp3 subunit are approximately 8 Angstroms apart. Since intramolecular crosslinking increases the mobility of the subunits, it is expected the mutants that crosslink with bis-maleimidoethane (BMOE) will travel faster on the sodium dodecyl sulfate polyacrylamide gel (SDS-page gel), and a lower molecular band will be shown on the western blot. The western blot uses gel electrophoresis to separate denatured proteins by the length of the polypeptides, and the proteins are transferred to a membrane so they can be stained with antibodies specific to the target protein. For the Arp2 mutants, the antibody specific to Arp2 subunit was used while the antibody specific to Arp3 subunit was used for the Arp3 mutants.

Results

The first objective of this project was to create plasmids and strains of *S. cerevisiae* that contain double cysteine mutations. The following table summarizes the all of the plasmids and strains that were made for this project using polymerase chain reaction (PCR) primers.

Table II. Plasmids and Strains of *S. cerevisiae*

Genotypes	Plasmids	Yeast Strains
R64C/T211C	pMR045	ScMR045
R64C/Q214C	pMR046	ScMR046
S61C/Q214C	pMR047	ScMR047
V103C/N294C	pMR048	ScMR048
L102C/K261C	pMR049	ScMR049

Subsequently, these Arp2/3 complex mutants were grown and harvested in 8 L of YPD media and purified with gel filtration column. Figure IV shows the coomassie gel from the final protein purification step of R64C/Q214C mutant, and a pure Arp2/3 complex protein was seen as indicated by seven bands each representing one subunit of the Arp2/3 complex. There was no evidence of degradation in these preps and they behaved normally on both the GST-VCA column and the SOURCE15Q column that were used to purify them.

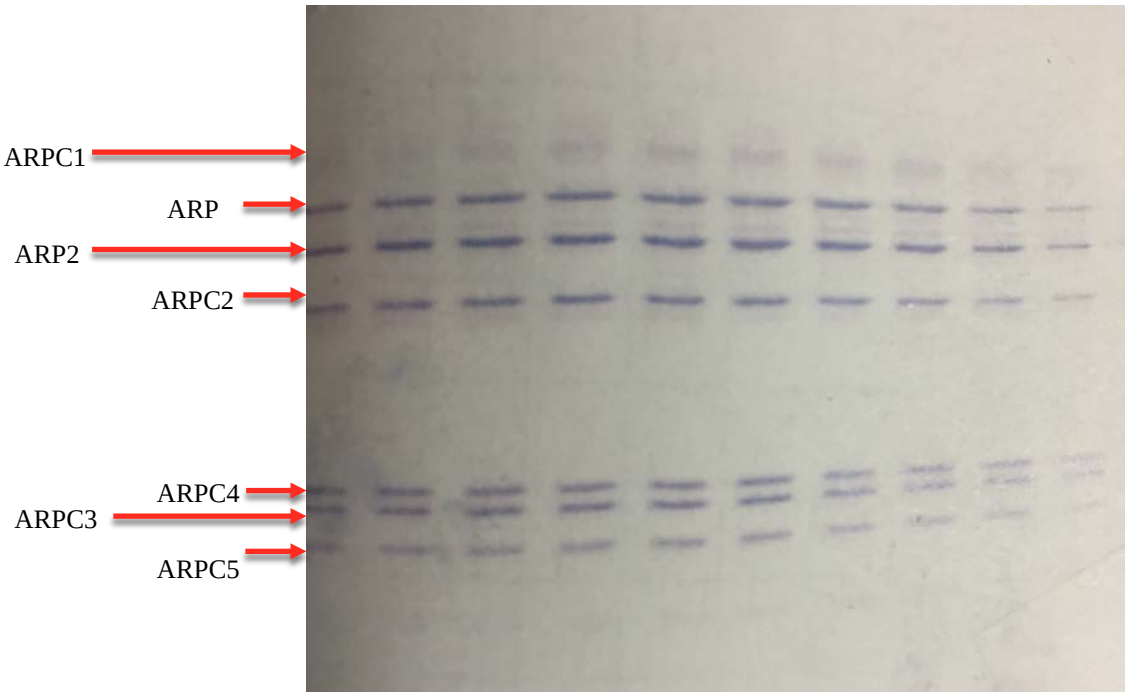


Figure VII. The coomassie gel of R64C/Q214C mutant from the final protein purification step. Each band represents a subunit of the Arp2/3 complex, indicated by different molecular weights. Each lane represents SDS-PAGE analysis of fractions from the SOURCE15Q column.

After all five constructs were purified, the pyrene assay was performed with these mutants to make sure introducing these substitution mutations did not disrupt their normal activity. Out of these five mutants, only R64C/Q214C mutant and V103C/N294C mutant showed similar activities as the wild-type Arp2/3 complex in pyrene assay, which are shown in Figure V. The nucleation activity of Arp2/3 complex is activated by members of the Wiskott-Aldrich syndrome family protein (WASP), and adding WASP enhanced the rate of actin polymerization when the wild-type Arp2/3 complex, R64C/Q214C mutant, and V103C/N294C mutant were present.

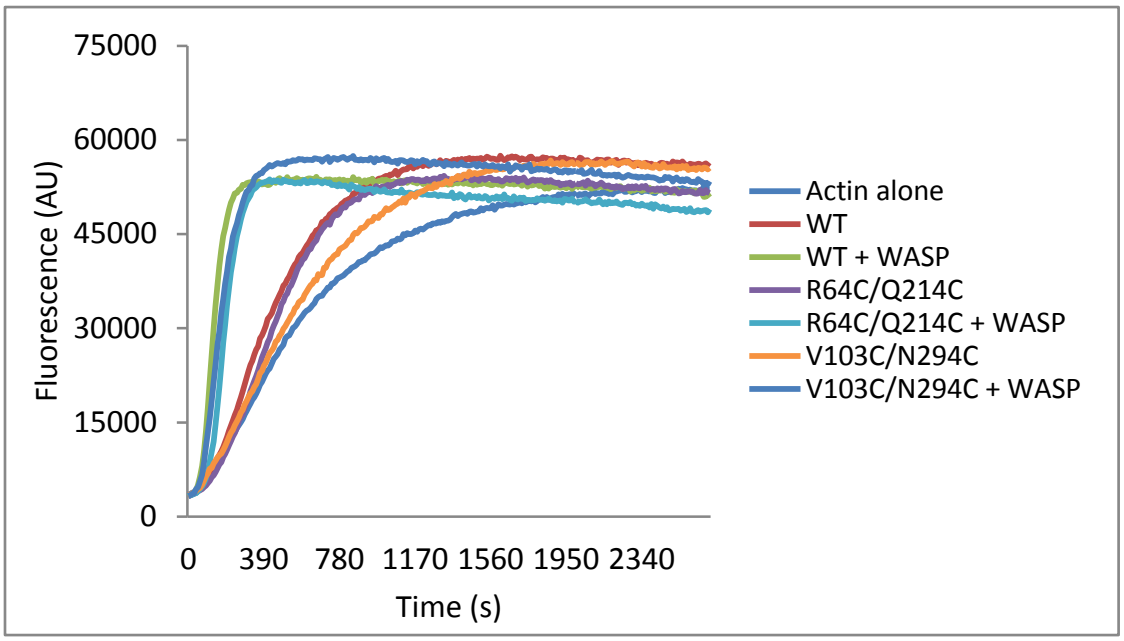
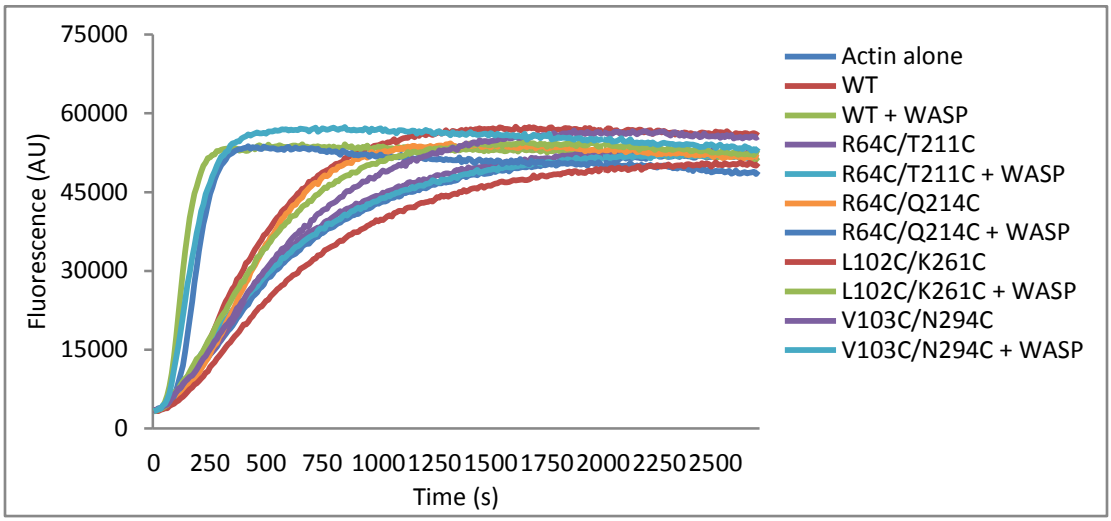


Figure VIII. Pyrene actin polymerization assay with R64C/Q214C mutant and V103C/N294C mutant. The wild-type Arp2/3 complex was used as the control. These mutants showed similar activities as the wild-type and adding WASp enhanced the rate



of actin polymerization.

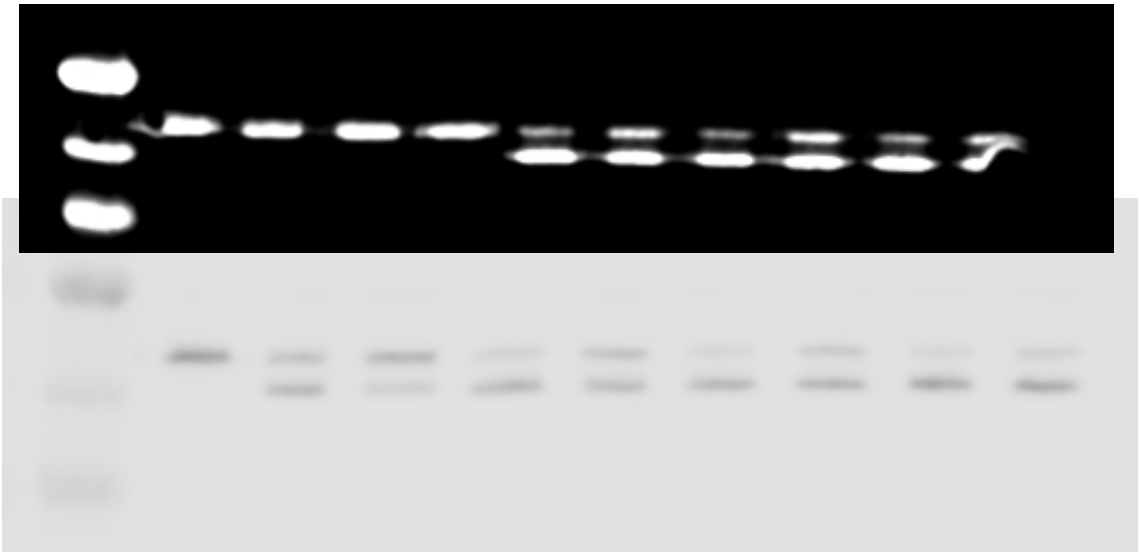
Figure IX. Pyrene actin polymerization assay with four flattening mutants of the Arp2/3 complex. The wild-type Arp2/3 complex was used as the control and only R64C/Q214C mutant and V103C/N294C mutant showed similar activities as the wild-type Arp2/3 complex. Other mutants showed similar baseline activity as the wild-type Arp2/3 complex, but adding WASp did not enhance their polymerization rate.

Subsequently, the R64C/Q214C mutant was used in the crosslinking reaction to test if BMOE can crosslink these two cysteine amino acids lining the nucleotide binding cleft. Since the wild-type Arp2/3 complex lacks cysteine in these residues, it was used as the negative control. The first lane contains the protein ladder that is used to estimate the size of proteins resolved by gel electrophoresis. As seen in the lanes 2-4 of the western blot in Figure VI, BMOE did not crosslink the wild-type Arp2/3 complex, and only one molecular weight band is present. However, BMOE successfully crosslinked the R64C/Q214C mutant regardless of the presence of absence of adenosine triphosphate (ATP). Although the presence of ATP was expected to increase the rate of crosslinking, the top band was more fainted when ATP was absent compared to the top band when ATP was present, indicating that the fraction of uncrosslinked Arp2/3 complex is higher when ATP was present.

	WT			R64C/Q214C						
BMOE	-	+	+	-	+	+	+	+	+	+
ATP	-	-	+	-	-	+	-	+	-	+

Figure X. The western blot showing the result of crosslinking R64C/Q214C mutant with BMOE. The wild-type Arp2/3 complex was used as the negative control, which are shown in the lanes 2-4 of this western blot. The fifth lane shows another negative control with the R64C/Q214C mutant and no BMOE. The sixth lane contains the R64C/Q214C mutant and BMOE while the seventh lane contains the R64C/Q214C mutant with BMOE and ATP. The reaction conditions in the lanes 6 and 7 were repeated in the lanes 8-11 to make sure this crosslinking was replicable.

To further quantify the kinetics of crosslinking and the effects of ATP on crosslinking reaction, the reaction was stopped at every 15 seconds until it reached one minute. As seen in Figure VIII, the crosslinking reaction was 50% completed

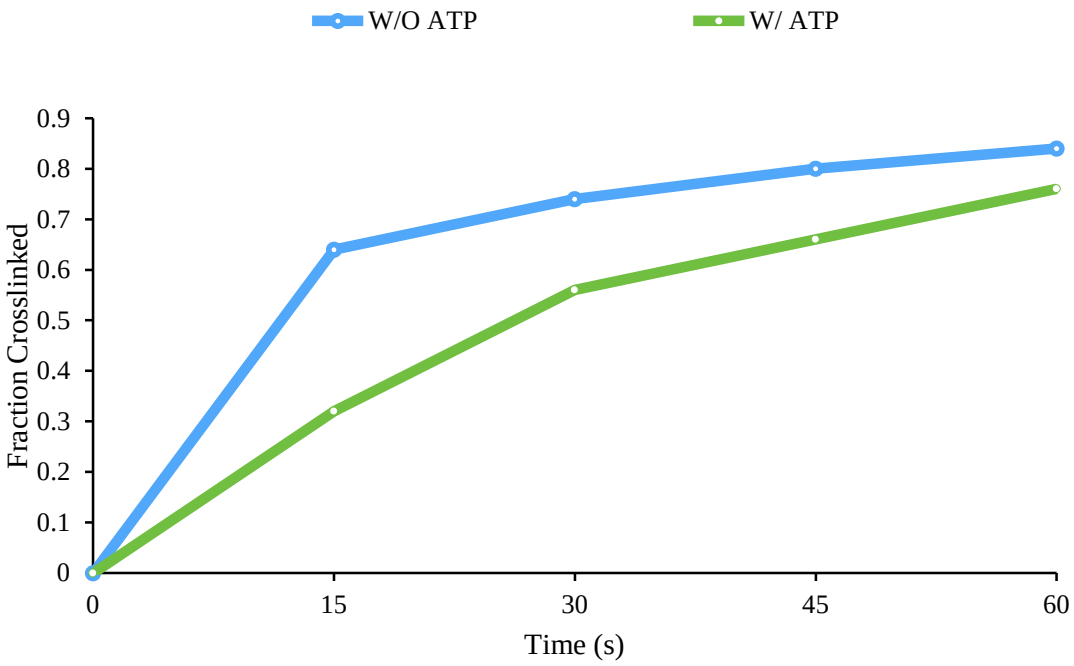


approximately at 25 seconds when ATP was present and approximately at 10 seconds without ATP. At one minute, the crosslinking reaction was 84% completed when ATP was absent and 76% completed when ATP was present. Although the presence of ATP was expected to increase the crosslinking rate, the opposite was observed because the binding of ATP in the binding pocket of the Arp2/3 complex caused structural rigidity. However, when ATP is not available, ATP binding pocket is not in a locked conformation, which allows more flexibility in the Arp2/3 complex. Since most protein molecules are not static, but are in varying degrees of motion, there would be more chance of these two cysteine residues being closer to each other and readily available for crosslinking. Nonetheless, the present of ATP significantly decreased the crosslinking rate.

Time (s)	0	15	15	30	30	45	45	60	60

Figure XI. The western blot showing the result of varying reaction time and ATP on crosslinking. The first lane is showing the protein ladder while the second lane is showing the R64C/Q214C mutant before crosslinking. The crosslinking reaction was stopped at every 15 seconds until it reached one minute. The lane 3 shows the result of crosslinking R64C/Q214C mutant with BMOE for 15 seconds when ATP was absent while the lane 4 shows the result of crosslinking R64C/Q214C mutant with BMOE for 15 seconds when ATP was present. The lane 5 and 6 are showing the result of crosslinking the mutant with BMOE for 30 seconds without and with ATP, respectively. The lanes 7 and 8 are showing the result of stopping the crosslinking reaction after 45 seconds without and with ATP, respectively. The lanes 9 and 10 are showing the result of crosslinking the mutant with BMOE for one minute without and with ATP, respectively.

Figure XII. The graph showing the fraction of the R64C/Q214C mutant that are crosslinked as the reaction time increased. Half of the R64C/Q214C mutant were



crosslinked approximately at 10 seconds when ATP was not present and at 25 seconds when ATP was present.

Table III. The fraction of R64C/Q214C mutant that are crosslinked with BMOE with and without ATP. At one minute of crosslinking, approximately 84% of the R64C/Q214C mutant were crosslinked without ATP whereas 76% of the R64C/Q214C mutant were crosslinked with ATP.

Time (s)	Crosslinking Without ATP	Crosslinking With ATP
0	0.00	0.00
15	0.64	0.32
30	0.74	0.56
45	0.80	0.66
60	0.84	0.76

One of the main goals for this project was to find a set of crosslinker and double cysteine mutants in which crosslinking will only occur if subunit flattening occurs. This study confirmed that introducing R64C/Q214C mutations in the Arp2 subunit and V103C/N294C mutations in the Arp3 subunit did not disrupt the normal function and activity of the Arp2/3 complex. Also, BMOE successfully crosslinked the R64C/Q214C mutant. However, this study only shows the preliminary data and further studies are needed to determine the effects of actin filaments on the crosslinking rate, which wold reveal the role of actin filaments in the activation pathway of the Arp2/3 complex.

Future Direction

These preliminary data suggest that there is a possibility of using the R64C/Q214C mutant to probe the formation of flattened conformation of the Arp2/3 complex. Since only two out of five potential mutants were active, a second attempt to purify these inactive mutants should be made in case they could be useful in probing the flattened conformation of the Arp2/3 complex. Although only BMOE was used as the crosslinker in this study, Eisenberg et. al. (2013) used MTS crosslinkers with actin, and showed successful crosslinking. Therefore, other crosslinkers with varying distances could be tested to determine the most reactive crosslinker with these mutants. Also, different reaction conditions could be tested that would optimize the crosslinking such as decreasing the amount of ATP added to the crosslinking reaction. Nonetheless, we have made a revolutionary step in developing a biochemical assay for probing the active conformation of the Arp2/3 complex.

Glossary

Actin: a globular multi-functional protein found in most eukaryotic cells
Adenosine triphosphate (ATP): the energy currency of life
Alzheimer's disease: a type of dementia that causes problem with memory, thinking, and behavior
Angstrom: a unit of length equal to 0.1 nanometer
Anneal: recombine the DNA in the double-stranded form
Bis-maleimidoethane: a short-arm, maleimid crosslinker for covalent, irreversible conjugation between sulfhydryl groups
Capping protein: a protein which binds in a calcium-independent manner to the fast-growing ends of actin filaments
Cell migration: a process in the development and maintenance of multicellular organisms which involves the translation of cells from one location to another
Chemotactic: the movement of a microorganism or cell in response to a chemical stimulus
Chemotherapy: the use of medicines or drugs to treat cancer
Conformational change: a change in the shape of a macromolecule often induced by environmental factors
Contractile apparatus: a force-generating apparatus composed of many contractile units called sarcomeres
Crosslinking: the process of chemically joining two or more molecules by a covalent bond
Cysteine: a sulfur-containing amino acid encoded by the codons UGU and UGC.
Cytokinesis: the physical process of cell division, which divides the cytoplasm of a parental cell into two daughter cells
Cytoskeleton: made up of microtubules, actin filaments, and intermediate filaments, which give the cell its shape and help organize the cells' parts
Denaturation: a process in which proteins or nucleic acids lose the quaternary, tertiary, and secondary structures, which is present in their native state
De novo: starting from the beginning
Dimer: a molecule or molecular complex consisting of two identical molecules linked together
Endocytosis: the process of capturing a substance or particle from outside the cell by engulfing it with the cell membrane and bringing it into the cell
Endothelial cells: a thin layer of simple squamous cells lining the interior surface of blood vessels and lymphatic vessels
Epithelial cells: cells lining the cavities and surfaces of blood vessels and organs throughout the body
Eukaryote: an organism with a nucleus and other organelles enclosed within membranes
Formins: a group of proteins that are involved in the polymerization of actin
Gel electrophoresis: a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size
Gel filtration chromatography: separates proteins, peptides, and oligonucleotides on the basis of size

Immune cells: the cells of the immune system that are involved in protecting the body against infectious disease and foreign invaders
In vitro: a process performed in a test tube, culture dish, or outside a living organism
Lamellipodium: a cytoskeletal protein actin projection on the leading edge of the cell
Membrane trafficking: the process by which proteins and macromolecules are distributed throughout the cell and released to or internalized from the extracellular space.
Metastasis: the spread of a cancer cell from one part of the body to another that is not directly connected with it
Microfilament: the thinnest filaments of the cytoskeleton, which is a structure found in the cytoplasm of eukaryotic cells
Microtubules: filamentous intracellular structures that are responsible for movements in all eukaryotic cells
Monomer: a molecule that binds to other molecules to form a polymer
Morphogenesis: the biological process that causes an organism to develop its shape
Mutagenesis: a process by which the genetic information of an organism is changed in a stable manner, resulting in a mutation
Myosin: a large motor proteins that move along actin filaments via ATP hydrolysis
Neuron: an electrically excitable cell that possesses and transmits information through electrical and chemical signals
Neuropathology: the study of disease of nervous system tissue
Neurotoxicity: damage to the brain or peripheral nervous system caused by exposure to natural or man-made toxic substances
Neutrophil: the most abundant type of white blood cells in most mammals and form an essential part of the innate immune system
Phagocytosis: the process by which a cell engulfs a solid particle to form an internal vesicle known as a phagosome
Plasmid: a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA
Polymerase chain reaction (PCR): a process in molecular biology to amplify a single copy of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence
Polymerization: a process of reacting monomer molecules together in a chemical reaction to form polymer chains or three-dimensional networks
Polypeptides: chains of amino acids. Proteins are made up of one or more polypeptide molecules
Primer: a strand of short nucleic acid sequences that serves as a starting point for DNA synthesis
Pyrene assay: a biochemical tool to measure the enhanced fluorescence that occurs during polymerization
Quenching: a process that decreases the fluorescence intensity of a given substance
Saccharomyces cerevisiae: a species of yeast. Also known as Brewer's yeast
Selective media: formulated to support the growth of group of organisms while inhibiting the growth of another

Sodium dodecyl sulfate polyacrylamide gel (SDS-page gel): a detergent that dissociates and unfolds oligomeric proteins into its subunits. Useful for molecular weight analysis of proteins
Tau: proteins that stabilize microtubules
Thin filament: one of the contractile elements in muscular fibers
Vesicle: a small structure within a cell consisting of fluid enclosed by a lipid bilayer
Western blot: analytical technique used to detect specific proteins in a sample of tissue homogenate or extract
Wild-type: a strain, gene, or characteristic that prevails among individuals in natural conditions
Wiskott-Aldrich syndrome protein (WASp): the founding member of a gene family which also includes N-WASP, and Scar. This protein is expressed in cells of the hematopoietic system and activates the Arp2/3 complex
YPD media: a medium for yeast growth consisting of yeast extract peptone dextrose

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