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„Development of a structural and mathematical model
of actin tails propelling pathogens “

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Abstract

Deutsch

Das Ziel dieser Masterarbeit war es sowohl eines mathematischen als auch eines strukturellen Modell zu entwickeln, mit Hilfe derer die auf Aktin basierende Fortbewegung von Pathogenen beschrieben werden kann. Mit einer 2 dimensional quasi-statischen Simulation versuchte ich die intrazelluläre Fortbewegung des Baculoviruses und die strukturellen Eigenschaften des Aktinschweifs zu imitieren. Mehrere Kräfte waren in meinem Modell inkludiert: die cytoplasmatische Reibung, die Brownsche Bewegung und die von den Aktinfilamenten ausgeübten Kräfte. Es wurden drei Varianten des Modells mit unterschiedlichen Formen des Zusammenhalts zwischen dem Aktinnetzwerk und dem Pathogen getestet. Deren Analyse deutete darauf hin, dass eine konstante Verbindung zwischen den Plusenden der Filamente und der Virusoberfläche notwendig ist, um die experimentellen Daten zu reproduzieren. Zudem wurde gezeigt, dass gerichtetes Verzweigen der Filamente zum Virus hin erforderlich ist, um das typische Fischgrätenmuster der Aktinfilamente zu imitieren. Die Modelannahmen waren ausreichend, um die Fortbewegung eines hypothetischen 10mal größeren Virus zu stabilisieren. Dieses Erkenntnis unterstützt die These eines gemeinsamen Mechanismus zur Aktin basierten Fortbewegung.

Elektronentomographie erzielte detaillierte strukturelle Erkenntnisse über den Aufbau des Kometenschweifs des Vaccinia Virus. Es gelang mir, als erste die Verzweigungen des Aktinnetzwerkes zu visualisieren, jedoch war die erzielte Auflösung nicht hoch genug um den exakten Verlauf der einzelnen Filamente innerhalb des Schweifs zu verfolgen.

English

This master thesis aims to develop a mathematical and structural model of actin based pathogen propulsion. Using a quasi-static approach in a two-dimensional model, I sought to mimic the intracellular movement of the baculovirus and the structural features of its comet tails. Several forces acting on the virus were assumed: cytoplasmic friction, Brownian motion, and the forces exerted by the actin filaments. Three model versions with different degrees of tethering the actin network to the pathogen were tested and their analysis suggested that constant tethering of the barbed filament ends to the virus surface is required to match the experimental data. Furthermore, it could be shown that directed filament branching towards the virus is necessary for reproducing the typical fish-bone like structure of the comet tail. The model assumptions were also sufficient to stabilize the movement of a hypothetical virus with 10 times larger dimensions, supporting the idea of a common underlying mechanism for actin based propulsion.

Electron tomography yielded detailed structural insights into the architecture of comet tails induced by vaccinia virus. For the first time, I could visualize branch junctions in the actin network of this pox virus, yet unfortunately the obtained resolution was not sufficient to track the filaments within the tail.

Introduction

Actin

Actin is a globular, highly conserved protein approximately 42kDa in size with the ability to form helical filaments.¹ It occurs at intracellular concentrations of up to several hundred micromolar.^{2,3} Actin is tightly connected to motility, since on one hand it is dominant in muscle cells, where actin together with the motor protein myosin facilitates muscle contraction.⁴ On the other hand, actin is one of the three main components of the eukaryotic cytoskeleton where it plays a major role in motile processes, such as cell motility, cellular protrusion, cell division, endocytosis and organelle transportation.⁵ The immune response⁶ relies on the actin-dependent mobilization of leukocytes and cells also use the actin system as a tension sensor in a cross-talk with their environment.⁷

Since the Hungarian scientist Brúno Straub was able to purify actin from muscles in 1942⁸ a long series of successful experiments has gained elaborated insights into the features and functions of this important molecule.

The protein survives acetone drying, which allows efficient and fast purification. Ensuing biochemical experiments revealed details of the polymerisation dynamics. Monomeric actin (also called G-actin) associates with other monomers to form a helical double stranded filament (F-actin) with a diameter of $\sim 7\text{nm}$ and a periodicity of 36nm ¹ (see Figure 1). The protein has an intrinsic ATPase function, thus it exists in an ATP and an ADP bound state with slightly different steric configurations and binding affinities. As a consequence an interesting dynamical phenomenon called “treadmilling” can be observed.⁹ ATP bound monomers are incorporated into one end of the filament (typically referred to plus or barbed end) followed by the hydrolysis of the bound nucleotide. Hence, the affinity between monomers decreases and depolymerisation occurs more likely at one end of the filament (referred to as minus or pointed end). Therefore actin filaments can polymerise at one end and depolymerise at the other simultaneously. At

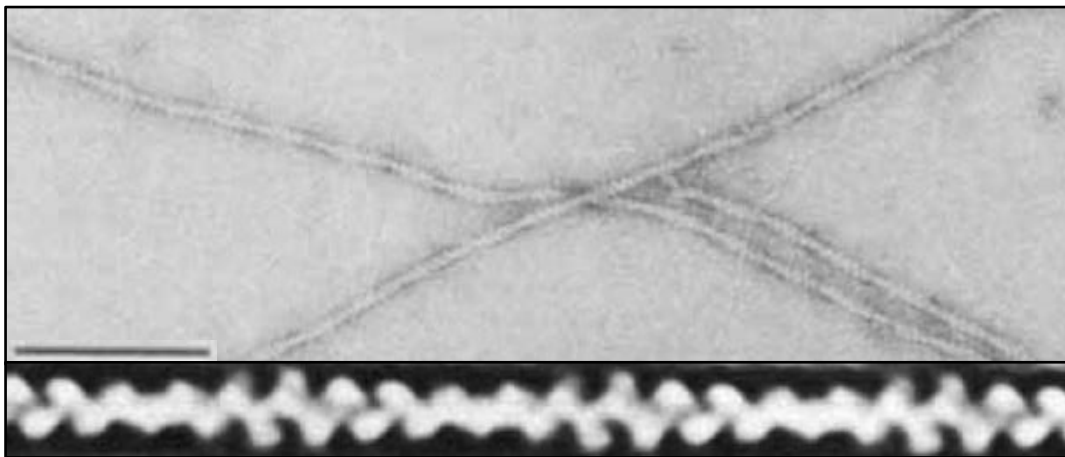


Figure 1: Electron Micrograph of negatively stained f-actin. Lower section shows the filtered structure of the helical actin filament. Bar: 100nm

steady state *in vitro* the G-actin and F-actin concentrations stay constant and polymerisation at the barbed end is matched by depolymerisation at the minus end, resulting in filaments with constant length¹⁰. In contrast to filament elongation, the nucleation is a thermodynamically unflavoured process, especially the assembly of the first three monomers.¹¹ This process can be catalysed by actin binding proteins.

Regulatory proteins

The properties and dynamics of actin are strongly influenced by a variety of regulating proteins. Their well-orchestrated interplay allows the occurrence of diverse processes at different locations within the cell. Giving a short overview of the distinct interaction partners and their purpose might be helpful to understand the dynamics present in my model system.

Firstly, there is a class of proteins promoting the formation of actin filament. As mentioned before, the limiting step in this process is the assembly of the first three monomers. Hence catalysing the formation of new filaments by accelerating the unfavourable step of seed formation is the common concept. Up to now there are three different mechanisms known, which are depicted in Figure 2.

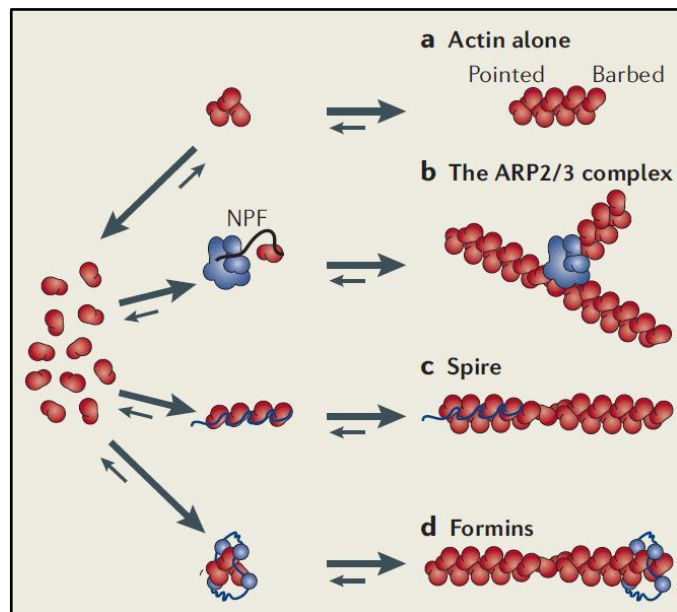


Figure 2: Schematic representation of four different mechanisms to initiate actin filament formation.

The most recent discovery is a group of **Spire** proteins first found in *Drosophila*¹². Their main feature is the actin binding domain WH2 which occurs four times in a row, facilitating the binding of four actin monomers. In this way polymerisation is launched and Spire remains at the pointed end preventing depolymerisation.

Alternatively, there is another group of proteins called the **formins**.¹³ Formin dimers are auto-inhibited in the cytosol and activated by recruitment to the plasma membrane. The doughnut-shaped formin homology 2 (FH2) domain will then promote filament nucleation. In opposition to Spire, formins remain processively associated to the barbed end and string-like extension containing a FH1 domain accelerate elongation by recruiting and incorporating profilin–actin complexes to the growing plus end. Formins have many

different binding partners including microtubules.¹⁴ Hence, formins also are engaged in kinetochore formation¹⁵ and steering cell movement by coupling actin polymerisation and microtubule stabilization.

The third mechanism is realised by a multi protein complex named **Arp2/3** complex. The name refers to two actin related proteins (Arp) 2 and 3, which share sequential and structural homology with G-actin and together with five additional subunits they form the heptameric complex.¹⁶ In contrast to Spire and formins, the Arp2/3 complex does not produce new independent filaments, but it rather forms daughter filaments, which branch from pre-existing mother filaments. The heptameric complex is incorporated in this branch and defines the fixed branching angle of approximately 74°.

In order to induce branch formation Arp2/3 needs to be activated by a nucleation-promoting factor (NPF). Members of the WASP-family (Wiskott-Aldrich Syndrome protein) are prime examples of such Arp2/3 activators, acting downstream of Cdc42¹⁷ and Rac.¹⁸ Their essential feature is a VCA domain regulated by auto-inhibition. Once the NPF is active it releases the VCA domain with its three distinct entities, the V (verprolin) motif, the C (connecting or central hydrophobic region) motif and the A (acidic) motif. The A region as well as the C region bind the Arp2/3 complex, thereby recruit it to distinct places inside the cell.¹⁹ Once it is there the Arp2/3 catalyses the unfavourable actin nucleation using its actin homologs as primer. The V domain of the NPF can bind G-actin and thereby contribute an actin monomer, which completes the nucleating trimer and facilitates elongation.²⁰ Arp2 and Arp3 have the same ATPase function as actin and once the bound nucleotide is hydrolysed and the surrounding actin depolymerises Arp2/3 complex is released and becomes available for recycling.²¹

Profilin has been linked to many aspect of cellular functions including proliferation, survival, motility, endocytosis and membrane trafficking, mRNA splicing and gene transcription.²² This actin binding protein can stimulate nucleotide exchange (ADP to ATP) on G-actin, which is an essential process in actin pool regeneration. While profilin stays attached to F-actin, ATPase activity is restricted and thus filament stability improves. The dissociation of the actin-profilin complex is mediated by PIP₂.²³ Due to the recycling GEF-like function profilin also is indispensable for all *in vitro* assays of actin filament dynamics.

ADF (actin depolymerizing factor)/ **cofilin** are also able to bind actin and thereby regulate actin dynamics. As the name suggests, ADF/cofilin can fragment and depolymerize filamentous actin. But degradation is by far not the only function. If the ratio of cofilin to actin is high enough, filaments become stabilized in a bent conformation. Additionally, cofilin can also promote actin polymerisation by severing and thereby provide new barbed ends.²⁴ Apart from regulating actin dynamics, cofilin is required for the nuclear import of actin.²⁵

Ena/Mena/VASP (vasodilator stimulated protein) proteins co-localize with various actin structures such as the tip of filopodia and lamellipodia, stress fibers²⁶ or focal adhesions.²⁷ Common features of this protein family are actin binding domains (EVH2) as well as profilin binding domains (PRO-region), suggesting two different mechanisms for promoting actin elongation. Remarkably, actin elongation in lamellipodia is solely dependent on the EVH2 domain, whereas the motility of *Listeria monocytogenes* requires only profilin-actin delivery by the PRO-domain. VASP is known to form tetramers, featuring four actin binding sides that can co-operate and under certain circumstances to facilitate processive elongation of actin filaments.²⁸

Capping Proteins (CP) form hetero dimers, which bind and cap the barbed ends of actin filaments. This capping process prevents further polymerisation and thereby limits the length of actin filaments.²⁹ Capping proteins are also essential for *in vitro* motility assays, since they limit G-actin incorporation to sites of high nucleation and elongations activity.³⁰

Bundling and cross linking proteins have minor impact on the actin dynamics but they rather influence the structural organization of the network. **Fascin** is a 55kDa large protein with two F-actin binding sides, capable of coupling of two parallel actin filaments. This bundling protein regulates finger-like actin protrusion as well as the stability of adhesion sites.^{31,32} Cross linking proteins can either interconnect actin filaments or link actin to other structures.

Actin structures

Actin can be found in many different places in the cell and under the influence of over 70 distinct classes of actin binding proteins it forms various structures.³³

Moreover, certain pathogens can invade cells and generate additional structures in the form of comet tails of actin to move in cytoplasm. Before discussing pathogens, I will first highlight one cellular structure, namely the lamellipodium, since it exhibits a function, structure and composition comparable to the actin comet tails formed behind pathogens.

Abercrombie *et al.* were the first to described the protruding sheets at the leading edge of a motile cell and called them lamellipodia.³⁴ Lamellipodia are 100-160nm thick and densely packed with an Arp2/3 derived actin network.³⁵ WAVE activates the branching complex locally at the leading edge and incorporation of actin monomers exerts a pushing force, which protrudes the membrane at the front.³⁶ It is believed that the actin based motility of pathogens employs the same mechanism of force generation hence investigating the motility of baculovirus can provide useful information for cell motility.

Other actin structures in non-muscle cells include filopodia and stress fibres. Filopodia, which generally coexist with lamellipodia, are slender rod-like protrusion filled with parallel actin filaments bundled by fascin that may function in a moving cell to explore the surrounding and sense chemokine gradients.³⁷

Stress fibres are required for traction during cell motility. The protrusion of a thin layer of cytoplasm in the front of a lamellipodium is only the first step in cell translocation. Next, the cytoskeleton needs to attach to the extracellular matrix in order to exert the required forces for traction of the cell rear. This takes place in the lamella the second structure, which Abercrombie found adjacent to the lamellipodium.³⁴ This area is much thicker, contains a sparser, less branched actin network with actin stress fibres. Such anti-parallel actin bundles comprise myosin, which allows these stress fibres to contract.³⁸ Linked to focal adhesions, which are connected to the extra cellular matrix, these contractile bundles drag the cell body forward.



Figure 3: **A** shows an electron micrograph of a plastic cross section of a B16 cell lamellipodium Bar 250 nm **B** This electron micrograph of the anterior region of a B16 cell displays the different cellular actin structures that can be found in protruding cell. LP lamellipodium; Fp filopodia; Sf stress fibres; Lm lamella.

Motility of pathogens and assays

A major aim of any pathogen is to enter the host organism and take advantage of its resources. A common strategy adopted by one class of pathogens is to invade cells to exploit the cellular cytoskeleton. This specialized group of parasites, includes the gram positive *Listeria monocytogenes*, the gram negative *Shigella flexneri*, but also other bacteria like EPEC and *Mycobacterium marinum* as well as viruses such as the baculovirus and the Vaccinia virus, all of which abuse the actin machinery to improve their dissemination within the host tissue.³⁹ The schematic in Figure 4 shows some examples of how the pathogens hijack the cellular actin network. Actin is used for cell invasion as well as intra- and intercellular movement. Focusing on the induced actin tails in the cytoplasm, one can compare the structure of the different networks generated by pathogens (Figure 5),⁴⁰ *Shigella flexneri*, and vaccinia generate highly branched tails with relatively short filaments, whereas *Rickettsia* sp. forms a tail consisting of long parallel filaments. The example of a *Listeria monocytogenes* tail shown in Figure 6 displays also a splayed actin network but other studies showed that addition of Fascin to cytoplasmic extracts containing *Listeria monocytogenes* induce a hollow tail with parallel filaments.⁴¹ The transition between these two forms seems to be linked to a velocity change.

Remarkably, pathogens have found several ways to generate actin comet tails for propulsion.

Firstly, there is the vaccinia virus, which exploits a receptor tyrosine kinase signalling cascade. The viral receptor A36R is a transmembran protein with phosphorylation sites for cellular Src family kinases. Once phosphorylated the viral protein recruits the downstream components Nck and WIP, which in turn recruit N-WASP to the membrane. The active N-WASP then activates Arp2/3 complexes, which induce the formation of an comet tail.⁴²

Vaccinia relies therefore on the existence of the actin machinery as well as the signalling proteins.

Similar to vaccinia also the related WheelYaba-like disease virus and EPEC stimulate actin polymerisation through the very same pathway, which is likewise used by the Nephrin receptor during slit-diaphragm morphogenesis and maintenance by podocytes in the kidney.⁴³

Secondly, *Shigella flexneri* interacts more downstream. The bacterial protein IcsA is capable of activating WASP which then recruits the Arp2/3 complex.⁴⁴

Listeria monocytogenes and Baculovirus bypass the whole pathway, since they express on their surface homologs of the activated WASP protein, ActA and p78/83 respectively, which directly activate the branching com-

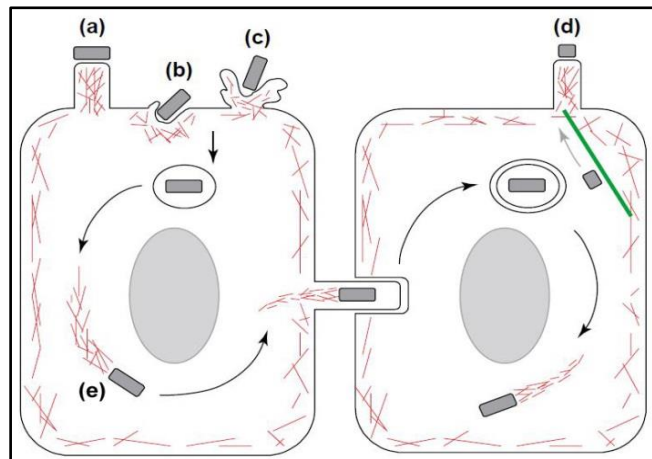


Figure 4: Actin based pathogen propulsion **a** EPEC the infectious type of *E. coli* induces an actin pedestal to surf along the surface and infect neighbouring cells. **b** *Listeria* and *Rickettsia* as well as **c** *Shigella flexneri* and *Burkholderia pseudomallei* induce rearranges in the actin cortex to facilitate invasion. Once they entered the host cell, they induce, likewise the baculovirus, actin tails propelling them through the cytoplasm **e**. Vaccinia induces its tail from the outside of the cell to reach the neighbouring cells **d**.

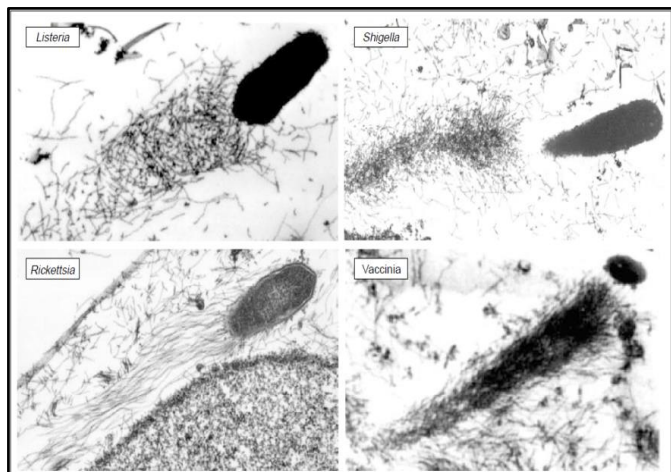


Figure 5: Electron microscopy of different actin tails decorated with myosin S1. *Shigella flexneri*, *Listeria* and *vaccinia* induce a branched network, whereas *Rickettsia* tails consist of long and unbranched filaments.

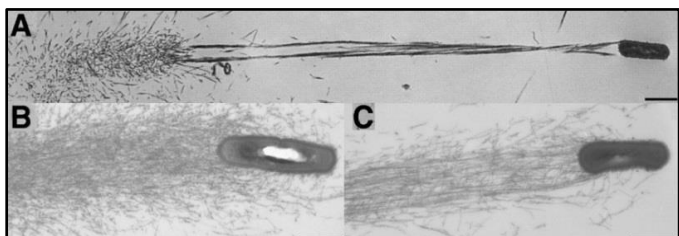


Figure 6: Thin-section electron micrographs: *Listeria* can induce two types of tail, either a dense branched(**B**, left side of **A**) network or a sparse tail of straight actin bundles (**C**, right side of **A**). Addition of fascin triggers the transformation shown in **A**. Scale bar in **A** 1µm in **B** and **C** 0,75µm.

plex Arp2/3 directly.^{40,45}

According to the complexity of actin tail initiation the pathogens can be used to investigate different aspects of actin based motility. The vaccinia virus system has served as a model to reveal a signalling pathway from a transmembrane protein to the actin machinery.^{46,47}

On other hand, *Listeria monocytogenes* has been usefully exploited to reconstitute the minimal set of proteins involved in actin based propulsion.³⁰ Loisel *et al.* proved with their *in vitro* experiments that five types of proteins are enough to produce sufficient force to propel a bacterium. Adding actin, Arp2/3, ADF, profilin and gelsolin to *Listeria monocytogenes* resulted in effective movement with comparable speed and persistence as observed *in vivo*. In follow-up experiments, this *in vitro* system was adapted to propel beads, vesicles, lipid droplets and rods. The results of these reductionist approaches stimulated the development of mathematical models of actin-based motility.

Baculovirus

The baculovirus, more precisely the Autographa californica nuclear polyhedrosis virus (AcMNPV) has evolved during the last few decades to a versatile tool in science. The most prominent member of the Baculoviridae family is widely used as a vector for protein expression in insect cells⁴⁸ but there are also attempts to use baculovirus for gene therapy.⁴⁹

Although the virus is able to invade a huge variety of cells including mammalian cells and induce the translation of its own genes, it can only replicate in the original host, namely Arthropods. The rod shaped virus with dimensions of 300nm length and a diameter of 50nm contains 80–180 kilo base-pairs of double stranded DNA.⁵⁰

There are two distinct forms of virions present during the life cycle of baculoviruses. On the one hand there are occlusion derived virions (ODV) and on the other hand there are the budded virions.⁵¹ The cyclic nature of insect populations, which requires a long-term storage form stable for several months, is one challenge the virus has to meet. For that purpose the virus is packed in stable occlusion bodies able to endure the changing conditions in nature. Through up-take of contaminated food the virus enters the host gut, where alkaline conditions dissolve the occlusion body. Thereby the occlusion derived virus (ODV) is set free. Its envelope is alkali resistant and the associated proteins allow penetration of the peritrophic membrane followed by the entry into epithelial cells via adsorptive endocytosis. Upon fusion of the envelope with the endosome membrane the virus escapes into the cytoplasm. Now the viral capsid, containing the protein P78/83 amongst others, is accessible to the cellular environment. Propelled by the actin comet tail the virions reach the nucleus and enter it through the nuclear pore complex. Approximately 6 hours after infection replication of viral DNA launches and new virions are as-

sembled. One fraction spreads the infection within the host. Budded viruses (BV) or also called non-occlusion derived viruses leave the cell wrapped in cellular membrane and easily taken up by neighbouring cells. After 20 hours the other fraction of virions is preferentially produced. These viruses named polyhedra-derived viruses form the next generation of occlusion bodies remaining inside the nucleus until host lysis.⁵²

Vaccinia virus

The vaccinia virus is a member of the poxvirus family that has a long and daunting history. Small pox had endangered humanity since approximately 10.000 BC and Ramses V was probably one of the first famous victims of this disease having a mortality rate of 30-35%.⁵³

Inoculation, the purposeful injection of smallpox under the skin was known to protect the patient from any further infection and this method was already used in the 17th century in Asia.⁵⁴ Jenner proved and refined the common belief stating that dairymaids are protected from smallpox. This research led to the discovery of vaccination. Yielded from fresh cow pox lesions of a dairymaid the viral probe was spread all over the world, after the medical doctor Jenner convinced his colleagues of the vaccine's efficiency. Thereby the vaccinia virus became the salvation of small pox. Extensive vaccination projects and the fact that humans are the sole host of small pox made the irradiation of this viral disease possible.⁵⁵

The vaccinia virus that we have today is a little mystery in virology. Although historically it is derived from cow pox, sequencing and restriction mapping indicates that there is a difference between these two species. Was it due to genetic recombination during prolonged serial passages or is the vaccinia virus a mixture between cowpox virus, variola virus and even horsepox virus, scientists are still puzzling.

What other features are known of this virus? The pathogen is one of the largest viruses with dimensions of roughly $360 \times 270 \times 250$ nm.⁵⁶ Pox viruses have the remarkable and unique ability to reproduce completely in the host cytoplasm independent of nuclear factors forming so called viral factories. The required enzymes, including DNA-dependent RNA polymerase, polyA polymerase and several capping enzymes are contributed by the virus itself. That explains the extended size of the pathogen. The viral core contains beside the reproduction proteins also a 200-kilobase-pair long double-stranded DNA protected by a lipoprotein core membrane.⁵⁷ During the complex life cycle the virus appears in four different variations each one equipped for its specific task (see Figure 7).⁵⁸

Starting at the viral factory in the host cytoplasm, where immature viruses (IV) are further processed to form intracellular mature virus (IMV) particles. The majority of these particles will stay in the host cytoplasm until they are set free during lysis. On the contrary a small fraction of the IMV will travel along microtubules to the trans-Golgi network (TGN) or an early endosome to get wrapped in a double layer of membrane. This form of vaccinia virus is then known as the intra-

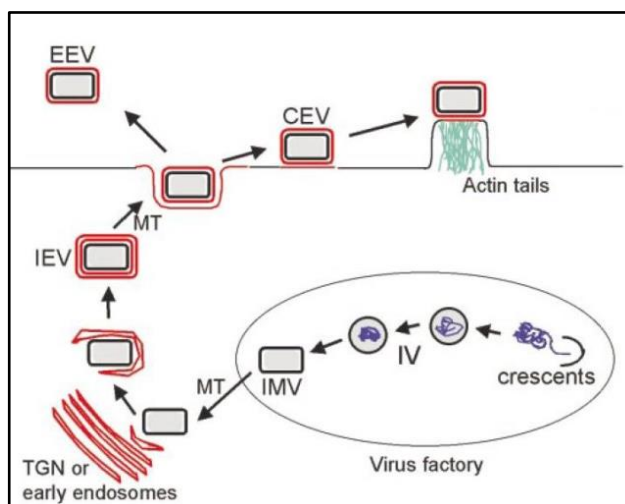


Figure 7: Schematic life cycle of vaccinia virus

cellular enveloped virus (IEV), which is now hidden in host material, able to evade the immune system. These particles are trafficked along microtubules to the cell periphery where the outer membrane fuses with the plasma membrane resulting in an early release of the pathogen.

Now there are two options, either the virus particle diffuses away from the cell and becomes an extracellular enveloped virus (EEV) or it stays attached to the host cell. These so called cell-associated enveloped viruses (CEV) are my focus of interest, since this regime of viruses is able to induce actin comets. Their 6.4-9.6 μ m long tails will propel the virus with an average speed of 2.8 μ m per minute.⁵⁹ EEVs as well as CEVs have one membrane layer and infect new cells from the same host organism. EEVs are meant to facilitate long range infection, whereas CEVs are rather specialized for direct cell-cell spread.⁵⁸

Mathematical Models

Interdisciplinary work is a new and successful trend in science, reflected for example by the impact of mathematical modelling on various fields in biology over the last few decades. Mathematical models that explain certain experimentally observed phenomena are useful in various ways. They may not only reproduce the behaviour of a certain system but also test competing hypothesis via simulations with prescribed parameter. Additionally one could also predict the reaction of the system under different conditions and prove the outcome by new experiments.

Locomotion exists in many different forms and many of them are well studied. In general one can say that motion is often coupled to some sort of motor. This is also true for biological motility where motor proteins convert chemical into mechanical energy to transport cargos along actin or microtubules, or facilitate the flexion of muscles.^{60,61,62}

In the case of lamellipodia protrusion and actin-based propulsion force is generated by filament polymerisation. Several theoreticians have tried to simulate these processes with mathematical with the aim of providing insight into the nature of the forces driving motion.^{63,64,65}

The *in vitro* reconstitution of *Listeria monocytogenes* with only 5 proteins proved that actin networks are capable of exerting forces and propelling pathogens.³⁰ This reduced system is a very suitable model for actin propulsion in general, since the bacterial protein ActA is a homolog of the nucleation-promoting factor WAVE at the membrane⁶⁶ and the actin tail can serve as an analogue of the lamellipodium. The number of proteins engaged in pathogen propulsion is limited and their characteristics are well studied, therefore there is also quite a number of models and simulations of this simplified process at different levels of resolution.^{67,68}

One macroscopic approach was inspired by the findings of Gerbal *et al.*, who measured the elastic features of the *Listeria monocytogenes* actin tail.⁶⁹ The basic idea is that the surrounding actin acts as an active elastic gel, which squeezes the pathogen forward. Additional studies with coated beads and droplets illustrated the distributions of stresses inside the actin continuum.^{70,71} The stresses are built up by polymerisation of new material at the virus surface and they are released by pushing the pathogen forward. These relaxations are not continuous hence the resulting movement is a discrete step-wise jumping. In this kind of model single filaments and their individual dynamics are not taken into account, which makes this approach less suitable for my model system. Larger tails such as comets from *Listeria monocytogenes* or *Shigella flexneri* can possibly propel pathogens by squeezing, but the comet tail of baculovirus is way too thin with 3-4 pushing filaments, hence I cannot use this kind of modelling approach.

Like others Mogilner tackled the question of pushing by actin from a microscopic point of view. His models are a very good example of how new insights change our thinking and he continuously adapted his theories. Based on the thermodynamical ideas of Kirschner and Hill⁷² the Brownian ratchet model from Perkin⁷³ was implemented consisting of a fluctuating load and a polymerizing actin network. The load is thought to be pushed away from the tail by Brownian motion and the actin uses the gained space and polymerizes until it touches the pathogen again, preventing thereby back fluctuations of the load. This model suggested that the velocity depends on the diffusion coefficient and thereby on the size of the pathogen. Such a relation was never measured experimentally hence Mogilner and Oster adapted the model in a way that the filaments were elastic and malleable and called it the elastic Brownian ratchet (EBR model).⁷⁴ In this model it is rather the thermic fluctuation of the filaments, which creates the gap. While the filaments are bent, there is enough space for them to polymerize and once they bend back, they push the load forwards. In this model the filaments are not connected to the virus

at all and the organization of filaments of the tail is not considered, as it was then unknown. Subsequent experiments showed that the tail must be attached to the load^{69,71,75} hence the EBR model had to be revised.

How could one include the filament tether into a model which relies on the gap formation between actin and load? Oster and Mogilner's answer was a transient linkage between the tail and the bacterial surface. Nascent filaments are assumed to be tethered through ActA to the pathogen and transiently slow down the movement; the connection then breaks and the untethered filaments are able to exert pushing forces on the bacterium. The model considers only the amount of tethered and untethered filaments ignoring their spatial arrangements and does not predict the pathogens trajectory. Nevertheless the predicted biphasic force-velocity relation resembles the measured data from⁷⁶. 9 years later Mogilner augmented his model once more and combined his microscopic model with the macroscopic elastic gel model taking into account single filament dynamics as well as global stresses derived from the surrounding actin shell. With this 2 dimensional model he included a prediction of the curvature of pathogen's tracks.⁷⁷

Overall all these models assume only transient or no tethering of the filaments although it is known that filaments can polymerise also during constant tethering.⁷⁸

In the clamped-filament elongation model from Dickinson and Purich⁷⁹ actin filaments are constantly tethered to the pathogen. Since Kuo and McGrath reported that *Listeria monocytogenes* moves in a saltatory fashion exhibiting a step size of 5.4nm(corresponding to the dimension of an actin monomer).⁷⁵ Purich and Dickinson's incorporated this feature into their model.⁷⁹ For this purpose a mechano-chemical motor was introduced, which coupled the ATP hydrolysis to actin polymerisation. In this so called "Lock, Load and Fire" mechanism a protein clamp binds actin with different affinities dependent on the phosphorylation level of the associated nucleotide. This clamp is strongly attached to the barbed filament end when new ATP-bound monomers are incorporated. By hydrolysing the ATP to ADP the affinity between the clamp and the monomer decreases and therefore the clamp releases the second to last monomer and binds to the new monomers at the prolonged barbed end. Possible candidates for this clamp protein are members of the Ena/Mena/Vasp family in combination with formins. Assuming that most of the filaments are under tension, the "Lock, Load and Fire" cycle leads to the same discrete movement with 5.4nm long steps. Interestingly the force-velocity prediction of this model differs significantly from Mogilner's model but it is also backed up by experimental data⁸⁰. Another observation could be explained by the extended clamped-filament model, namely the right-handed trajectories reported for *Listeria monocytogenes* motion.⁸¹ The twist of the constantly tethered actin filaments leads to torsional tensions, which result in helical twisting of the bacterium.

This torque was also a necessary component of another dynamical model, which aimed to reproduce the curvy trajectories of *Listeria monocytogenes*.⁸²

A more complex approach was taken by Alberts and Odell⁸³ who reconstituted the *Listeria monocytogenes* system *in silico*. An immense effort was put into simulating every chemical and mechanical process present, starting from protein concentrations, ATP/ADP bound actin, turnover rates and binding affinities. The drawback with this analysis was the demand for computing power satisfied by a cluster of 80 Linux processors. Furthermore it is not easy to distinguish between important and less important mechanism and parameters.

In the present work I took an alternative approach made possible by our access to the first structure of an actin comet tail derived by electron tomography, namely that driving a baculovirus. Rather than focusing on the molecular interactions I aimed to develop a mathematical model that would simulate comet tail architecture and the paths adopted by the baculovirus in cytoplasm.

Aims

I am a trained molecular biologist and my additional interest in mathematics brought me in the lucky position to carry out a joint project in Dr. Schmeiser's and Dr. Small's groups. At the time when I started my master project, Jan Müller was already in the middle of his studies about the actin comet tail of Baculovirus and detailed information was already available. Equally, a mathematical simulation of the protruding lamellipodium was developed from Christian Schmeiser. Starting from this initial position, I had the following aims:

Firstly, I planned to extract and interpret all the information about the actin comet tail delivered from light microscopy (LM) and electron tomography (ET) and utilize them for developing a mathematical model of the pathogen propulsion. This simulation should mimic all the structural and dynamical features observed in experiments. The approach of choice was a quasi-static simulation describing all the main players and their interactions. The computation was performed using the MATLAB program.

Subsequently, I aimed to use this mathematical model to gain further insights into the mechanism of propulsion and test various hypotheses. By questioning purposefully the necessity of various assumptions and mechanisms I could define a minimum set of model features. The quality of each result was determined by comparison with the experimental observations.

From a biological point of view it was interesting to test the relevance of this model system "baculovirus" by relating it to other motile systems such as the actin driven vaccinia virus. Hence, I used the same experimental approach with which the Baculovirus tail was investigated, namely electron tomography. I acquired tomograms of vaccinia virus comet tails embedded in negative stain and in vitreous ice to obtain new structural information about filament architecture. A comparison between this data and that obtained for baculovirus should show whether or not different pathogens use the same basic mode of propulsion by actin.

Methods

The method section is divided into the biological part describing the experimental work and a detailed description of the mathematical model.

Biology

Baculovirus

The wild type baculovirus was kindly supplied by Venugopal Bhaskara working at the Institute of Molecular Pathology (IMP), Vienna, Austria whereas the baculovirus particles tagged with m-Cherry were kindly provided by Taro Ohkawa.⁴⁵

To amplify the baculovirus, a member of an insect virus family, Sf9 cells were infected with either wild type or mCherry-tagged baculovirus. After 3-5 days the cells were harvested by scraping them of the cell culture dish, centrifuged at 2,500g for 5min, filtered through a membrane with 0.45µm pore size and stored as a stock. Straight before the experiment the virus was centrifuged at 18,000g for 30min and re-suspended in 150µl of baculovirus buffer (10mM HEPES, 0.5M KCl, pH 7.4).

To infect subconfluent cells in a 12 well plate 150µl of the virus containing solution were added to the 2ml of media. After 1-3h of incubation the samples were either used for light microscopy or fixed for electron tomography.

Vaccinia virus WR

The vaccinia virus WR was kindly provided from Dr. Huemer working at the Medical University Innsbruck.

To amplify the virus HeLa cells were infected and after 48-72h the adherent cells were scraped off and re-suspended in 1ml medium. After 3 cycles of freezing and thawing the stock was ready to use. The titer of a virus stock is given as plaque forming units (pfu) per volume, estimated using the plaque forming assay.

HeLa cells were plated onto a 6-well plate such that they would form a confluent layer the next day. A dilution series (10^{-3} to 10^{-8}) of the virus in media was performed and 1ml of each mixture was added to one well. The medium was removed after 2 hours of incubation at 37° and the cells were overlaid with a 1:1 mixture of 1xMEM/8%FCS and 3% carbomethyl-cellulose (VWR) in ddH₂O. Then the 6-well dish was kept in the 37°C incubator for 2 - 3 days. Afterwards the embedding mixture was removed and the cells were fixed and stained with 0.1%crystal violet in 15% ethanol for 1 hour. After a brief washing the unstained plaques were counted. The clusters of dead cells correspond to the number of infectious virus particles, which was around 10^8 - 10^9 pfu.

Cell culture, transfection and infection

An especially thin type of HeLa cells was kindly provided by Michael Way's lab from London. These cells were cultured in Minimum Essential Medium (MEM, from Invitrogen) with Earle's salt, 1% L-Glutamine, 1x nonessential amino acids, 5% fetal bovine serum (PAA) and 1% Penicillin/Streptomycin at 37°C in the presence of 5% CO₂.

For fluorescence light microscopy the cells were plated on 15mm coverslips coated with human fibronectin (Roche) at a concentration of 20µg/ml. The same procedure was performed before plating the cells on filmed metal grids used for electron microscopy.

HeLa cells were infected with the vaccinia virus using a multiplicity of infection (MOI) of 3 – 8 which corresponds to ~0.1 to 1µl of the virus stock solution. The samples were fixed 7-9h after infection.

For light microscopy the cells were fixed with 4% Paraformaldehyde for 15min followed by 3 times washing in 1x Phosphate buffered saline (PBS) for 5min. Subsequently, the sample was extracted with 0.2% Triton x-100 in CB pH 6.1 for 2min and again 3 times washing with PBS ensued. 4% Bovine serum albumin (BSA) in 1x PBS was used for blocking for at least 20 minutes. The next step included the staining of the actin filaments with Alex-labelled Phalloidin and additional DAPI staining. Finally, the cover glass was mounted on a cover slide and light microscopy was performed on an inverted Zeiss Axio-scope equipped with epifluorescence optics using either a 100× total internal reflection fluorescence (TIRF) lens in wide-field mode or 40x and 63x Ph3 lenses and a halogen lamp (Zeiss). The images were recorded on a Roper Micromax, 512×512 rear-illuminated, cooled CCD camera controlled by Metamorph.

Preparation of gold colloid

975µl of 10nm gold colloid (BBI) were mixed with 25µl of 200mM Sodium-pyrophosphate (NaH₂P₀₄) buffer pH 5.0. 5mg/ml BSA was dissolved in 5mM NaH₂PO₄ buffer pH 5.0 and added to the gold colloid in a dilution of 1:20. The mixture was vortexed and incubated for 15 minutes before the colloid suspension was centrifuged for 60 minutes at 26.000g and 4°C. The supernatant was removed and the pellet was resuspended in ddH₂O in the same volume as before the centrifugation step. Now the sol was centrifuged once more using the same parameters. As much as possible of the supernatant was then removed and the pellet was resuspended in the remaining ddH₂O. The prepared gold colloid suspension was stable for several weeks if stored at 4°C.

Electron microscopy

For routine inspection with an 80kV transmission electron microscope (Morgagni, FEI) 200 mesh hexagonal copper palladium grids (Agar Scientific) were coated with a Formvar film (1.5% Formvar powder dissolved in chloroform). These grids were then coated with fibronectin (20µg/ml) and the cells were allowed to spread at least 5h. For vaccinia infection the virus was added with an MOI of 3 – 7 and after 7 to 9 hours the cells were extracted with 1% Triton in cytoskeleton buffer (10mM MES buffer, 150mM

NaCl, 5mM EGTA, 5mM glucose, 5mM MgCl₂, pH 6.1) for 1min and then fixed with 2% Glutaraldehyde and 10µg/ml Phalloidin in cytoskeleton buffer for at least 20min. Afterwards the sample was stained with 70µl of 2% sodium silicotungstate (SST).

Electron tomography

For negative stain electron tomography the grids were stained with 70µl of 6% SST and 1:10 BSA-gold colloid.

For cryo-electron tomography cells were plated on Quantifoil R2/2 perforated carbon films on 200mesh Au grids coated with fibronectin. The cells were fixed and the grids subsequently transferred to forceps in a grid-plunging device (Leica EM GP), supplemented with 4µl of CB and 1:2 BSA-saturated colloidal gold. During mounting and blotting, the grid was held in a chamber at constant humidity of 99% and a fixed temperature of 23°C to avoid evaporation. The samples were blotted automatically for 1.5-2s with humidified Whatman No.1 filter paper applied to the backside of the grid preventing any contact with the cells. Samples were frozen by quickly plunging them into liquid ethane cooled to -184°C by liquid nitrogen, in which the grids remained for storage and also during microscope loading.

The FEI Tecnai F30 Helium Polara microscope was cooled to -190°C and operated at 300kV. The tilt series was acquired using SerialEM 3.x. and the average tilt range was -60° to +60° using the Saxton tilt scheme based on 1° increments for negative stain and 2° increments for cryo samples from 0° tilt, at a nominal defocus value of -5µm for negative stain and -15µm for cryo samples. For negative stain samples two tilt series around orthogonal axes were recorded on a Gatan UltraScan 4000 CCD camera at on-camera magnifications typically from x23,000 to x31,000. For frozen hydrated samples zero-loss images were recorded on a Gatan MSC 2k camera on a GIF 2002 using a slit width of 15eV. The total electron dose for the cryo tilt series was less than 130 electrons per Å² the primary on-screen magnification was from x20,000 to x29,000.

Image processing and analysis

Re-projections from the tilt series with gold particles as fiducials for alignment and contrast transfer function corrections were performed using IMOD software from the Boulder Laboratory for 3D Electron Microscopy of Cells. A typical tomogram comprised a z-stack of 60-120 sections of 0.4-1.5nm each.

Using IMOD, filaments were manually tracked and models generated. A typical tomogram comprised a Z-stack of 60-120 sections of 0.4-1.1nm each.

The coordinates of filaments and branch points were extracted from the model using the model2point function implemented in IMOD. Ensuing measurements such as filament length, branching angle and inter-branch distance performed in MATLAB whereas more sophisticated measurements were kindly accomplished by Christoph Winkler.

Mathematics

Outstanding work from my lab members gave me access to detailed information concerning the structural architecture of the actin network. The aim was to include and reproduce all the experimental data and extract the essential mechanisms. Hence I have taken a quasi-static approach to model the key players engaged in actin-based propulsion. In this type of approach it is assumed that the system seeks a level of minimal energy whereby slow processes can be approximated by series of states infinitesimally close to equilibrium. In order to simulate such a series of balanced states one needs two distinct parts.

In the stochastic part actin dynamics and Brownian forces arising from thermal fluctuations take place. These processes shift the system away from the energetic equilibrium. In the following deterministic part the new position of the virus is calculated by balancing the existing forces. The duration of two such consecutive parts is fixed to $\Delta t = 0.9\text{ms}$. The combination of these stochastic and deterministic calculations is iterated in an alternating way.

Objects

The virus

The model is 2-dimensional and hence the shape of the virus is simplified as a rectangle with length L and width $2r$, with two semicircles of radius r attached

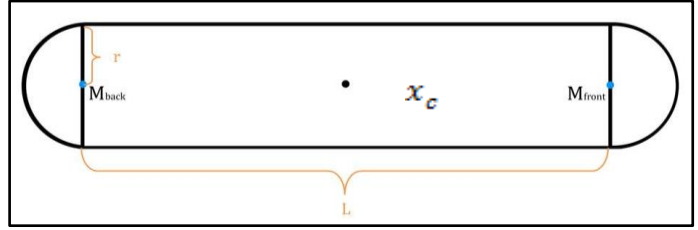


Figure 8 is showing the approximated dimensions of the Baculovirus used in the simulation.

(Figure 8). The size of the baculovirus' rod-shaped nucleocapsids appears to be in a range of 30–60nm x 250–300nm, hence parameters of the conventionalised model virus are $L = 200\text{nm}$ and $r = 25\text{nm}$. The virus is taken as a rigid body, where the notation of the centre of mass is x_c and the two centres of the flanking semicircles are designated as M_{back} and M_{front} .

The position of the virus is given in terms of its centre of gravity $x_c \in \mathbb{R}^2$ and an orientation angle α , whereby $\Omega_0 \subset \mathbb{R}^2$ includes all the point within the virus shape.

Every possible position of the object is given relative to the reference position

$x_c = \begin{pmatrix} 0 \\ 0 \end{pmatrix}$ and $\alpha = 0$. Hence the virus in any configuration with its parameters x_c and α contains the following points $x_c + R(\alpha)x$ with the rotation matrix

$$R(\alpha) = \begin{pmatrix} \cos \alpha & -\sin \alpha \\ \sin \alpha & \cos \alpha \end{pmatrix}.$$

Actin filaments

Actin filaments are modelled as rigid straight rods and described as lines with the length f_l and the rotation angle f_α starting from the position of the pointed end f_{PE} . These filaments are considered as immobile relative to the cytoplasm, which avoids laborious calculations. Barbed ends can either be tethered to the virus surface or be detached. This tethering connection is facilitated by the viral protein P78/83 and potentially also by VASP. Similar assumptions were made for the *in silico* reconstitution of the *Listeria monocytogenes* actin tail.⁸³ For this reason the anchoring of the filaments is limited to the rear of the baculovirus, where the P78/83 is localized. This region is simplified as a semi-circles, hence the attachment point of a filament is described by the angle β between the radius through this point and the centre line (Figure 9), whereupon $\beta \in [-\beta_{\max}, \beta_{\max}]$.

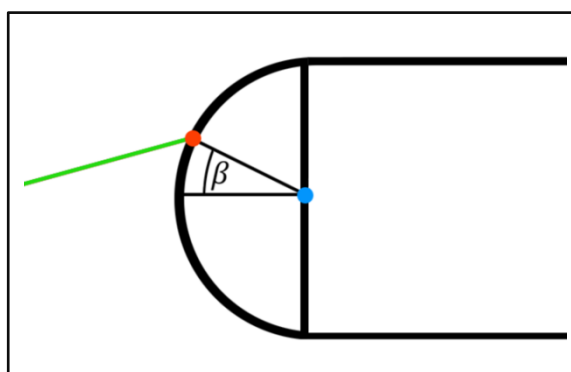


Figure 9 displays the rear of the virus in black and an actin filament in green. Angle β characterises the position of the attachment point in red

Filament Dynamics

Each actin filament can grow, branch and become capped at the barbed end and it can depolymerise from the pointed end. All these stochastic processes take place with discriminative rates λ [event/time step]. At the beginning of each time step the decision about the occurrence of such an event is made by comparing its probability $\lambda\Delta t$ with a randomly chosen number. If the drawn number is smaller than the rate, the event takes place. These varying numbers are generated by an integrated MATLAB function, which produces evenly distributed random numbers between 0 and 1.

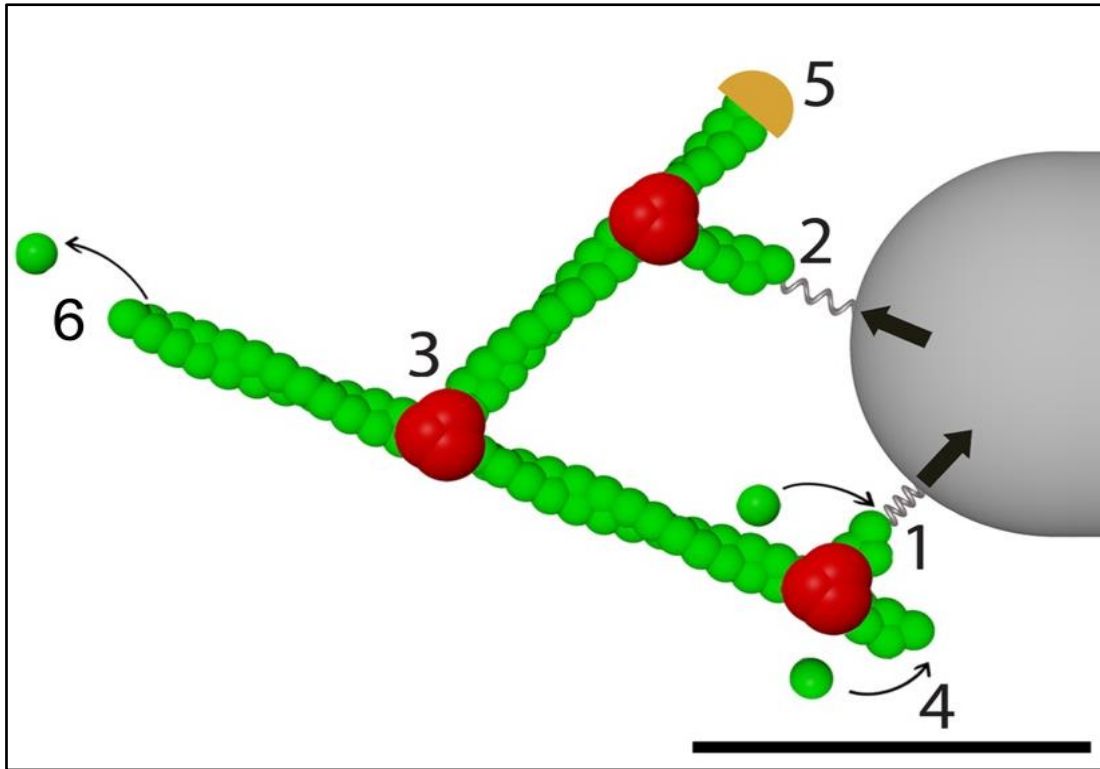


Figure 10 showing the essential components of the model. Actin filaments (green) are tethered to the virus and engage in pushing (1) or pulling (2) regimes, modelled by spring connections. Branches (3) are initiated by Arp2/3 complex (red) recruited to the plus ends of actin filaments at the virus surface. Elongation of filaments that can no longer push (4) continues until they are capped (5) and subsequently depolymerized (6).

Polymerisation (see Figure 10.1,4):

Actin filaments grow by incorporating monomers at the barbed end. In my simulation the elongation is not modelled on the scale of discrete monomer steps but continuously. Every uncapped barbed end is extended by $v\Delta t$ [nm] per time step. The polymerisation speed v of tethered filaments is dependent on the exerted counter force. Filaments which do not interact with any obstacle grow with a maximal speed of $v = v_{max}$.

Tethered filaments grow with the same maximal velocity until they touch the virus surface. Then the decline of the speed is related to the angle φ between the filament and the normal to the surface. This angle serves as a proxy for the counter force exerted by the pathogen. A filament growing normal to the virus surface will therefore slow down to a minimal velocity of $v = v_{min}$.

Hence, I used the formula $v = (v_{max} - v_{min}) \sin \varphi + v_{min}$ to calculate the speed of every filament approaching the virus surface. Resulting from this the growth speed varies between 160 and 390 nm/sec in my simulation.

Branching (see Figure 10.3):

Branching happens only at tethered barbed ends with a fixed branching angle $\gamma_b = 74^\circ$. In the 2-D model there are two possible branching directions, either left or right. In reality

there are infinite constellations of a daughter filament enclosing a branching angle $\gamma_b=74^\circ$ with their mother filament. Judging from the structural data, there seems to be a favoured branching direction toward the virus surface. Thus the program compares the minimal distance between the centre of the rear semicircle M_{back} and the barbed end of a newly formed daughter filament, which is branching either to the left or to the right side of the mother filament. The version with the shorter distance is then realized.

It is known that the viral capsid protein P78/83, a homolog of WASP is capable of recruiting and activating the Arp2/3 complex⁸⁴. The same protein is thought to link the filaments to the rear of the virus, which results in a limited capacity of branching and tethering new filaments. This consideration was taken into account by locally and globally adapting the branching rate. Within a very crowded region the likelihood for a newly branched filament decreases. I have implemented this by assuming that each attached filament partially inhibits the branching of other filaments in its close neighbourhood. This suppressing effect is mathematically described as a triangular hat function ϱ with a maximal width 2 and maximal height of 1. At the peak of this function the probability for a new actin filament branch from a different actin filament is zero. All the local inhibitions of different filaments (Figure 11 A) sum up to an inhibition profile, which reflects an inverted probability density along the semicircle (Figure 11 B).

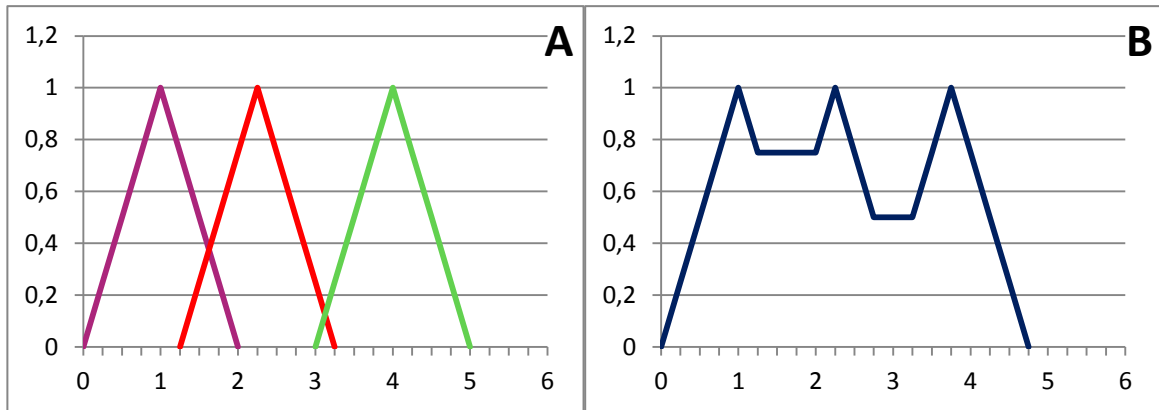


Figure 11 **A** displaying the triangular hat functions ϱ of three barbed ends, representing the individual inhibiting effect. **B** Showing the cumulative effect of the present filaments. In both cases the x-axis represents the position along the semi-circle and the y-axis the inhibiting effect or the inverted probability for a new branch.

To account for the global influence of existing filaments the probability of a branch decreases with the number of pre-existing filaments. The branching rate λ_b for the mother filament i is computed by reducing its maximal value $\lambda_{b,max}$ by a factor depending on the total number of attached filaments $(N-n/N)$ and by the additive inhibition effect ϱ of the other tethered barbed ends (with numbers j) close by.

$$\lambda_b = \lambda_{b,max} \frac{N-n}{N} \left(1 - \sum_{j \neq i} \varrho \left(\frac{\beta_i - \beta_j}{\Delta\beta} \right) \right)_+$$

N is the maximal number of tethered barbed ends and n is the number of actually attached filaments. The position of the barbed end of the i^{th} filament along the virus surface is given in terms of the angle β_i (Figure 9) $i = 1, \dots, n$. The parameter $\Delta\beta$ can be interpreted as an inhibition range of one barbed end. In the final simulations a value of $\Delta\beta = 18^\circ$ was used, which corresponds to 15.7 nm along the semicircle.

Capping (see Figure 11.5):

The polymerisation of actin filaments can be stopped by capping. So called capping proteins bind and mask the barbed end and prohibit addition of further actin monomers. I have assumed that filaments tethered to the pathogen are protected from this process due to constricted accessibility. Only detached barbed ends can become capped with a rate λ_c . In reality there is also a small chance of uncapping⁸⁵ but in the model the process of capping is set to be irreversible. This simplification has little influence on the relevant results.

Depolymerisation at pointed ends (see Figure 11.6):

Degradation of actin filaments includes various processes such as depolymerisation, severing and debranching.^{86,87} Tomography and also light-microscopy do not provide enough information to understand this entire process completely. The degradation of the actin tail does not influence the movement of the virus, since the pool of available proteins is large enough to allow unrestricted incorporation of actin⁸⁸ and therefore depolymerisation is not relevant for the questions addressed to the model. Thus I decided to model the degradation in a very simplified and basic way. Once a filament is capped and has reached a certain age t_d depolymerisation starts with a rate λ_d . Thereafter the filament shrinks from the pointed end with fixed speed v_d . The age threshold is chosen to mimic the approximate length of the tail as observed experimentally.

Nucleation of new filaments

Actin filaments can be nucleated de-novo at the rear of the virus providing new mother filaments for tail initiation but also during propulsion.

The underlying mechanism is thought to be an activated Arp2/3 complex recruited to the rear of the pathogen. Instead of binding a pre-existing mother filament a new filament seed is formed. Since this alternative process is surely less frequent than classical branching I have limited the de-novo nucleation to one new filament per time step at most.

Another restriction implies that nucleation only occurs if the number of tethered filaments falls below a certain threshold n_0 . If all these requirements are fulfilled the process takes place with a nucleation rate λ_n . The position of the new filament is chosen along the anchoring region according to the distribution of the pre-existing filaments. A probability density arises from the inhibitory triangular hat functions

$$f(\beta) = c \left(1 - \sum_{j=1}^n e^{\left(\frac{\beta - \beta_j}{\Delta\beta} \right)} \right)$$

where the constant c is chosen to make f a probability density. Thus a homogeneous spreading of the filaments along the semicircle is favoured. I assume that each nucleated filament will branch immediately, which allows one to determine the orientation of the filaments to the virus. Assuming that after the branching event both filaments grow with the same speed, one will observe an isosceles triangle with both filaments including the same angle with the virus surface.

Virus Dynamics

Brownian motion

Brownian motion is a ubiquitous feature of for microscopic objects in solution. The principal idea of atoms and molecules wiggling and colliding due to thermal energy might be quite easy to imagine but predicting the movement of larger non spherical objects is far beyond triviality. Hence I have again used a simplified approximation of the real process. Assuming that the forces in all different directions are normally distributed one can divide the resulting movement of the virus into a translation (by at most Δx) and a rotation (by at most $\Delta\alpha$). In each deterministic part of the time step the direction and the length of translation as well as the amount of rotation is determined by drawing random numbers between 1 and 0. Diffusivity measured in m^2/sec describes the mobility of a substance under certain conditions. The diffusion coefficient of a purified baculovirus in TE buffer is known to be $(2.89 \pm 0.74) \times 10^{-8} cm^2/sec$.⁸⁹ Since in the simulation's case the virus is located in the much more crowded cytoplasm and additionally the pathogen is connected to the actin tail (which is approximately 10 folds longer than the virus itself) the mobility is surely reduced. To avoid artefacts through overestimating the Brownian forcing values for Δx and $\Delta\alpha$ were chosen in such a way that they correspond to a diffusion coefficient of $4.94 \times 10^{-10} cm^2/sec$.

The second part of each time step consists of the quasi-stationary force balance process. According to the theory of quasi-static processes the system is always close to the energetic minimum. Therefore the new position of the virus is chosen by looking for an energy minimum. There are three main forces acting on the virus and influencing the energy level of the system: Filaments push the virus forward via steric interactions, pulling forces are exerted by spring like tethers and the friction of the virus against the cytoplasm opposes any movement in general.

$$E(x_c, \alpha) = E_{steric}(x_c, \alpha) + E_{pull}(x_c, \alpha) + E_{friction}(x_c, \alpha).$$

Steric interactions

The underlying mechanism of filaments pushing a pathogen is the steric interaction between two objects. Since the virus as well as actin filaments are thought to be rigid one possible way of simulating this interaction would include a side condition prohibiting the penetration of the virus by filaments.

But side conditions impede the minimization function therefore another solution was to convert possible penetration into a penalizing energy term.

In the first place the length of the filament part extended into the virus (L_j) was used as a measure for steric interaction. However this approximation seemed too imprecise and led to several artificial behaviours. Thus the area between the filament and the virus surface, which includes more information about the location of the filament relative to the virus, is used instead. This approach appears to be an appropriate alternative to the side condition because one can transform the penalisation of the penetration into an energetic contribution to the potential energy. To do so, I integrate over the filament segment inside the virus (using an arc length parameterization $\{x_j(s): 0 \leq s \leq L_j\}$) and calculate the distance between each point of the segment to the viral surface. The function $d(x; x_c, \alpha)$ describes this distance, chosen in such a way that the resulting integral can be computed easily. This resulted in the following formula:

$$E_{steric}(x_c, \alpha) = \mu_{steric} \sum_j \int_0^{L_j} d(x_j(s); x_c, \alpha) ds ,$$

where the sum goes over the numbers j of all filaments penetrating the virus. Details concerning the function $d(x; x_c, \alpha)$ and its integral can be found in the appendix 1.

Pulling forces

Assuming that filaments are tethered to the virus, they can exert pulling forces. This linkage is modelled as a linear elastic spring force. If the spring is extended a counter force is induced, but this spring like connection can also break, if the deflection is too large. The maximal deflection $z_{threshold}$ and the spring constant μ_{tether} (a measure for the stiffness of the spring) are the two essential features of the tether. The potential energy stored in a spring is defined as

$$E_{spring} = \frac{1}{2} \mu_{tether} (x - x_0)^2$$

, where $x - x_0$ is the deflection of the spring and μ_{tether} the elastic constant.

In the simulation the deflection of the spring is defined as the distance between the barbed end of the tethered filament (denoted by $x_{b,1}, \dots, x_{b,n}$) and the respective nearest points on the virus (denoted by $x_{v,1}(x_c, \alpha), \dots, x_{v,n}(x_c, \alpha)$).

The total pulling energy is the sum of the energy contribution of all tethered filaments.

$$E_{pull}(x_c, \alpha) = \sum_{j=1}^n E_{tether}(|x_{b,j} - x_{v,j}(x_c, \alpha)|),$$

And the spring potential is

$$E_{tether}(z) = \begin{cases} \mu_{tether} \frac{z^2}{2}, & z < z_{threshold}, \\ 0, & else. \end{cases}$$

where $z = |x_{b,1} - M_{back}| - r$ defines the minimal distance between barbed end and virus surface.

Once a tether rips off, it cannot regenerate. After the new virus position is defined all freshly detached filaments are labelled as untethered and from now on they are accessible for capping.

In the case of the branch tethered simulation the connecting linkage exists between the Arp2/3 complex and the virus rear. The only difference in the implementation is to substitute the coordinates of the branch point with the ones from the barbed filament end.

For the untethered simulation there is no pulling force assumed, hence the elastic constant is set to zero.

Friction

A solid body which moves through a liquid phase is dragged by the skin friction. This friction follows the drag equation:

$$F_D = \frac{1}{2} \rho v^2 C_d A$$

F_D , the drag force, is linearly dependent on the density ρ , on the drag coefficient C_d and the reference area A . Additionally the drag force goes with the square of the velocity v . What actually is needed for the 2-dimensional simulation is not the force but rather the energy.

For a given centre of gravity x_c^{old} and orientation α^{old} at the previous time step, the frictional contribution to the potential energy which was necessary to move the virus to the current position x_c, α is described as follows:

$$E_{friction}(x_c, \alpha) = \frac{\mu_{friction}}{2\Delta t} \int_{\Omega_0} |x_c + R(\alpha)x - x_c^{old} - R(\alpha^{old})x|^2 dx,$$

where $\mu_{friction}$ is the friction coefficient. Assuming very small Δt the friction energy can be approximated as follows.

$$\nabla_{x_c} E_{friction} \approx \dot{x}_c \mu_{friction} Area(\Omega_0),$$

$$\partial_{\alpha} E_{friction} \approx \dot{\alpha} \mu_{friction} \int_{\Omega_0} |x|^2 dx .$$

Simulation

The sample size, namely the simulation time required to obtain a significant result, was first estimated by observing the development of selected features of the model over time.

The following examples illustrate how this was done to judge the stability of my simulation:

Panel A in Figure 12 shows the number of actin filaments touching the virus rear over the first 65 seconds. The mean is 4.03 and the standard deviation is 1.26 and the diagram suggests that the values behave uniformly during the whole simulation. A serial comparison of standard deviation with an interval of 10 seconds yields a low variance of 0.0058, which indicates that the simulation is stable.

Panel B shows the average length of the filaments during the first 600 milliseconds. The simulation starts with two filaments both on average 10nm long, which grow and branch in order to build up the actin tail. After 300msec the average filament length reaches a plateau that stabilizes between 300 and 600 milliseconds is with 101nm very close to the overall mean length of 106nm.

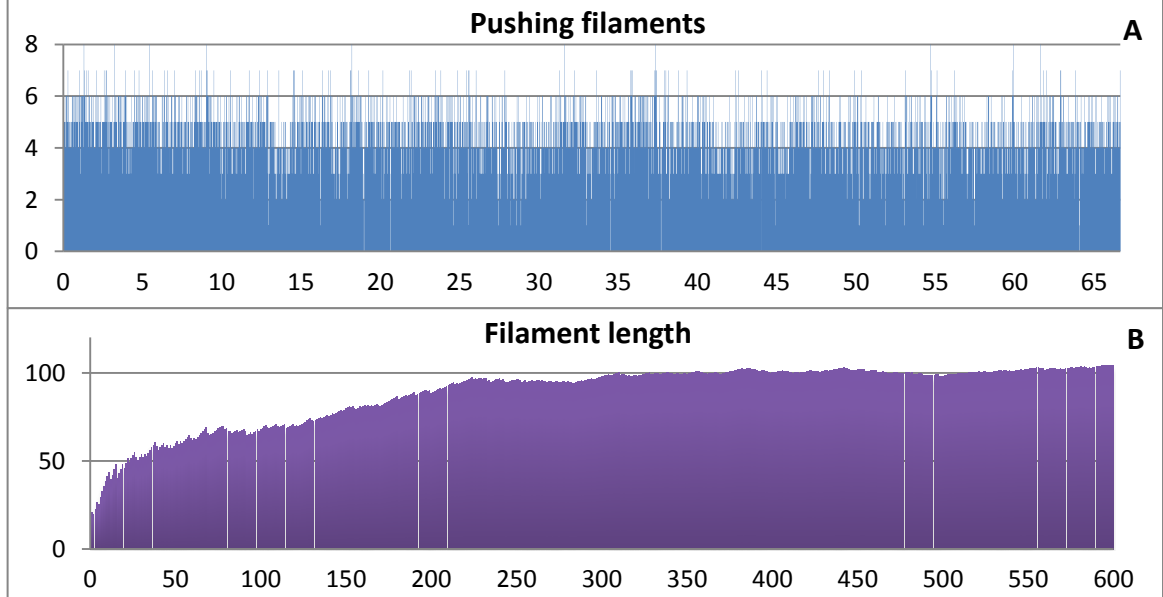


Figure 12: **A** shows the number of pushing filaments (y-axis) during 65 seconds (x-axis). **B** shows the average filament length (y-axis) during the first 600 seconds of the simulation (x-axis).

A full simulation, including on average of 300.000 to 800.000 iterations for 4.5-12 minutes simulation time, required 2-4 days to perform the calculations. For evaluation of a simulated phenotype at least 30 minutes were analysed, to ensure a significant and reliable result. Statistical analysis was restricted to the mean, the standard deviation and histograms depicting relative frequencies.

Measurements used for comparison

Choice of the type and magnitude of parameters is a primary consideration in any modelling analysis as these have a primary impact on the outcome.

The following parameters were used for comparing and fitting the simulation to the experimental data.

Features such as the tail length and the speed of the pathogen were already known from literature⁴⁵ and could be confirmed using light microscopy and conventional electron microscopy.

Using electron tomography more detailed structural information at single filament resolution we could obtain data on filament length, filament density, the number of filaments per μm tail or the total length of all filaments within $1\mu\text{m}$ tail.

A second feature was the spacing of the daughter filaments along one mother filament corresponding to the inter-branch distance and a measure for branch frequency. This distance was one of the main parameters, which I tried to reproduce in my simulations. Since branches are associated with filaments, the branch density within the tail and the average filament length per branch were additional features to be considered.

To describe the connectivity of the actin network I have to introduce the term sub-set. A sub-set is a group of filaments connected to each other via branches. This means that all filaments are derived from one initial mother filament. The size of such sub-sets reflects the efficiency and the stability of Arp2/3 dependent network.

Furthermore, the fish-bone like arrangement of the actin filaments within the tail can be quantified by measuring the angles between each filament and the virus trajectory (angle to trajectory).

Christoph Winkler performed the measurements of the angle to trajectory using cryo-tomograms. The coordinates of the filaments as well as the tail axis were derived from the manual tracking of the tomograms. A smoothing curve was laid through the tracked points and the angle was measured between the filament and the subtending segment of the tail. This measurement approach was applied for the 3-dimensional cyro tomograms as well as for the model.

A perfect symmetrical tail would show filaments subtended at an angle of 37.5° to the trajectory, which is half the branching angle of actin filaments. For the experimental data and the simulations, this value was variable and depicted in a histogram (Figure 24).

To analyse the mechanism of propulsion we need to know how many filaments interact with the virus at any time. To estimate the number of pushing filaments a tube with a diameter of 50nm , corresponding to the dimensions of the virus, was laid through the central axis of the tails in our tomograms. The number of pushing filament deduced from the number of filaments that crossed tube swept by the virus. These measurements were also performed by Christoph Winkler.

Another characteristic of the baculovirus' movement is the persistence of the covered track. Quantifying the straightness of a path turned out to be no easy task, especially from a mathematic point of view. Many scientist e.g. the group from Welch⁴⁵ have described the straightness as a ratio between effective displacement and the length of the travelled path. I have applied the same method to our light microscopy data as well as to my simulations. The temporal resolution of our microscope was restricted to 2 seconds, thus the average distance between

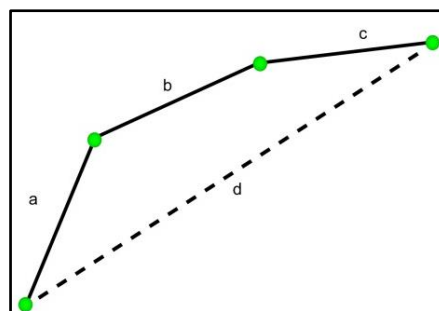


Figure 14: One possible way to describe the straightness of a path: Divide the sum of sub steps (a+b+c) by effective displacement (d).

two consecutive points (shown in green in the Figure 14) is theoretically 500nm. Then I took the sum of 20 sub-steps which relates to 40sec of movement (schematically a+b+c) and divided it by the total displacement within this time frame (distance d, Figure 14). The resulting ratio varies between 1, for a straight line and 0 for any path where the start and end point are identical. Unfortunately this method is not highly robust, since the outcome depends on the ratio between sub-step length and total displacement. Figure 13 shows this relation. 40 seconds are an arbitrary time frame, which I adopted from existing studies to allow comparison⁴⁵.

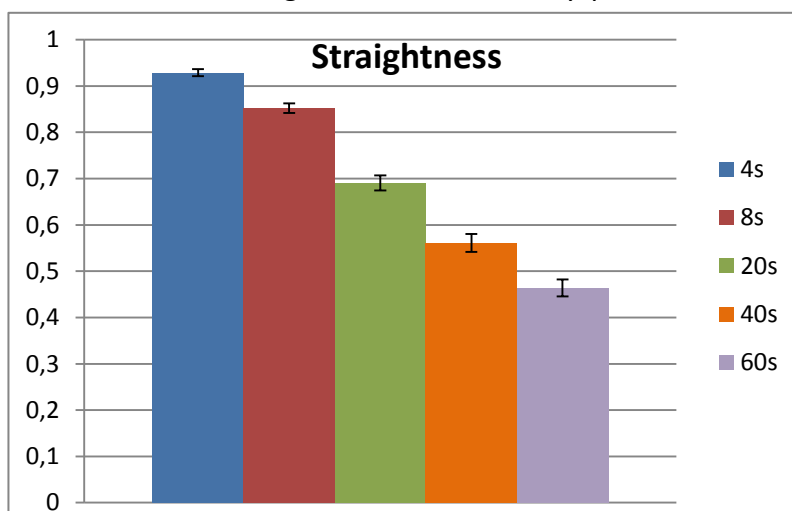


Figure 13: The values for the straightness of the very same track decrease with increasing times chosen for the total displacement.

Another disadvantage of this measurement is that a straightness value provides only information about the effective displacement and the length of the travelled path, but hardly any details about the persistence and tortuosity of the path. One value of straightness describes many different trajectories.

Therefore, my mathematical colleague Christoph Winkler tried to invent an improved automatic way of measuring the straightness. The aim was to reduce the track to a polygon in which the number of vertices was chosen automatically by analysing the evolution of an energy function. Even this method had limitations, because the energy function and its analysis varied and influenced the outcome.

Thirdly, I used the turning angle to describe the deviations in the path. Electron micrographs of comet tails acquired with an average magnification of 28000x were compared to the simulations. The tails were simplified by consecutive edges of 200nm. The angle between two successive lines is defined as turning angle φ (shown in Figure 15).

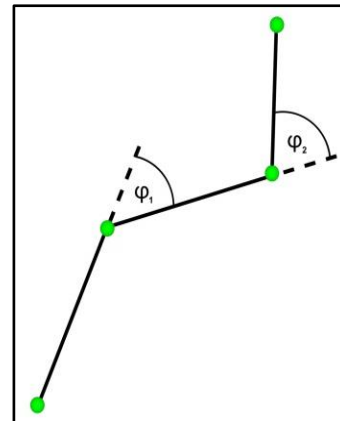


Figure 15: Turning angles φ describe the amount of direction change between two sub steps.

Fitting the model to the experimental data

The purpose of the primary simulation was to reproduce the observed experimental data as close as possible. Once all the objects, their interactions and dynamics were implemented the behaviour of the simulation was optimized by fine tuning the input parameters. During this process I sought to match the velocity, the inter-branch distance, the size of the sub-sets, the filament length and to some extent the straightness of the track. The most relevant input parameters had the following effects: The filament growing rate influenced the velocity of the baculovirus; the branching rate in relation to the growing speed affected the inter-branch distance; and the size of the sub-sets was influenced to some extent by the elastic spring constant and its maximal deflection. The stability of the system relied on various parameters, such as the balance between friction and pushing force, the *de-novo* nucleation rate and the pulling forces counterbalancing the Brownian motion.

In contrast, the capping rate and the tail length had no influence on the model result.

The final input parameters used for all simulations are shown in Table 1.

Results

Mathematics

The mathematical model provided two different kinds of results.

The primary analysis provided a close fit to the experimental data, referred to here as “wild type”. In a second series of analyses I tested various predictions and hypotheses by varying the model assumptions.

Wild type simulation

In the wild type simulation the velocity of the model was 3% higher and the inter-branch distance was 5% shorter than experimentally observed. The number of pushing filaments was 4.1, slightly higher than the value 3.9, derived from the cryo tomograms. The number of filaments in a sub-set estimated from the tomograms was also 3 more than the simulation. The angles to trajectory were not significantly different between *in vivo* and *in silico* ($P = 0.8270$). Likely was the straightness of the model measured in turning angle not significantly different than the experimental data ($P > 0.9999$).

The number of filaments per μm tail in the simulation was 60% of that observed and the total filament length per comet length was 55% of the *in vivo* situation. A comparison of the data is presented in table x and visual similarities between wild type simulation and experimental data can be seen in Figure 16 and Figure 20.

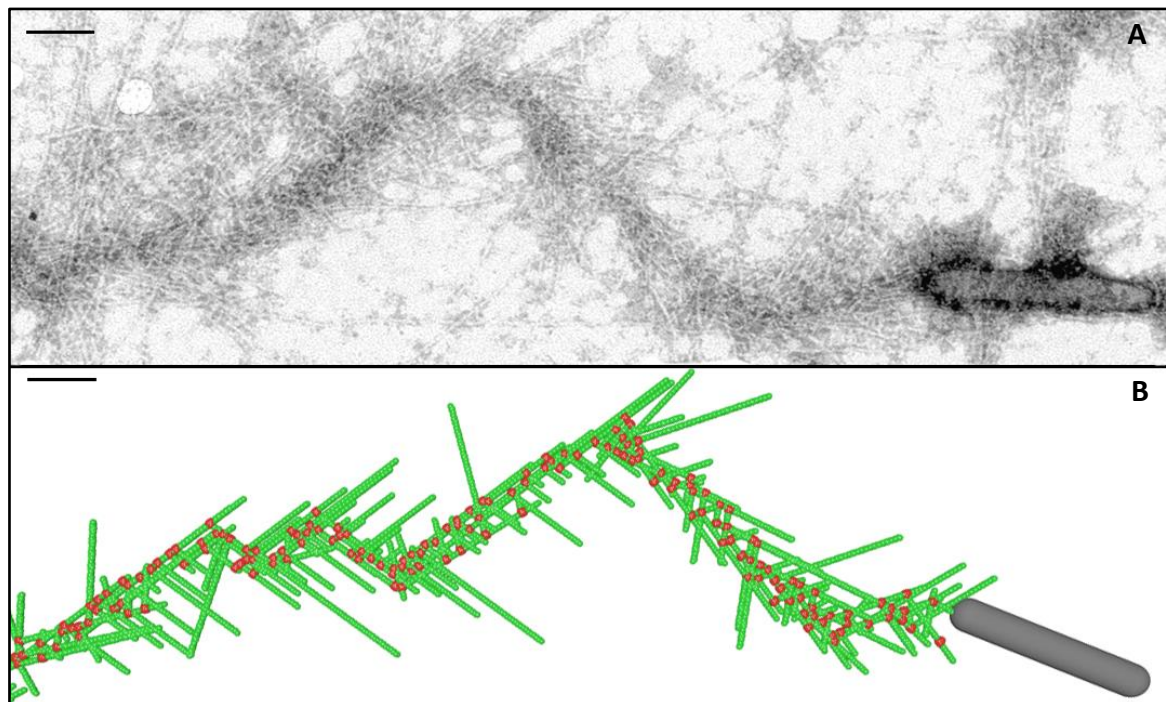


Figure 16: The model results fit the observed data. **A** Electron micrograph from a negatively stained baculovirus in a CAR fibroblast. **B** Simulation matching the example in **A**, filaments in green and branch points in red. Bars: 100nm

De-novo nucleation

One of the first findings was that branching alone is not sufficient as a source of new actin filaments. If filaments only branch from pre-existing mother filaments, the virus flips over sooner or later and when the last filament loses contact with the rear of the virus movement stops (Two examples are shown in Figure 17).

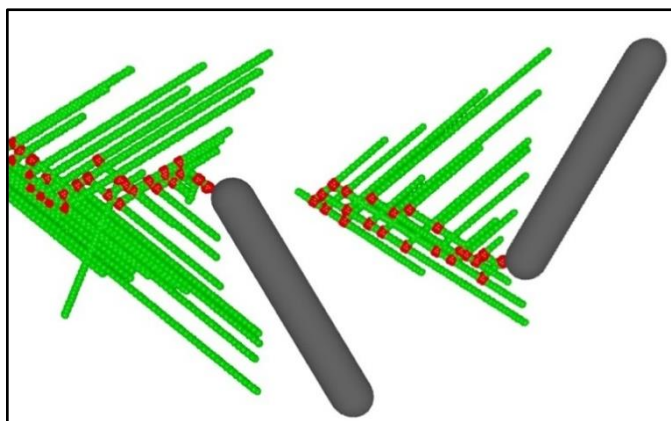


Figure 17: Two examples of simulations without *de-novo* nucleation. Note that branching is no sufficient source of filaments during pathogen propulsion

50 simulations were analysed, each starting with one branched filament in the middle of the virus rear and using the parameters listed in Table 1 except that the nucleation rate was set to 0. The virus moved on average for 6 seconds and reached a mean displacement of $1.5\mu\text{m}$, with a standard deviation of $1.2\mu\text{m}$. The average resulting tail consisted of 152 filaments all connected to each other via branches in one sub-set with 14 fold more filaments than in the wild type simulation. The speed and the filament density were the same as for the wild type simulation. In conclusion one sub-set of filaments was insufficient to reproduce continuous movement.

Steric interactions

Untethered and uncapped filaments continue with polymerisation and since they do not experience a counter force they grow with maximal speed. Thus, they can overtake the virus end and stabilize the virus track by prohibiting narrow curves. As the model is only two dimensional and the filaments are modelled as stiff and fixed rods, some unrealistic artefacts were observed. One long single filament counteracts the rest of the actin tail without bending or sliding over the virus (see Figure 18a). In such cases the influence of untethered filaments was clearly over estimated. This led to the question whether the restriction of the overtaking filaments is necessary at all.

To this purpose the steric interaction of untethered filaments and the virus were excluded (Figure 18b). The resulting trajectories show that their stabilizing contribution is not re-

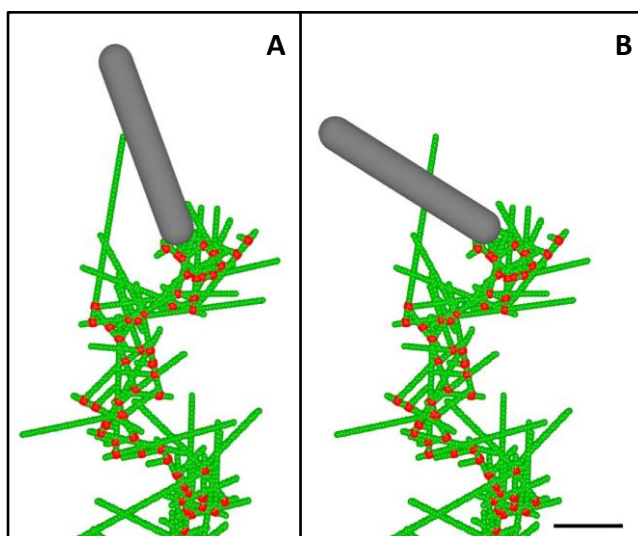


Figure 18: Two model variations. **A** Unproductive filaments have a steric interaction with the virus **B** Unproductive filaments do not interact with the pathogen. Bar: 100nm

quired. Without this restriction the virus could cross its own tail resulting in a looped trajectory, which was also observed in electron microscopy images (Figure 19).

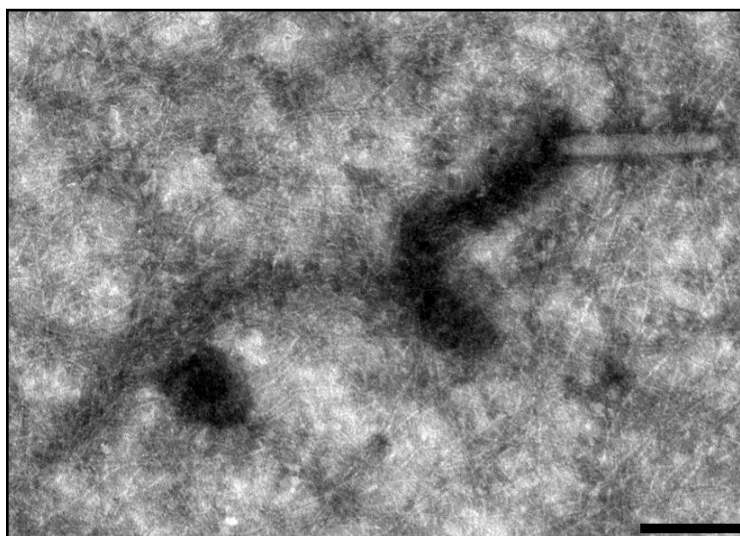


Figure 19: Electron micrograph of a negatively stained baculovirus induced actin tail inside a B16 cell. Scale bar 200nm.

Branching direction

One of my working hypotheses was that branching of the filaments occurs exclusively towards the virus surface. In order to challenge this assumption the branching directions were randomized by using the MATLAB random number generator, such that the filaments branch in half of cases towards, in the other cases away from the virus.

From analyses of 48 minutes of simulation of virus movement the following deviations were observed: The speed of the virus slightly decreased to $12.8\mu\text{m}/\text{min}$ and the straightness of the path exhibited an average turning angle of 25° similar to the wild type simulation (Figure 25). The branch density rose to 115 branches per μm tail and the inter-branch distance changed slightly to 32 instead of 35nm. Moreover the average size of a sub-set of filaments dropped to 6 and the average number of pushing filaments at any time diminished to 3.5. Notably the number of filaments per μm tail was considerably higher (136) than the wild type (114) indicating that many filaments are unproductive since they grow away from the virus surface and never push the pathogen.

The mean angle between filaments and trajectory was 59° (Figure 24), significantly different than the standard simulation and the experimental data ($P < 0.0001$).

In 29% of all cases the angle between filament and trajectory exceeds 90° , which means that they cannot contribute to the propulsion of the virus. In comparison, in the wild type simulation only 7.5% of the filaments form an angle larger than 90° with the tail axis. Random branching led to an unusual structure of the tail (Figure 23c).

Tethering

Many studies suggest that the actin filaments of comet tails are tethered to the pathogen⁹⁰. It is not clearly understood by now if these tethering connections are indispensable

ble for effective movement. To this end I abolished the tether in the model by reducing the maximal deflection of the spring to zero. The outcome differed clearly from the wild type simulation and the experimental data in many aspects. Generally the tracks appeared more curvy and random. Due to the absence of a link between tail and virus the pathogen wiggled around and lost contact, since the filaments could not correct for displacements caused by Brownian motion. (Figure 23d)

Movement was only continuous if the number of new filaments was increased resulting in significantly lower number of filaments per sub-set (3.6) as compared to the wild type (11). This meant that more than every third filament was derived from *de-novo* nucleation instead of branching. The interrupted propulsion led to a less persistent path with decreased straightness, which was reflected in an average turning angle of 51° (Figure and Figure 25). Although a higher filament density was measured within the tail (140 filaments per μm tail compared to 113) the average number of pushing filaments at any time point was only 2.5 instead of 4.1. The instability was also reflected in the distribution of the angles of filaments to the trajectory (Figure 24) with a mean value of 60° . All these factors had an impact on the velocity of the pathogen ($12.9\mu\text{m}/\text{min}$) and the average filament length which was 14nm shorter than the wild type.

Branch tether

The exact mechanism of tethering is yet unknown. Theoretically, there are several options how the connection between the filaments and the pathogen could occur. In the wild type simulations the tether linked the barbed ends to the virus.

Yet it could also be the case that tethering only exists between the Arp2/3 complex and the virus via the p78/83. This kind of connection would be transient occurring only during branching events.

To test this variation of my model the tether was implemented in a way that the nature of the tether itself stayed the same, but it linked the branch point instead of the barbed filament end to the virus surface.

The resulting simulations drew a clear picture. Analysis of 55 min simulations revealed clear differences to the wild type. (Figure 20 and Figure 23) The persistence of the trajectories with tethering during branching diminished al-

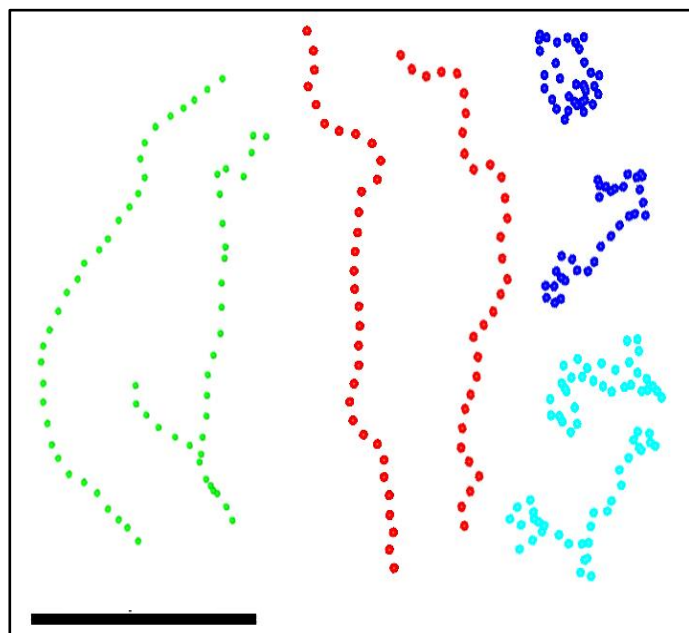


Figure 20: Comparison of comet tail tracks observed by live cell imaging (green) with simulated tracks for the cases of wild type (red), untethered (dark blue) or filaments tethered during branching (light blue). Bar: $10\mu\text{m}$.

most to the level of the untethered simulation. The straightness dwindled to a level of 0.34 and the turning angle increased to 44.4 (Figure 25). Also the appearance of the actin tail was more similar to the non-tethered version. (Figure 23b,d) The mean angle between filaments and trajectory was 65° (Figure 24) and the size of an average sub-set was with 3.9 filaments only a third of the wild type. The number of pushing filaments (2.7) was also too low.

In conclusion that the branch tethered simulation was similar to the untethered simulation and both differed from the experimental data as well as from the wild type simulation.

Up scaling

Given that the wild type simulation mimicked the experimental data most accurately the question arose as to whether the parameters of this system were applicable to tails propelling pathogens of different sizes. For this purpose I simulated hypothetical giant baculovirus with a length of 3 μ m and a diameter of 500nm. The same input parameters were used (see table 1), except that the number of maximal tethered filaments N was increased to 100 instead of 10, to allow for the 10 times increase in surface area of the pathogen's rear. Additionally, the Brownian motion was also adapted according to the pathogen's size. Although the virus area was

100 times larger than the normal baculovirus, the speed of the giant virus increased to 18.9 μ m/min. Instead of 4 pushing filaments 10 actin filaments were engaged in propulsion (Comparison of wild type and enlarged virus is depicted in Figure 21). The architecture of the tail and also the curvature of the track were reasonable. The trajectory was almost straight with an average turning angle of 7° (see Figure 22 and Figure 25) and therefore hardly any new mother filaments had to be created *de-novo*. The mean sub-set consisted of 435 filaments, which meant that only every 8 μ m a new sub-set was initiated. The orientations of the filaments in respect to the core tail axis were almost as expected from an ideal tail. The mean value was 40° and the distribution displayed in the histogram could be approximated by a symmetric exponential curve (see Figure 24).

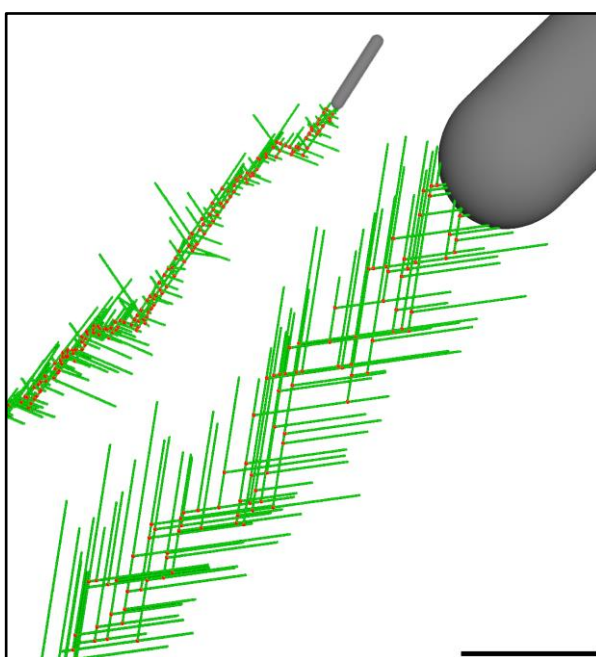


Figure 21: Normal sized and enlarged virus with their actin tail. Filaments are shown in green and branch points in red. Bar: 500nm.

The average filament length increased to 368nm, although the capping rate was unchanged. Also the inter-branch distance rose to 105nm, whereas in contrast the branch density decreased to 50%. A result of an increased filament length and inter-branch distance was a lower filament density as compared to the values obtained for a normal sized virus.

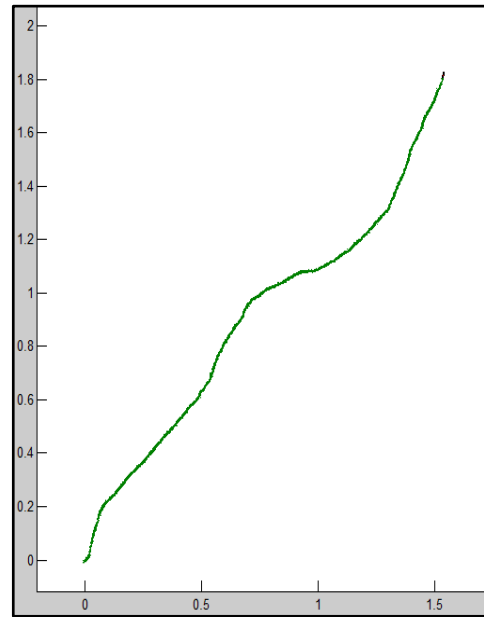


Figure 22: Track of the giant baculovirus. The axes are labelled in $10^2\mu\text{m}$ and the total time was 13.5minutes.

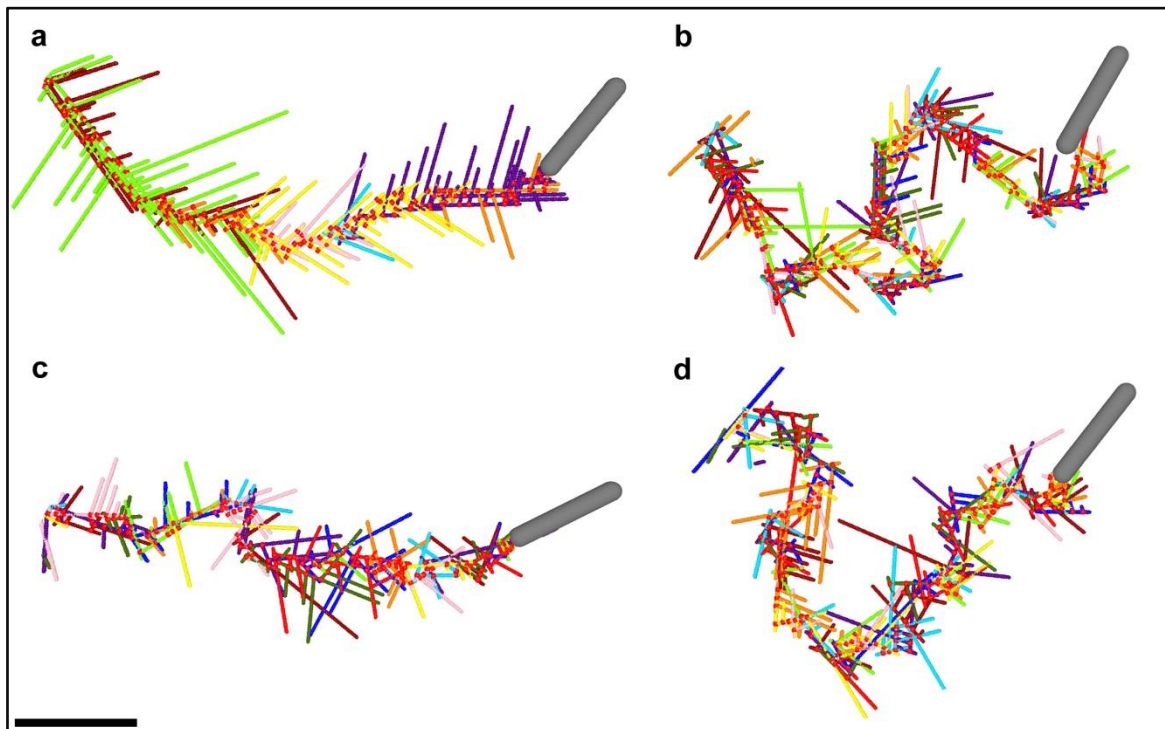


Figure 23: Simulated actin tails **a** wild type **b** branch tethered **c** random branching **d** untethered. Different sub-sets are depicted in different colours. Bar: 300nm

Histograms of angle to trajectory

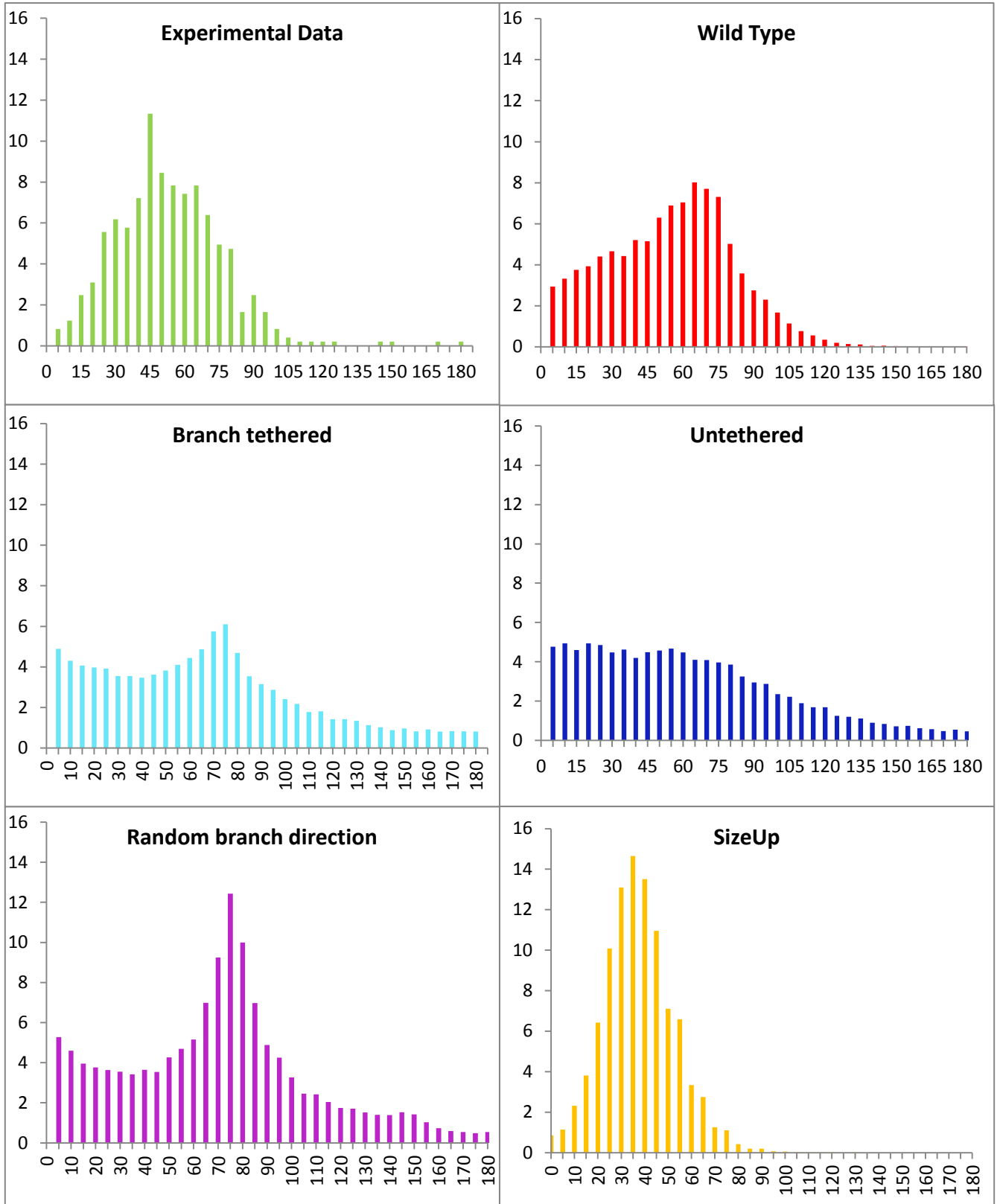


Figure 24: Histograms of angles between filament and trajectory from various simulation types. X-axis angles to trajectory measured in degree, y-axis relative frequency.

Histograms of turning angles

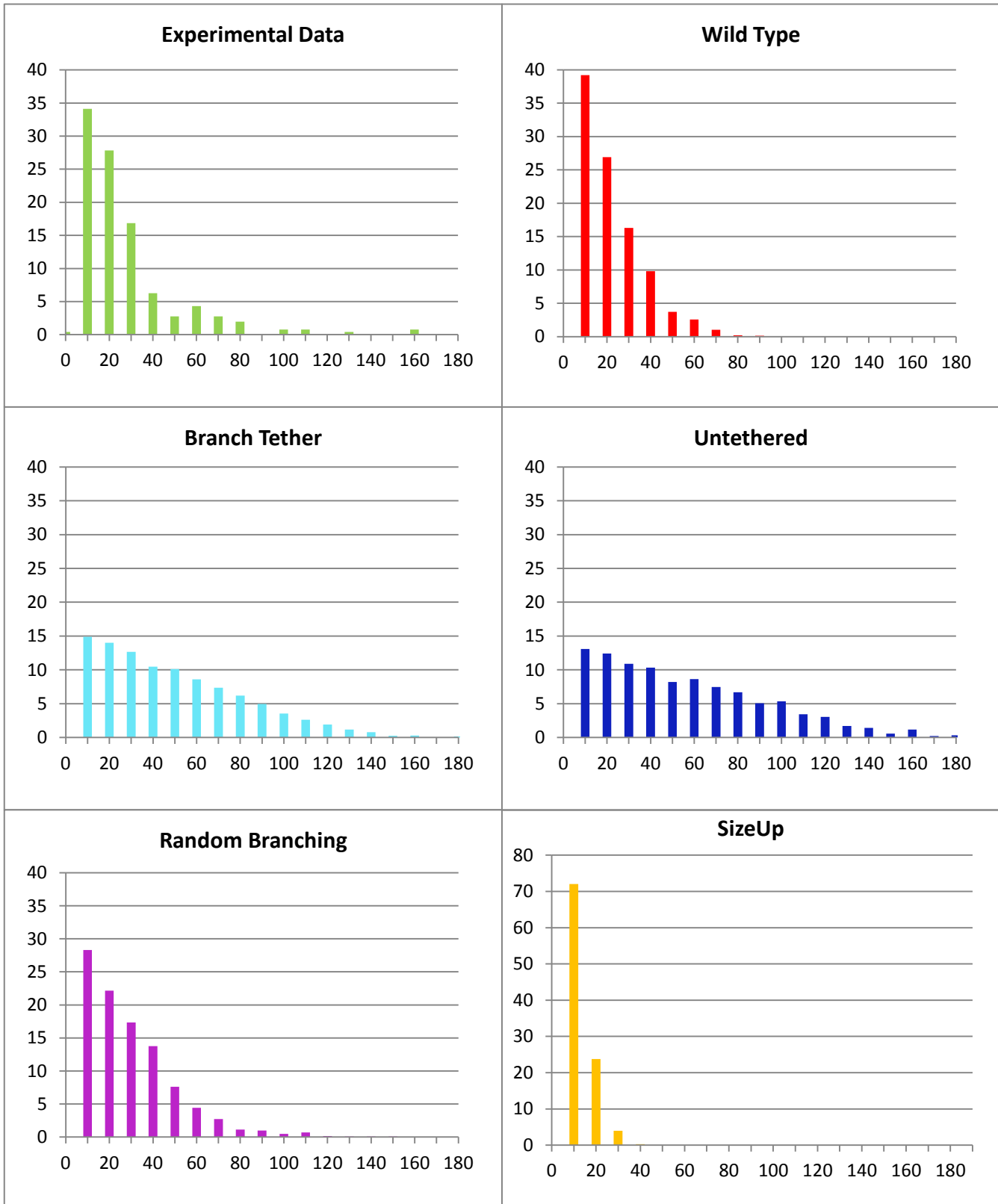


Figure 25: Histograms of turning angles of various simulation types. X-axis depicts the turning angle in degree and on the y-axis the relative frequency is plotted.

Biology

In order to confirm or reject the hypothesis of a common underlying mechanism of actin based propulsion I aimed to resolve the architecture of the vaccinia virus comet tail.

The vaccinia virus requires a safety regulation level 2, yet our laboratories lack access to S2 fluorescence light microscopes. Hence I was limited to fixed samples stained for actin using Alexa-Phalloidin. This method was sufficient to judge the tail formation and evaluate the optimal incubation time. The images provided also an overview of the nature and curvature of the actin tail. Figure 26 depicts HeLa cells after 7 hours after infecting the cells with vaccinia virus with a multiplicity of infection (MOI) of 7.

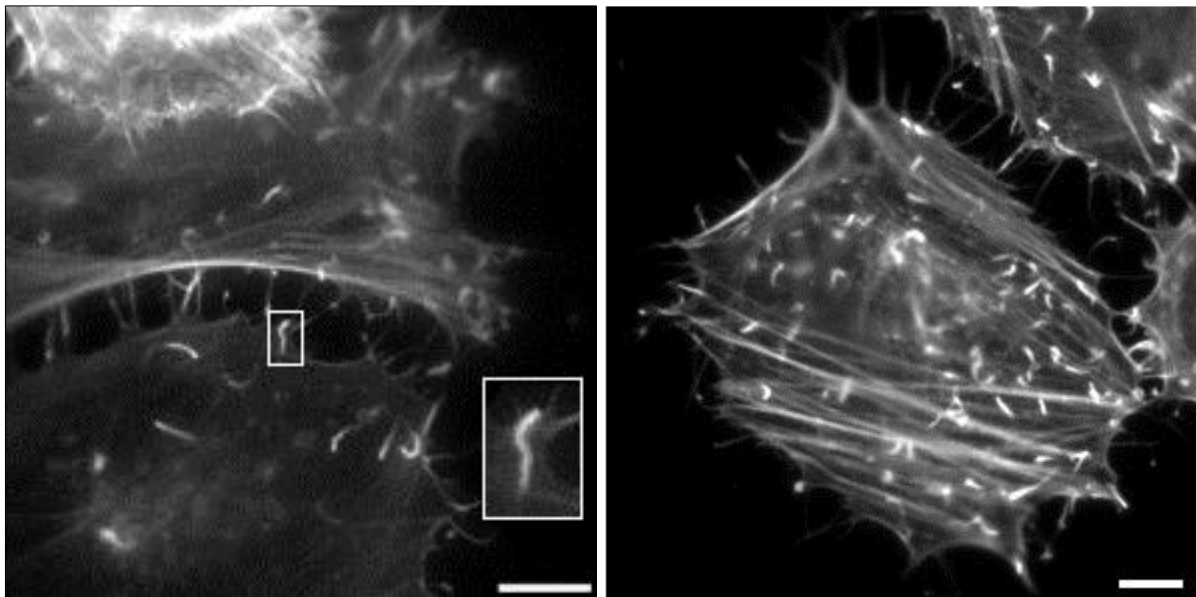


Figure 26: Alexa-488 Phalloidin stained HeLa cells 7 hours after infection with vaccinia virus. Bars: 10 μ m

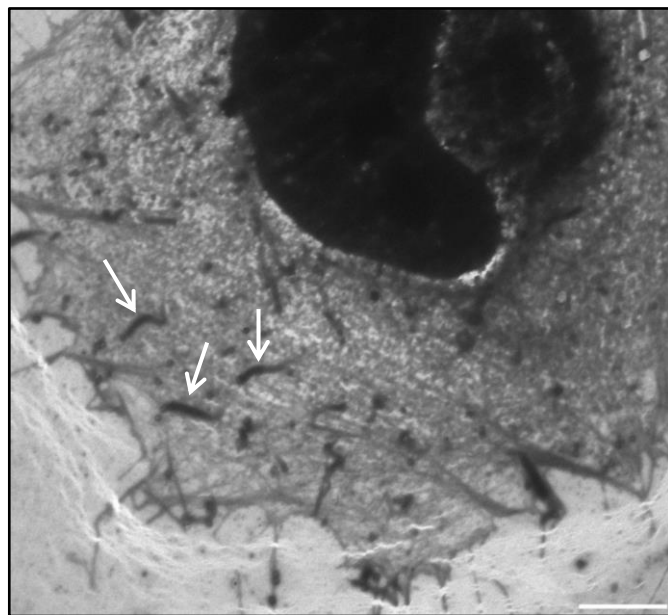


Figure 27: Electron micrograph of a negatively stained HeLa cell infected with vaccinia virus. Actin tails are indicated with white arrows. Bar: 5 μ m.

Negatively stained samples

In the next step the extraction and fixation for electron microscopy was optimized. It transpired that the vaccinia actin tails were very stable and they survived the strong extractions required to minimize background in the electron microscope. Figure 27 depicts a HeLa cell after 1 minute extraction with 1% Triton-X100 and 20 minutes of fixation with 2% Glutaraldehyde and 10 μ g Phalloidin. Some of the dense actin comet tails are indicated with white arrows.

Figure 28 shows a negatively stained vaccinia virion at a magnification of 93,000x. The virus particle was found outside the cell hence its protecting lipid layer might have been removed during the extraction procedure. The dappled surface is also clear to see in Figure 30. The dark black spots are 10nm gold fiducials. The inner structure of the virus can be seen in tomograms (Figure 36 and Figure 35).

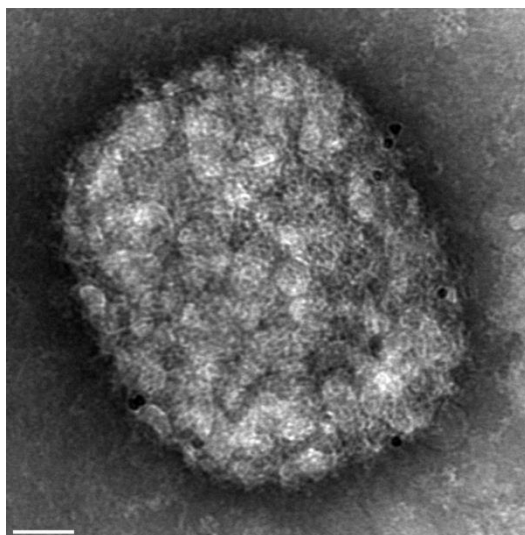


Figure 28: Electron micrograph of a negatively stained vaccinia virion. Bar: 50nm

In the beginning, I started with negatively stained samples for double tilt electron tomography. This method delivers a very strong contrast combined with a high electron-dose tolerance. Figure 29 is an electron micrograph showing two viruses and one actin tail. The virus, depicted on the left side of the image and marked with a white star, exited the cell, induced the tail, grew upwards and during fixation the tail flipped on top of the cell body. Figure 30 shows a 1 nm thick section of a tomogram; note that the virus in the tomogram was not the one associated with the actin comet. The tail was in general very dense and this feature was enhanced by the collapse of the whole structure. Vaccinia virus is at least 250nm across and therefore induces tails of similar dimensions. Yet the tail was only 100nm thick, which indicated that the structures were collapsed to half their height. Due to the densely packed actin network tracking of single filaments was not possible.

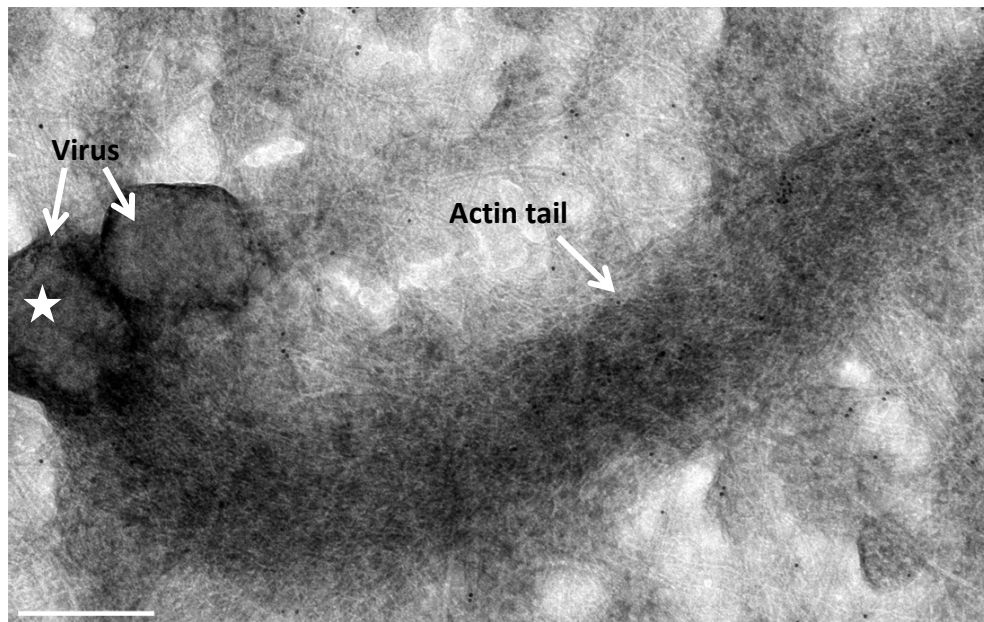


Figure 29: Electron micrograph of two viruses and one actin tail. The virus which induced the comet is marked with a white star. Bar: 300nm.

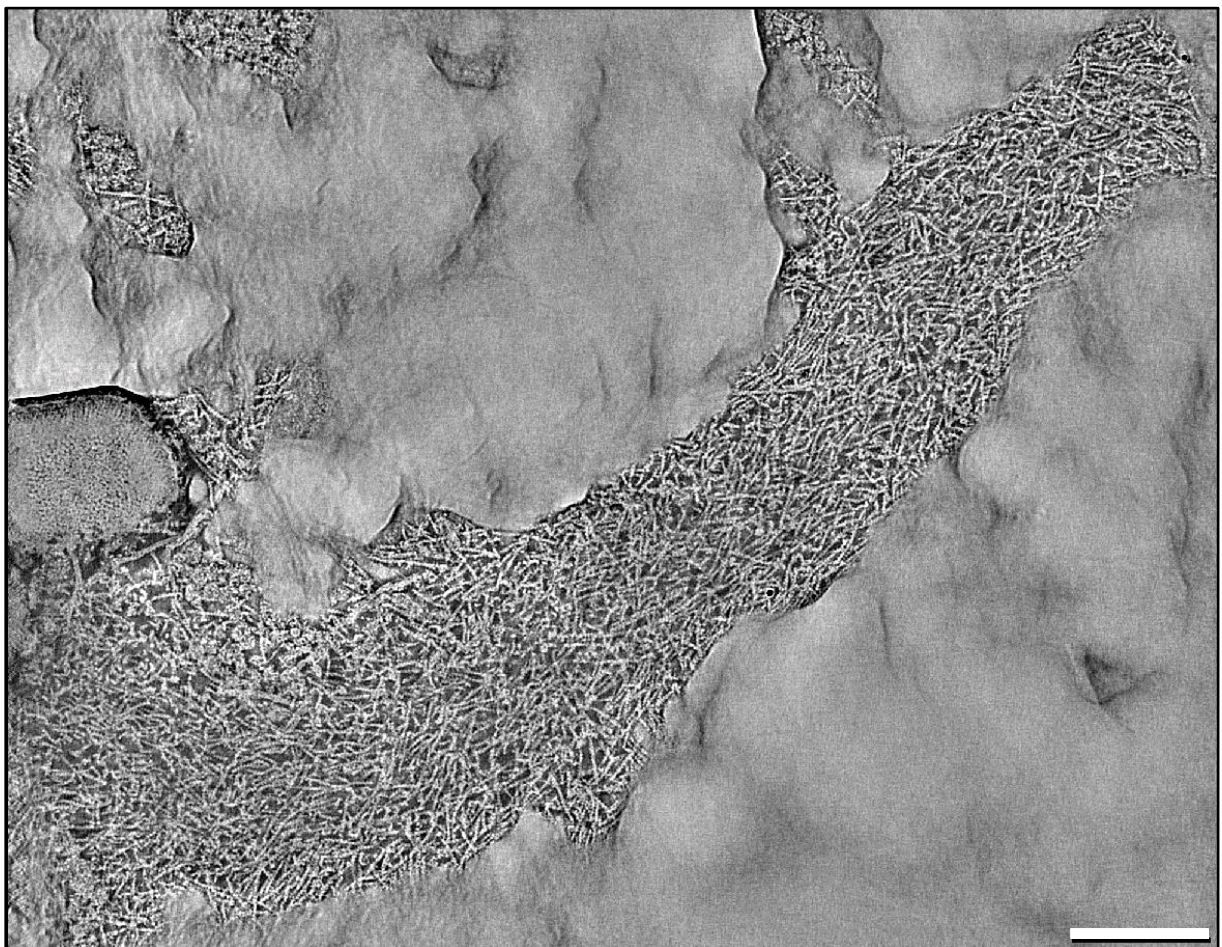


Figure 30 Section of a negatively stained double tilt tomogram showing a vaccinia virus and its actin tail. Bar: 200nm

One option to improve the quality of the tomogram was to acquire a tail, which grew laterally out of the cell and hence was not superposed on cytoplasmic structures. Figure 31 shows such a tail and the region of the acquired tomogram, depicted in Figure 32. The virus particle detached from the tail, possibly due to the Triton treatment. Note that the comet tail was flanked by a bundle of parallel actin filaments. The image in Figure 32 displays a 22nm thick section of the tomogram. Even though the tail height was collapse to 70nm the resolution within the tail was better and one could already recognise branch points (encircled in red). However the manual tracking as well as the automatic tracking program from Christoph⁹¹ delivered unreliable results.

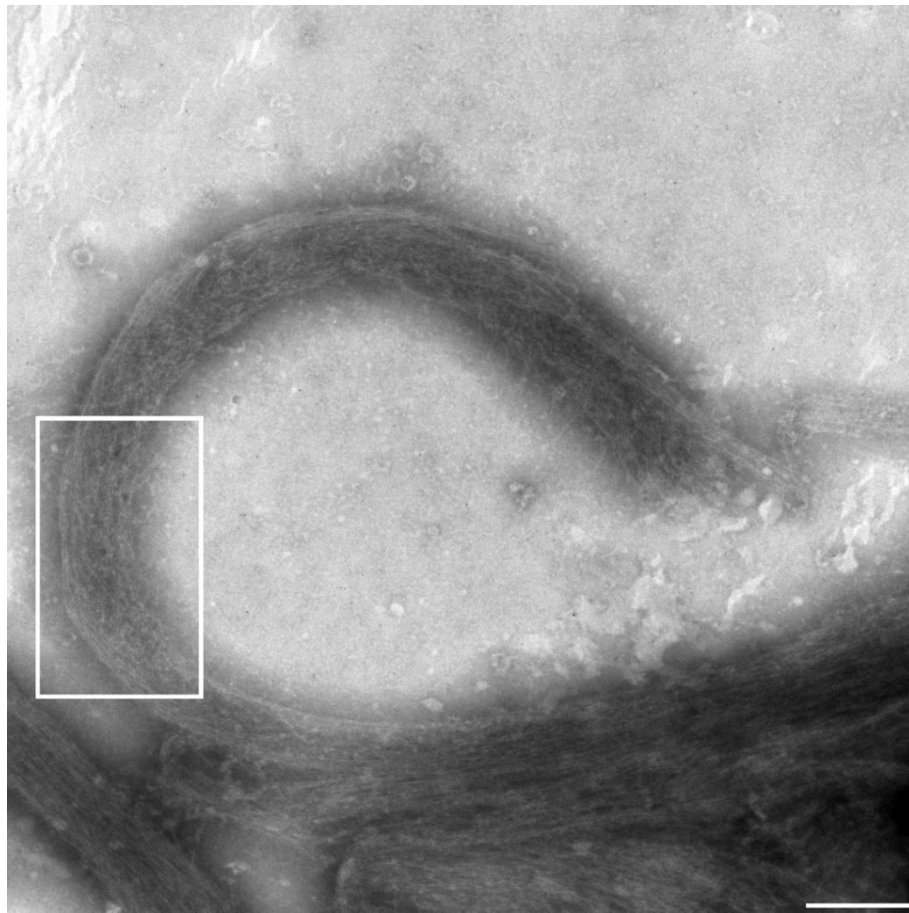


Figure 31: Electron micrograph of a comet tail protruding at the cell edge. The white frame marks the area used for tomography. Bar: 500nm.

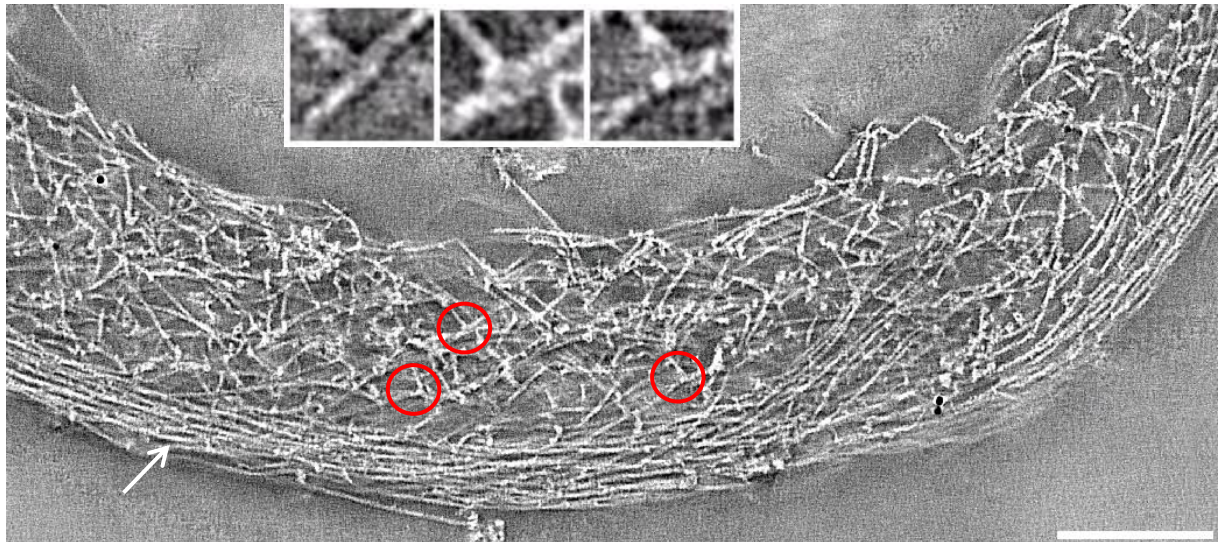


Figure 32: 22nm thick section of a negatively stained tomogram. The branched comet tail is flanked by a bundle of parallel actin filaments. Branch points, which are depicted in the inset, are marked with red circles the parallel bundle is indicated with a white arrow. Bar: 200nm.

Another strategy for optimizing the traceability of actin filaments was to focus on the rear of the tail, where the network gets already sparser. Figure 33 indicates such an area, and the corresponding tomogram is displayed in Figure 34. The slice thickness is 10nm and besides actin there are also microtubules, indicated with black stars. Although this tomogram had the least collapse, the tail is still 110nm thick, one could clearly see the quality difference between cytoplasmic actin and virus induced filaments. While the twist of the actin helix could be seen in the surrounding filaments, the actin within the comet was still not clearly enough depicted to track single filaments.

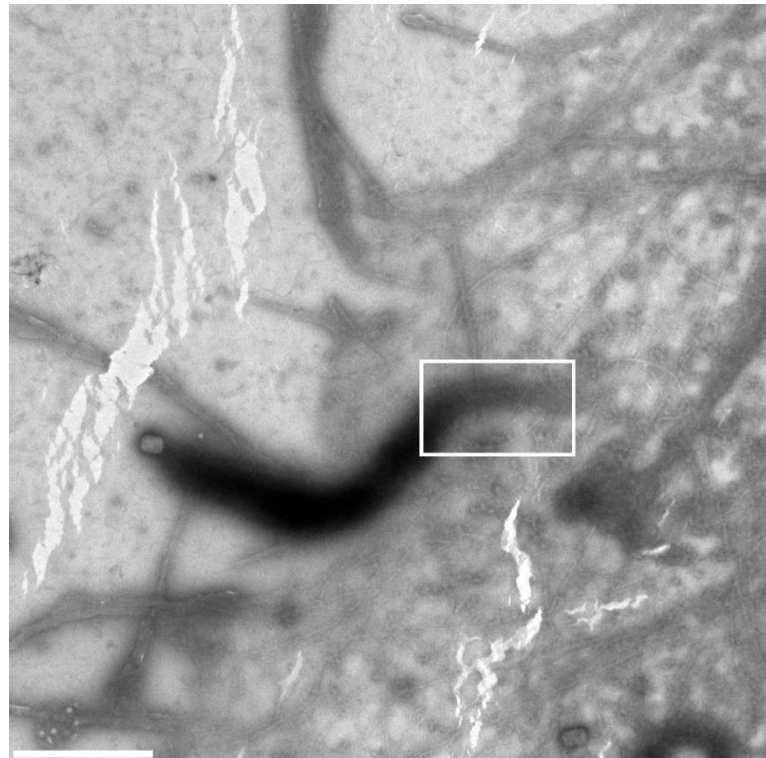


Figure 33: Electron micrograph of a vaccinia virus and its actin tail protruding at the cell edge. The white frame indicates the area used for tomography. Bar 500nm

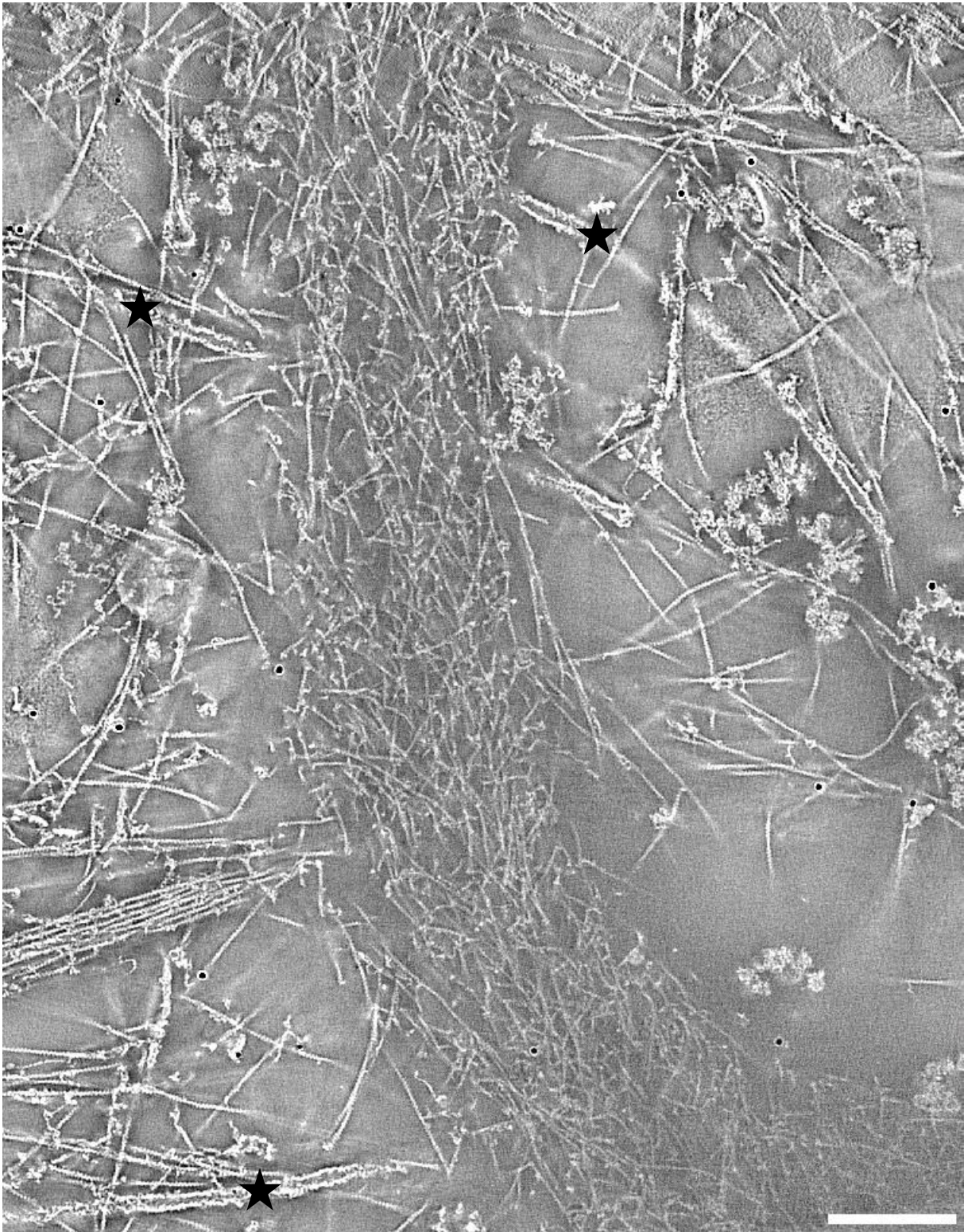


Figure 34: Section of a negatively stained tomogram of the actin tail's rear. Microtubules are indicated with black stars. Bar: 20nm

Cryo samples

Since negative stain compresses the samples in the z-dimension and therefore the filament density becomes artificially increased, tracking single filaments was impossible. Cryo preparation of the samples should prevent the collapse observed after negative staining, yet the drawback is a weaker contrast and a higher sensitivity to radiation damage.

Figure 35 displays a 10 nm thick section of a cryo tomogram. The thickness of the actin tail was 180nm, which confirms a better sample conservation in all three dimensions. Note the bundle of parallel actin underneath the comet tail. Single filaments were easily recognised, yet there was still too much back ground signal derived from proteins, which remained in the centre of the tail.

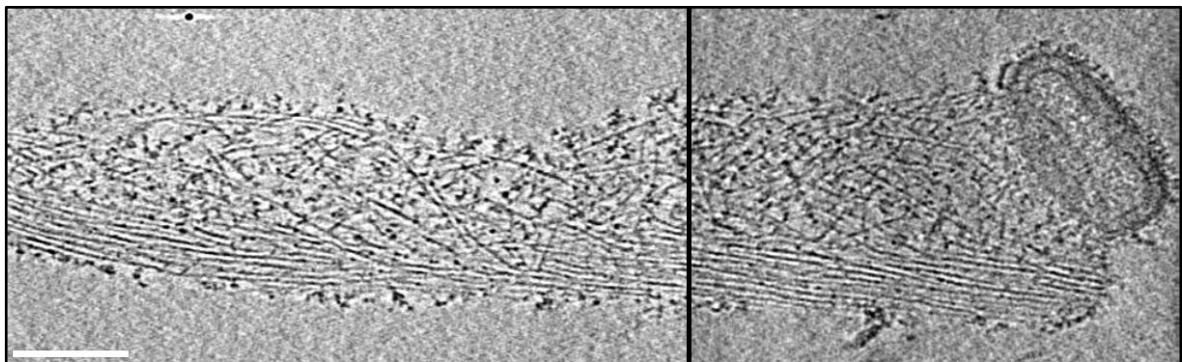


Figure 35: 10nm thick sections of a cryo tomogram. The z-level of the left part of the picture is 8.7nm below the one of the right part. The tomogram includes a Vaccini virus with its comet tail and an adjacent filament bundle below. Bar: 200nm

Figure 36 shows a series of section through a 225nm thick tomogram. Each section is 7.5nm thick and the difference between two consecutive parts is 37nm. Again there is an adjacent filopodia above the actin tail indicated with a white arrow. Cryo electron tomography revealed also detailed information of the virus and its surrounding coat. Black arrows indicate the envelope and the orange arrow indicates the interface between the virus and the actin tail where the viral nucleation-promoting factor A36 is located.

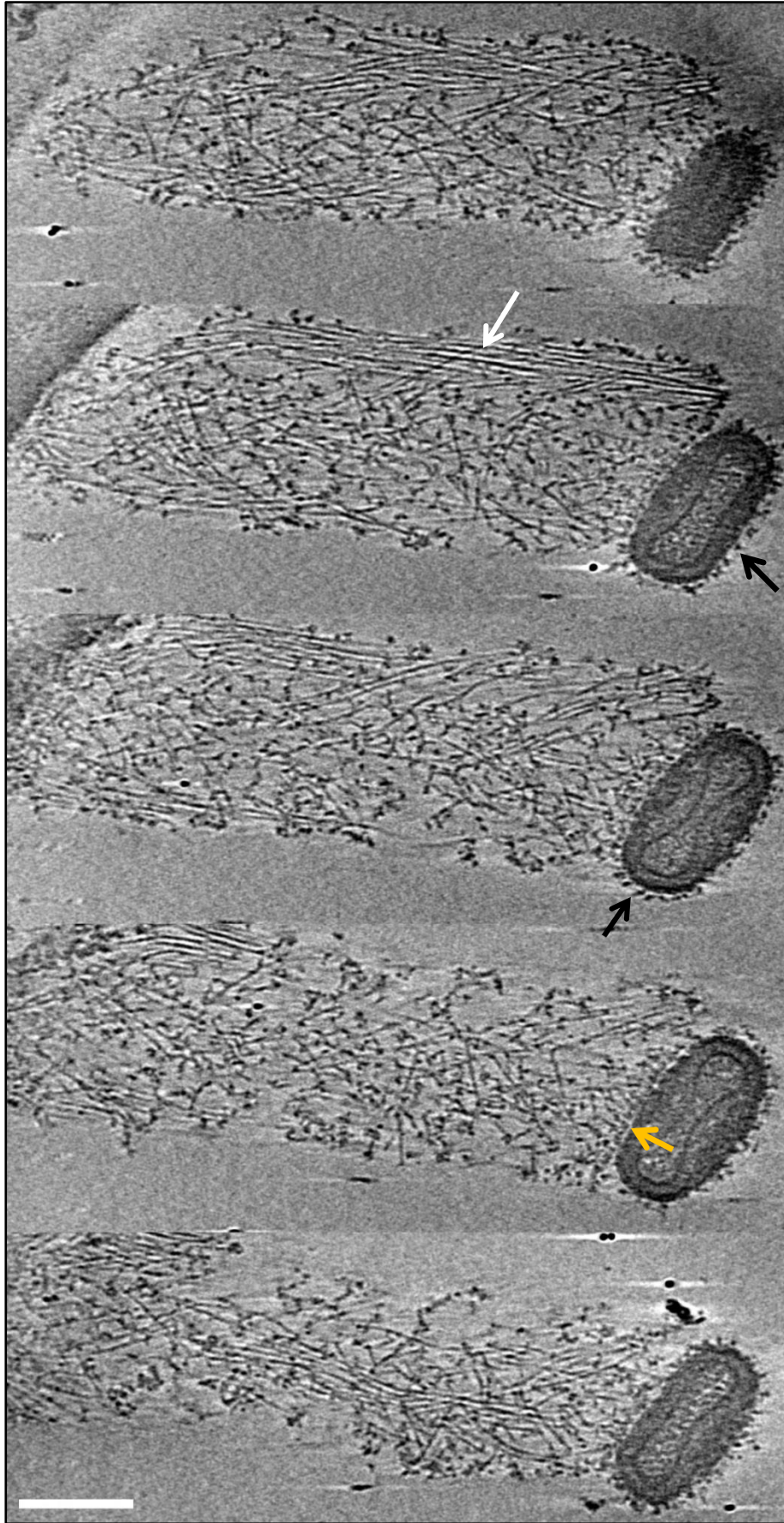


Figure 36: 5 sections of a cryo tomogram, each section is 7.5nm thick and the difference in z-direction between two consecutive pictures is 37nm. The parallel bundle is indicated with a white arrow. Note the coat surrounding the virus particle (black arrow). Bar: 200nm.

The adjacent parallel bundle was a common feature in many of the comet tails. Its appearance might be similar to filopodia or microspikes. In order to test this hypothesis I acquired a cryo-tomogram of a parallel actin bundle protruding from the cell edge. The well-aligned filaments indicated with a white arrow showed indeed high similarities with the structures found beside comet tails. Figure 37 displays a 10nm thick section of the tomogram, which had a total height of 120 nm.

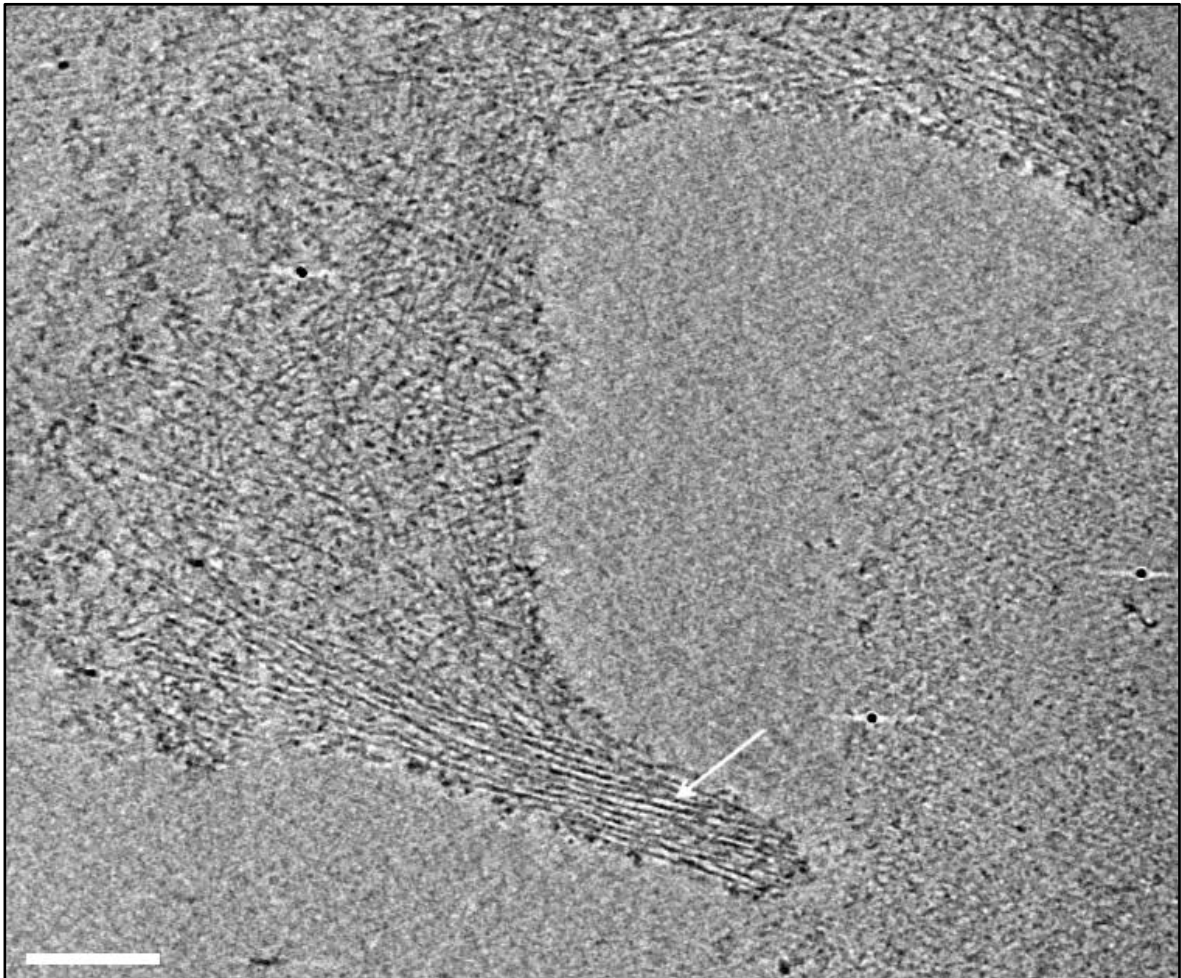


Figure 37: 10 nm thick section of a cryo tomogram. The area includes the cell edge with a small filopodia/microspike. The white arrow indicates the parallel actin bundle. Bar 200nm.

Discussion

Biology

For almost 20 years it has been known that vaccinia virus induce actin tails to improve its cell to cell dissemination. The average comet is 6-10 μ m long⁵⁹ and propels the pathogen with a mean velocity of 280nm/sec.⁹² Recent studies have shown that actin polymerisation is not only required for the translocation of the virus but it plays also a role in releasing the virus from the cell membrane.⁹³

Although the importance of the comet tail has long been known, there limited information on its structural organization. Cudmore *et al.*⁵⁹ presented an electron micrograph of a section of an embedded comet tail (see Figure 38). Actin filaments, decorated with S1 myosin, are recognizable at the edge of the tail, but the internal structure is unclear.

Therefore, my aim was to acquire electron tomograms to gain detailed structural information at single filament resolution of the actin tail architecture.

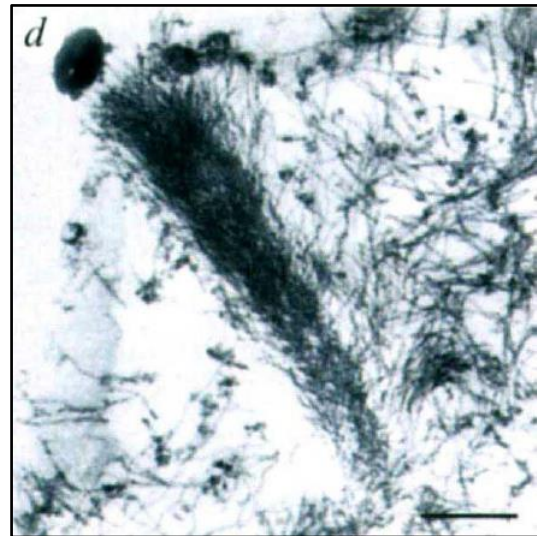


Figure 38: Electron micrograph of an actin tail decorated with S1 myosin embedded in EPON. Bar: 400nm

Negatively stained samples of vaccinia induced actin tail confirmed the suggested fish-bone like structure⁵⁹ and revealed the presence of branch points in the network (Figure 38). The branch junctions were very similar to those found in the lamellipodium.⁹⁴

As previously reported the process of negative staining leads to a 50% collapse in the z-dimension of lamellipodia.⁹⁵ The same effect was observed in my tomograms of comet tails, where the negative stain procedure reduced the mean thickness to 93nm in contrast to the cryo samples with 207nm. This collapse is less critical for regions, which are naturally thin and Vinzenz *et al.* showed that filament tracking is possible in negatively stained lamellipodia⁹⁴ as Mueller *et al.* did in baculovirus tails (Mueller *et al.*, submitted). Unfortunately, this was not true for comet tails of vaccinia virus. Since the dimensions of vaccinia are 250*270*360nm one would expect a 250nm thick tail. This exceeds the optimal range for electron tomography. In Figure 34 one can clearly see the difference between cytoplasmic filaments close to the cell edge and filaments within the comet tail. The centre of the tail is unfortunately too thick and does not allow sufficient penetration of the electron beam. Even though the tomogram in Figure 34 is acquired at the end of the actin tail, where depolymerisation already takes place, the contours of the filaments inside the comet are unresolved.

One possibility to obviate this problem would be to use sectioned material. This approach requires either plastic embedding or sectioning in vitreous ice, however, both procedures are yet unsuitable for visualising cytoplasmic actin. Therefore, I hoped to obtain better results using plunge-freezing and cryo electron tomography^{96,97}. In this case, the structures are surrounded by vitreous ice, which avoids collapse in the z-direction.

Figure 35 and Figure 36 are good examples of what I was able to obtain. The preservation of the natural configurations compensates for the lower contrast. Improvement was especially observed regarding the virus. Interesting details of the surrounding envelope were revealed. After exiting the host cell, the virus is only wrapped in one membrane, which contains various proteins.⁹⁸ It is questionable how much of this lipid layer survives the Triton extraction, yet one can clearly see some structures surrounding the virus.

Additionally, the interface between virus and actin tail is well preserved. In Figure 4 the orange arrow highlights a line of particles with high electron density. The particles could contain the viral protein A36, which is essential for actin tail formation. Note that the actin filaments are in contact with this surface, which supports the tethered filament hypothesis I suggested earlier.

Unfortunately it was not possible to obtain sufficient filament resolution to track all the filaments and compare the tail architecture with the one from the baculovirus (Mueller *et al.*, submitted). Either a new embedding approach, which conserves filament better, or an improved automated tracking algorithm could bring the required improvements.

A very interesting observation was the adjacent bundle of parallel filaments, which was found beside the vaccinia induced comet tail in approximately 40-50% of the cases. From the tomograms in Figure 35 and Figure 36 it is clear that the virus itself does not initiate the unbranched actin structures.

A comparison with filopodia revealed high similarities, which suggest that the actin tail is sometimes flanked by a filopodia-like structure. There are different possible mechanisms describing the initiation of filopodia^{37,99} but nevertheless it is known that formins are found at the tip of these structures.¹⁰⁰ With my existing data I am not able to distinguish whether the filopodia is formed first and promotes tail formations or comet tails facilitate filopodia formations. Filopodia/microspikes move sideways along the cell edge and if they would meet a vaccinia virus, which is recruiting all the necessary proteins, the filopodia could trigger the protrusion of the tail. On the other hand, it is also possible that during actin tail initiation also formins are close by and the membrane curvature induced by the comet tail could facilitate filopodia formation. Future experiments like fluorescence live cell imaging could answer this question. In the next step one could also perform immune staining for formins and examine the existence of formins at the tip of these parallel bundles.

Mathematics

Cell motility has attracted the attention of scientists from various fields including mathematics and biophysics. Actin based propulsion of pathogens is an acknowledged model system and together with the *in vitro* reconstitution of this motility system¹⁰¹ provided insights also into the mechanism of lamellipodia protrusion^{102,103}. A diversity of ensuing theoretical models describing the mechanism of actin propulsion was proposed^{104,68}. Some models focused on actin dynamics⁶⁵ others on the mechanism of force transduction^{105,79,69} and some tried to cover the whole range of existing processes and simulate all of them at once⁸³. So why do we need another model? First of all, none of the existing models took into account the structural organization of the comet tail, since it has not been known. In light of the new structural information I could re-evaluate the existing models.

The elastic gel model^{69,106} could be excluded immediately. This model of an actin continuum squeezing the pathogen forward is based on the assumption that there is a dense filament network surrounding the pathogen. This mechanism cannot be excluded for larger pathogens, such as *Shigella flexneri* or *Listeria monocytogenes*, which provided the experimental background¹⁰⁷, but cannot explain the movement of the baculovirus, since only 4-5 filaments are involved in pushing.

The extensive approach used by Alberts and Odell⁸³ has the disadvantage that the value of many parameters was assumed from *in vitro* data and analysis required extensive computing power. I chose to use fewer parameters in order to simplify the model and avoid biased assumptions about protein kinetics.

I also had to consider the question of whether or not filaments are tethered to the pathogen. Dickinson and Mogilner support opposing theories, yet several experiments suggest a stable connection between pathogen and comet tail⁹⁰.

To shed more light on this discussion I decided to test both assumptions and examine the role of tethering in virus motility.

Measurements

On one hand, I aimed to simulate the organization of the tail to generate values for the inter-branch distance, the angle to trajectory and the size of sub-sets.

On the other hand it was also important to reproduce the dynamics of the trajectories. For this purpose, the velocity of the virus and the straightness of the tracks were taken into account.

From my analysis the filament length and the tail length had no influence on the model outcome (not shown).

Wild type simulation

The wild type simulation fulfilled its purpose to reproduce the observed experimental data quite well. For the features which were used during optimization (velocity, inter-branch distance, size of the sub-sets and filament length) this was no surprise, but the accuracy of other parameters, which were predicted by the model and cross-checked afterwards, confirmed the quality of the model. The turning angles of the tracks, as well as the filament angles to trajectory showed no significant difference between measured and simulated data.

The only large mismatch was found in filament and branch densities, which was explained by the inherent limitations of matching a 2-dimensional model to a 3-dimensional structure. Nevertheless, the simulation mimicked the structural and dynamical features of the comet tails well enough to test several hypotheses and to explain the main processes driving pathogen propulsion.

***De-novo* nucleation**

Why is *de-novo* nucleation of new mother filaments necessary?

Pushing an elongated object at one end is an unstable process, comparable with the task of balancing a broom on its tip with one finger. One solution is to correct small fluctuations immediately and bring the object back to the instable equilibrium position with a minimal application of force. In contrast to a person balancing an object, the virus system lacks any kind of perceiving control mechanism. Hence the comet tail must solve this instability problem in another way.

The solution can be found in the architecture of the tail. Due to the fish-bone like structure filaments push with an average angle of 51 ($n = 485$) to the core axis. Since approximately half of the applied force of such a filament twists the virus while the other half pushes the virus forwards, minor direction changes can be corrected easily.

The distribution of filaments along the rear semicircle depends on intermittent branching events and since this process is a stochastic event, fluctuations are unavoidable. If the pushing force is on one side larger than on the other, the virus starts turning. Once all the tethered filaments are located on one half of the semicircle, there is no way to regain the stability through branching. The turning of the virus continues until the last filament detaches from the P78/83 covered region.

However the baculovirus can move effectively and my simulations suggest that additionally to the branching also new filaments are recruited to the virus surface.

The source of such new filaments could either be nucleation at the virus surface, or capturing filament seeds from the surrounding potentially provided by recycling of short filaments from the depolymerised end of the tail. Since the exact process is not clear all these processes are collected under the term *de-novo* nucleation, in order to distinguish them from filaments branched from the pre-existing tail.

Several reasons back up the theory of additionally recruited filaments.

Firstly, the actin tail has to be initiated somehow in the first place and the very same mechanism can act as a source for filaments during propulsion.

Secondly, tracking of the filaments in our tomograms shows that the actin network is build up by more than one sub-set. New sub-sets can be found especially in regions of narrow curves.

Interestingly, two other models also require filament generated through both branching and *de-novo* initiation^{77,83}.

Steric interaction

At the beginning of my simulations we speculated about the stabilising mechanism, which allows persistent trajectories. Dealing with 4 to 5 pushing filaments the balance of our system seemed more fragile than for example the actin tail of *Listeria monocytogenes*. Inspired by a similar problem, namely how to balance a raw egg, I thought that the untethered filaments, which overtake the virus, might act as an eggcup-like support. Electron micrographs showed that filaments often protrude beyond the virus rear and it seemed possible that they could restrict wobbling of the virus.

Therefore steric interaction was modelled initially between all filaments and the virus. The resulting tracks looked promising, but some artefacts were generated.

In cases where the virus was already turning a single fast growing filament would push the pathogen and correct its path (Figure 18a). However these long filaments exerted forces far above that considered possible for a single actin filament. This feature highlighted one limitation of the 2-D model. In reality filaments would rather slip above or below the virus instead of sliding along its long side. By incorporating this modification in the model comets could form loops, like those observed for tails *in vivo* (Figure 19).

Furthermore it avoided unrealistic situations in which the virus could crash into its own comet tail and stop.

Without steric interactions between unproductive filaments and the virus the simulation's results were surprisingly good, because all features of the tail matched the experimental data nicely, also the straightness of the trajectories. From this observation one can conclude that the stabilizing factor of the actin cup surrounding the virus is not essential for persistent movement.

To test this hypothesis one could perform an *in vitro* assay with increased capping protein concentration to inhibit the outgrowth of filaments. Unfortunately the *in vitro* assay carried out by Christoph Le Clainche did not produce any motile baculoviruses, although other assays with bacteria, beads or lipid vesicles work nicely. Actin comet tails were formed behind the baculovirus but movement was not detectable under the light microscope.

Branch direction

I initially assumed that actin filaments branch always towards the virus. Electron microscopy supported this idea, since there are hardly any filaments growing backwards relative to the tail.

In an ideal tail the filaments would grow with an angle of $\pm 37^\circ$ to the main tail axis. This implies that every mother filament, which is orientated in a way that its plus end is pointing to the right side of the tail ($+37^\circ$) must always produce a daughter filament branching towards the virus with an angle of -37° . A branch in the wrong direction would result in a filament with an angle of 109° and this is barely ever observed. Only 1.65% of all filaments in the tomograms exceed an angle of 110° and these exceptions might be derived from analysis mistakes.

Nevertheless, I tested the necessity of directed branching. Instead of choosing the branching side defined by the shorter distance to the centre of the rear semicircle, I allowed branching randomly towards and away from the pathogen. The results reveal some interesting insights. The virus moved against my expectations still quite fast and straight, which indicates that the stability of the movement did not exclusively rely on the biased branching direction. It seemed as if the system could compensate for filaments growing backwards by increasing the number of total filaments.

But notably the histogram of the filament angles to trajectory showed clear alterations compared to the wild type simulation (Figure). More than half of the filaments formed an angle of $50\text{-}100^\circ$ with the tail axis showing a peak at around 75° . These filaments can either generate branches towards the virus leading to filaments which have a very small angle towards the trajectory or branches away from the virus with high angles to the trajectory.

Overall, the simulated architecture did not mimic the experimental data. Therefore the existence of favoured branch directions toward the virus is confirmed.

The exact mechanism of directed branching is still unknown. How is the decision made in which direction the filament should branch? Vinzenz *et al.*⁹⁴ showed that there is a periodicity in the inter-branch distance found in lamellipodia. These thin sheet-like structures can be simplified as a plane hence, filaments can only branch left or right. Analysis of the inter-branch distances showed that many measured lengths are a multiple of the 36nm, which is the period of the actin helix. From this one can conclude that the direction of the branch is determined by its position within this helix and the limited height of the lamellipodia restricts the minimal inter-branching distance.

On the contrary, inside the actin tail there are no structural limitations set by a surrounding membrane, which would hinder a filament from branching in any direction with unrestricted branch spacing. Nevertheless the branches and their orientation did not occur by chance.

One possible factor, which might facilitate this selection, is the viral capsid protein P78/83. Unfortunately there is very little known about the structure and the exact inter-

action of this NPF during branch formation. Sequence similarities suggest that this protein is closely related to members of the WASP-family as well as to the bacterial ActA found in *Listeria monocytogenes*⁶⁶ and immuno-labeling indicates that the capsid protein P78/83 is only found at one end of the virus¹⁰⁸. In order to activate an Arp2/3 complex the NPF has to bind it and then an actin filament has to be attached. It is possible that existing structural limitations and steric restriction favour newly branched filaments growing towards the virus. Fletcher suggests that actin bending could influence the branch localization¹⁰⁹, but it is questionable whether the same mechanism applies to baculovirus propulsion.

Tethering

Tethering or not tethering is an important consideration when it comes to modelling actin based propulsion. Models have been developed, which support one or the other theory but experimental data suggest that there is a stable connection between actin tail and load⁹⁰ favouring the tethered situation. VASP is such a potential linker between actin comet tails and pathogens.⁷⁹

Accordingly I included the spring like tether in my simulation, but what happens if there is no tethering link and no pulling forces acting on the virus? Constant turning and wiggling disconnected the virus partially from the propelling comet tail. Hence it seems that one of the main functions of these tethering linkages is to prevent detachment of the virus from the actin tail in the presence of Brownian forces.

In the untethered simulation the pathogen diffused away from newly branched filaments and even increased polymerisation speed could not always compensate for the Brownian motion acting on the baculovirus. In order to counterbalance the lack of pushing filaments new mother filaments had to be created in the model to maintain a constant number of pushing filaments. This resulted in an increased number of sub-sets per tail (39 instead of 10) and in fewer filaments per sub-set. On average sub-sets contained only 3 filaments, which indicated that after every second branch the filament network lost contact to the pathogen and was no longer engaged in pushing.

Interestingly, the virus velocity was not dramatically affected, but this could be an artefact of my simulations. Since I could not find any reliable rate of *de-novo* nucleation of Arp2/3 it might be that the process of new sub-set initiation is less frequent in reality and longer breaks would slow down the overall movement.

I should also mention another aspect, which could explain the mild effect on the speed. Whenever a new sub-set is formed, a mother filament and a daughter filament appear at the virus rear and start pushing the pathogen. But in the cell filaments would be rather pushed back, instead of moving the much larger virus forward. The filaments are modelled as immobile, because I assumed that as soon as they are connected to the tail and supported by other structures in the cytoplasm, they have enough grip to exert pushing forces. This assumption might be plausible for larger sub-sets within an intact

highly connected tail but not necessarily for such networks comprising only 3 filaments linked together in a sub-set.

An intermediate tethering situation was also tested, namely tethering during branching, where the linkage existed between the Arp2/3 complex and the viral surface. The Brownian tethered filament model from Mogliner¹⁰⁵ assumes also a rather short time of linkage compared to the untethered pushing state of a filament.

Yet in my simulation this kind of tethering was not sufficient to produce straight trajectories. The phenotype looked very similar to the untethered simulations and did not mimic the experiments, since the connecting tether existed only for a very short time. The maximal deflection of a spring was set to 2.75nm therefore the tether had to break as soon as one or two monomers were inserted at the barbed end. Statistically this corresponded to a mean life time of 10msec per branch tether and since there were approximately 1350 branches per second the virus was linked maximally during 22% of the time to its tail. This was apparently too short to stabilize virus in a persistent track.

Filament angles to trajectory

Besides the filament length and the number of filaments within one sub-set, there was another feature, which described the appearance of an actin tail quite well. The angles between the filaments and the main axis of the tail are a measure for their orientation. Without prescribing any range of angles of filaments I checked the distributions produced by the simulations. Interestingly the experimental data derived from 3 cryo tomograms fitted rather closely the values obtained from the simulation (mean of 51° for the experiments and 53° for the simulations: Figure 39).

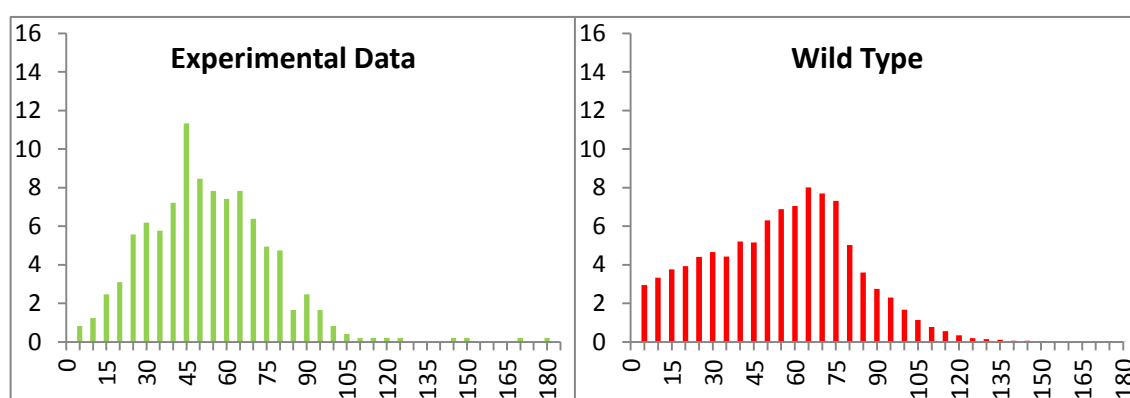


Figure 39: Histogram of filament angles to trajectory comparing experimental with model data. X-axis depicts angles to trajectory measured in degree, y-axis describes their relative frequency.

In both data sets hardly any filaments showed an angle higher than 100° , but the fraction of filaments almost parallel to the main core axis was much higher in the simulation than in the wild type. These filaments grew very long and created daughter filaments with angle between $70-90^\circ$. The mother filaments stayed attached to the virus while the daughter filaments grew ineffectively sideways and the resulting structure reminded of a rose stalk with thorns (see Figure 40).

Such structures were rarely seen in the tomograms, so why did I find them in my simulations? One explanation could be the rigidity and immobility of the filaments. If a filament grows straight against the virus rear it will most likely bend and slide a bit along the pathogen's surface. Thereby the angle subtended with the core axis increases and after the creation of the next daughter filament both will have intermediate angles.

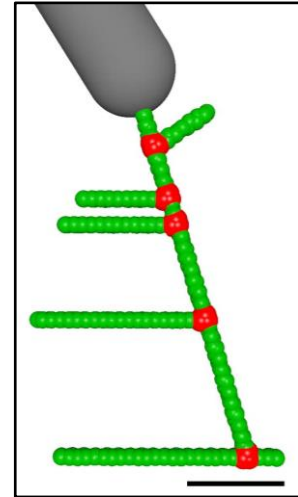


Figure 40: Rose like filament arrangement.
Bar: 50nm

Up scaling

Finally I tested whether the same mechanism, which stabilizes the baculovirus, is also able to allow a persistent movement of larger objects. For this purpose the length and width of the virus were multiplied by 10, which results in a 100 larger area. The speed was surprisingly increased and the persistence of the virus was much higher. One can conclude that more filaments provide a more stable system and fluctuations have less disturbing effects on the pathogens motility. Especially the histogram of the angles to trajectory was symmetrical reflecting a regular fish-bone array. 41% of all filaments were subtended at an angle of 30 and 45° to the trajectory with a peak value at 35° .

Interestingly the filament length was 50% longer than the wild type filaments, although the same capping rate was chosen. Actin filaments are protected from capping as long as they are tethered to the virus rear and with a larger particle the time of attachment is correspondingly longer. In the cell VASP could prevent filament capping, since experiment with purified proteins confirmed the protective role of VASP¹¹⁰.

This observation was consistent with the shorter filaments produced by the untethered and branch tethered simulation for the native virus. In these scenarios the virus detached much faster from the tail, hence the filaments were sooner available for capping.

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Appendix

Appendix 1

Calculation of the steric energy of one filament:

First trials showed that the total length of the penetrating segment does not serve as a good proxy for the steric interactions. Hence I took an approximation of the area between the filament and the surface (red dashed area) as a measure for steric interaction and its penalisation. There are several different configurations how a filament can penetrate the virus. In any case the contribution from the semicircle and the rectangle are calculated independently.

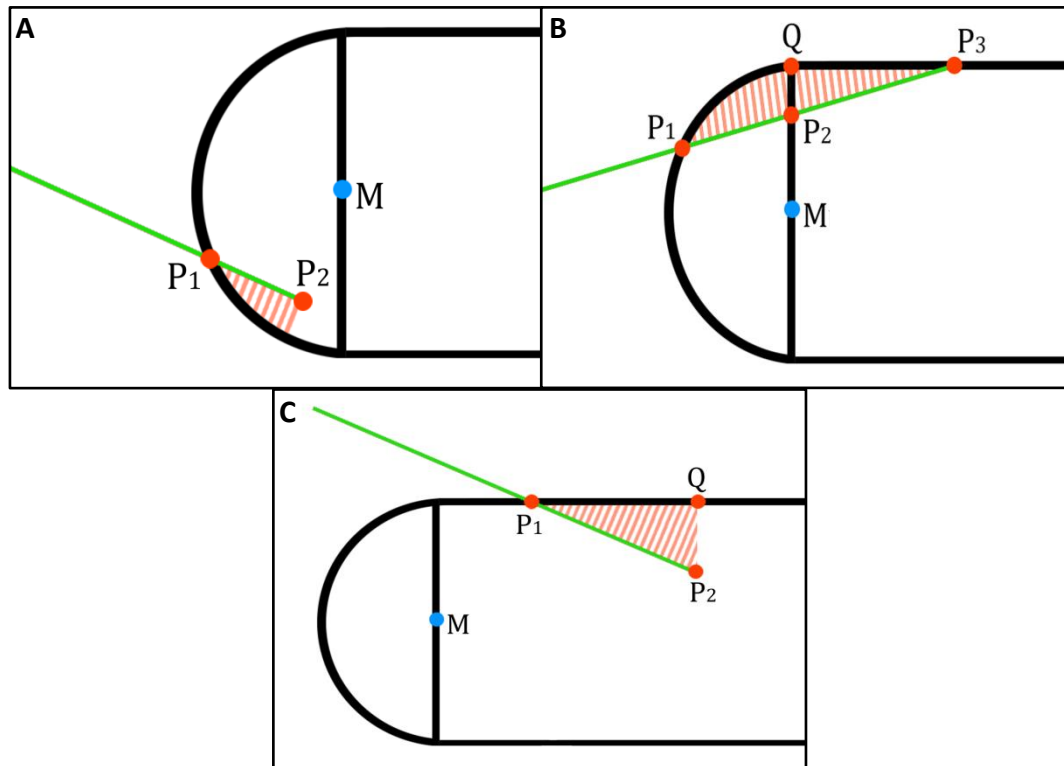


Figure 41: Scheme of three different cases how a filament (green) penetrates the virus. **A** Filament only penetrates the semicircle, **B** Filament penetrates the semicircle and the rectangle **C** Filament only penetrates the rectangle. The red dashed area indicates the area equivalent to the penalising potential.

Energy contribution from the filament segment penetrating the semicircle in case **A** and **B**:

$$E_{steric} := \mu_{steric} \int_0^L r^2 - |x|^2 ds$$

$$f(s) := P_1 + s * v$$

$$v := \frac{(P_2 - P_1)}{|P_2 - P_1|}$$

$$L := |P_2 - P_1|$$

$$s \in [0, L]$$

$$|x|^2 := (M_x - (P_{1x} + sv_x))^2 + (M_y - (P_{1y} + sv_y))^2$$

$$|x|^2 = (M_x - P_{1x})^2 + (M_y - P_{1y})^2 - s(2((M_x - P_{1x})v_x + 2((M_y - P_{1y})v_y) + s^2v_x^2v_y^2$$

$$a := (M_x - P_{1x})^2 + (M_y - P_{1y})^2$$

$$b := (2((M_x - P_{1x})v_x + 2((M_y - P_{1y})v_y)$$

$$v_x^2v_y^2 = 1$$

$$E_{steric} = \mu_{steric} \int_0^L r^2 - (a - sb + s^2) ds$$

$$E_{steric} = \mu_{steric} L(r^2 - a) - \int_0^L sb + s^2 ds$$

$$E_{steric} = \mu_{steric} L(r^2 - a) + \frac{L^2b}{2} - \frac{L^3}{3}$$

Energy contribution from the filament segment penetrating the rectangle of the virus in case **B**:

$$E_{steric} := \mu_{steric} \int_0^L r^2 - (r - \hat{d})^2 ds$$

$$E_{steric} = \mu_{steric} \int_0^L 2r\hat{d} - \hat{d}^2 ds$$

$$y = |P_2 - Q|$$

$$x = |P_3 - Q|$$

$$v = \frac{y}{x}$$

$$\hat{d} = y - sv$$

$$\hat{d}^2 = y^2 - 2svy + s^2v^2$$

$$\begin{aligned}
E_{steric} &= \mu_{steric} \int_0^L 2r(y - sv) - (y^2 - 2svy + s^2v^2) \, ds \\
E_{steric} &= \mu_{steric} L(2ry - y^2) \int_0^L -2rsv + 2svy - s^2v^2 \, ds \\
E_{steric} &= \mu_{steric} \left(L(2ry - y^2) - rvL^2 + vyL^2 - \frac{L^3v^2}{3} \right) \\
E_{steric} &= \mu_{steric} \left(-\frac{L^3v^2}{3} + L^2(-rv + yv) + L(2ry - y^2) \right)
\end{aligned}$$

Energy contribution from the filament segment penetrating the rectangle of the virus in case **C**:

$$\begin{aligned}
v &= \frac{y}{x} \\
\hat{d} &= sv \\
\hat{d}^2 &= s^2v^2 \\
E_{steric} &= \mu_{steric} \int_0^L 2rsv - s^2v^2 \, ds \\
E_{steric} &= \mu_{steric} \left(rvL^2 - \frac{L^3v^2}{3} \right)
\end{aligned}$$

Tables

Table 1 Input Parameters used for mathematical

Symbol	Meaning	Value	Justification
β_{max}	Describes the region on the virus surface, where p78/83 is present	73.8°	Observed in EM, ¹⁰⁸
β_{max}	Maximal polymerisation speed	388nm*s ⁻¹	Fitting parameter
v_{min}	Minimal polymerisation speed	166nm*s ⁻¹	Fitting parameter
γ_b	Branching angle	74°	Observed in ET
λ_b	Maximal branching rate	28nm*s ⁻¹	Fitting parameter
N	Maximal number of tethered filaments	10	Observed in ET
$\Delta\beta$	Inhibition range of tethered barbed ends	18°	Related to the filament diameter
λ_c	Capping rate after detachment	6.4s ⁻¹	Fitting parameter
λ_d	Rate of activation of depolymerisation after capping and aging	555s ⁻¹	Fitting parameter (tail length)
v_d	Depolymerisation speed	388nm	Fitting parameter (tail length)
n_0	Threshold number of tethered barbed ends for activating nucleation	4	Observed in ET
λ_n	Nucleation rate (if $n < n_0$)	9.4s ⁻¹	Fitting parameter
L	Length of the rectangular part of the virus	300nm	Observed in ET
r	Radius of the semicircles forming the ends of the virus	25nm	Observed in ET
$\mu_{friction}$	Friction coefficient	5*10 ⁻⁶ g*s ⁻¹ *nm ⁻²	Fitting parameter
μ_{steric}	Penalization of steric interactions	1g*s ⁻² *nm ⁻¹	Compromise: accuracy vs. computability
μ_{tether}	Elastic constant of tethers	0.7g*s ⁻²	Fitting parameter
z_{tether}	Maximal stretching of tethers	2.75nm	Fitting parameter
Δx	Maximal translation per time step by Brownian forcing	0.2nm	Small compared to published data, qualitative effect
$\Delta\alpha$	Maximal rotation per time step by Brownian forcing	1.62°	Small compared to published data, qualitative effect

Table 2 Matched parameters

Averaged quantity	Unit	Experimental data	Wild type	Untethered	Branch tethered	Random Branching	Size up
Virus speed	μm/min	14.3	14.8	12.9	12.8	13.7	18.9
Number of pushing filaments	#	3.9	4.1	2.5	2.7	3.5	9.8
Inter-branch distance	nm	37.1	35.1	31.7	31.7	32.0	105.5
Filament length	nm	121	109	95	97	101	368

Table 3: Predicted and observed parameters

Averaged quantity	Unit	Experimental data	Wild type	Untethered	Branch tethered	Random Branching	Size up
Filaments / tail	#/μm	190	114	140	142	136	52
Filament length / tail length	μm/μm	22.4	12.4	13.2	13.7	13.6	19
Filaments / subset	#	14.2	11.3	3.6	3.9	6.7	435
Filament length / branch	nm	169	120	132	130	118	369
Branches / tail length	#/μm	122	103.5	100	106	115	52
Subsets / tail length	#/μm	13	10.3	39	36	20	0.12
Turning angle	degree	22	17.6	51.2	44.4	24.5	7.2
Angle to trajectory	degree	50.9	52.6	60	65	59	40

Curriculum vitae

Julia Pfanzelter

I. Education

2011 Master thesis “Developing a mathematical and structural model of actin comet tails propelling pathogens”
2010 Enrolment at University of Vienna Master Program in Molecular Biology with emphasis on Cell Biology (1.3)
2010 Graduation: Bachelor of Sciences, A-Level (1.2)
2007 Enrolment at University of Salzburg/Linz Bachelor Program Molecular Biology
2007 Graduation: “Matura”, excellent leaving certificates
1995 Primary and secondary school Reithmannngymnasium Innsbruck

II. Working Experience

2009 One month internship at the Institute of Biomedical Aging Research
Supervisor: Dr. Berger, Innsbruck
2011 Two months internship at the Institute of Molecular Biology Austria.
Supervisor: Dr. Thomas Marlovits, Vienna
2011 Master Thesis at the Institute of Molecular Biology Austria, Vienna.
Electron Tomography, Mathematical Modelling (MATLAB) Supervisors: Dr. Small und Dr. Schmeiser

III. Selected Qualifications, Trainings and Social Skills

2005 Participation at the EU Project “Forum of Politics” lasting 2 years,
Travelling to Germany, England, Malta, Poland
2005 Certified Sport Instructor by the City of Innsbruck, Austria. Excellent grade
2006 Internship at the Bank Austria, Innsbruck
2009 Elected committee representative of the Faculty of Natural Sciences, University of Salzburg
2011 Programming Course Perl, University of Vienna
2012 Lab exchange with the Institute of Cancer Research London, UK
2012 Rhetoric Course at the University of Vienna
2012 EMBL Course “Microscopy, Modelling and Biophysical Methods”, Heidelberg
2012 Actin Summer School, Regensburg
2012 Poster presentation at the International Actin Dynamics Meeting of DGZ, Regensburg

IV. Languages

German native speaker
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V. Awards

2006-2010 Scholarship “Michael von Zollerstiftung”
2010 Award for the bachelor degree

Figures

Figure 1: **Aebi, U., Millonig, R., Salvo, H. & Engel, A.** The three-dimensional structure of the actin filament revisited. *Annals of the New York Academy of Sciences* 483, 100–19

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Figure 3a: **Urban, E., Jacob, S., Nemethova, M., Resch, G. P. & Small, J. V.** Electron tomography reveals unbranched networks of actin filaments in lamellipodia. *Nature cell biology* 12, 429–35 (2010)

Figure 3b: **Koestler, S. A., Auinger, S., Vinzenz, M., Rottner, K. & Small, J. V.** Differentially oriented populations of actin filaments generated in lamellipodia collaborate in pushing and pausing at the cell front. *Nature cell biology* 10, 306–13 (2008)

Figure 4: **Gouin, E., Welch, M. D. & Cossart, P.** Actin-based motility of intracellular pathogens. *Current opinion in microbiology* 8, 35–45 (2005).

Figure 5: **Gouin, E. et al.** A comparative study of the actin-based motilities of the pathogenic bacteria *Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia conorii*. *Journal of cell science* 112 (Pt 1, 1697–708 (1999)

Figure 6: **Brieher, W. M., Coughlin, M. & Mitchison, T. J.** Fascin-mediated propulsion of *Listeria monocytogenes* independent of frequent nucleation by the Arp2/3 complex. *The Journal of cell biology* 165, 233–42 (2004).

Figure 7: **Smith, G. L., Vanderplasschen, A. & Law, M.** The formation and function of extracellular enveloped vaccinia virus. *The Journal of general virology* 83, 2915–31 (2002).

Figure 38: **Cudmore, S., Cossart, P., Griffiths, G. & Way, M.** Actin-based motility of vaccinia virus. *Nature* 378, 636–8 (1995)

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