# Nascent adhesions shorten the period of lamellipodium protrusion through the Brownian ratchet mechanism

Keith R. Carney<sup>1,2</sup>, Akib M. Khan<sup>1, 2</sup>, Samantha Stam<sup>1,2</sup>, Shiela C. Samson<sup>1,2</sup>, Nikhil Mittal<sup>3</sup>, Sangyoon J. Han<sup>3</sup>, Tamara C. Bidone<sup>4,5</sup>, and Michelle C. Mendoza<sup>1,2,4</sup>\*

<sup>1</sup>Department of Oncological Sciences, University of Utah, Salt Lake City, UT

<sup>2</sup>Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

<sup>3</sup>Department of Biomedical Engineering, Michigan Technological University, Houghton, MI

<sup>4</sup>Department of Biomedical Engineering, University of Utah, Salt Lake City, UT

<sup>5</sup>Scientific Computing and Imaging Institute, University of Utah, Salt Lake City, UT

\* Corresponding author: Michelle Mendoza (<u>michelle.mendoza@hci.utah.edu</u>)

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Abbreviations: (BR) Brownian ratchet, (MC) molecular clutch

#### Abstract

Directional cell migration is driven by the conversion of oscillating edge motion into lasting periods of leading edge protrusion. Actin polymerization against the membrane and adhesions control edge motion, but the exact mechanisms that determine protrusion period remain elusive. We addressed this by developing a computational model in which polymerization of actin filaments against a deformable membrane and variable adhesion dynamics support edge motion. Consistent with previous reports, our model showed that actin polymerization and adhesion lifetime power protrusion velocity. However, increasing adhesion lifetime decreased the protrusion period. Measurements of adhesion lifetime and edge motion in migrating cells confirmed that adhesion lifetime is associated with and promotes protrusion velocity, but decreased duration. Our model showed that adhesions' control of protrusion persistence originates from the Brownian ratchet mechanism for actin filament polymerization. With longer adhesion lifetime or increased adhesion density, the proportion of actin filaments tethered to the substrate increased, maintaining filaments against the cell membrane. The reduced filament-membrane distance generated pushing force for high edge velocity, but limited further polymerization needed for protrusion duration. We propose a mechanism for cell edge protrusion in which adhesion strength regulates actin filament polymerization to control the periods of leading edge protrusion.

Significance statement:

- Cell migration involves the conversion of oscillating edge motion into sustained periods of protrusion. Actin assembly and adhesion to the substrate drive edge velocity, but how adhesions impact the protrusion period is unknown.
- Combining computational modeling with experimental tests, the authors show that nascent adhesions at the cell edge shorten the protrusion period. The regulation occurs through adhesions' tethering of actin filaments near the edge, which results in reduced probability of further polymerization.
- These findings explain how signals that simultaneously drive actin polymerization and adhesion turnover produce forward motion and cell migration.

#### Introduction

Cell migration is essential to physiological and pathological biology. During development, wound healing, and cancer, chemical signals direct cells to move towards or away from specific locations. Directional migration is associated with the velocity and duration of outward motion of the leading edge, termed lamellipodium protrusion (Harms et al., 2005; Riaz et al., 2016; Heck et al., 2020). The protrusions emerge from pushing and traction forces, which are generated by actin filaments polymerizing against the plasma membrane and transmitting their motion to a substrate through adhesions (Pollard and Borisy, 2003; Gardel et al., 2010). While longer periods of protrusion are needed for persistent migration, how adhesions control the period is unknown.

Protrusion periods are composed of phases of initiation and reinforcement, characterized by changes in membrane tension and actin polymerization rate (Prass et al., 2006; Ji et al., 2008). Protrusion initiation occurs with the un-tethering of actin filaments from the membrane, which decreases membrane tension and increases the likelihood of monomer addition (Mogilner and Oster, 1996; Bisaria et al., 2020; Welf et al., 2020). According to the Brownian ratchet mechanism, fluctuations in the membrane and the actin filaments create gaps between the two structures that allow for new monomer addition (Mogilner and Oster, 2003). The polymerization of new actin monomers onto actin filaments abutting against the membrane pushes the edge membrane out (Abraham et al., 1999; Lacayo et al., 2007; Pollard, 2007; Gardel et al., 2010). Thus, the actin polymerization rate correlates with the velocity of the cell edge (Ponti et al., 2004; Ponti et al., 2005). Tension against actin filaments decreases their polymerization (Brangbour et al., 2011; Zimmermann et al., 2012). As the protrusion progresses, the membrane is stretched and membrane tension increases, which pushes back on the actin filaments and

decreases the likelihood of actin monomer addition (Raucher and Sheetz, 2000; Ji et al., 2008; Gauthier et al., 2011; Houk et al., 2012).

In the reinforcement phase, increased actin filament polymerization against the plasma membrane counters membrane tension to move the cell edge forward (Abraham et al., 1999; Lacayo et al., 2007; Pollard, 2007; Gardel et al., 2010). The reinforcement phase is also named the power phase in reference to the increased number of actin filaments pushing against the membrane (Machacek and Danuser, 2006; Ji et al., 2008; Lee et al., 2015; Mendoza et al., 2015). Recruitment and activation of ARP2/3 leads to reinforcement and sustained membrane motion (Amann and Pollard, 2001; Suraneni et al., 2012; Wu et al., 2012; Lee et al., 2015). ARP2/3 increases net actin polymerization by initiating branching, the formation of new filaments off the sides of existing filaments (Mullins et al., 1998; Achard et al., 2010). Despite the increased pushing force, the continued increase in membrane tension and actin polymerization causes edge velocity to slow during protrusion reinforcement (Ji et al., 2008; Mendoza et al., 2015). As a result, the positive correlation between actin filament polymerization and edge velocity reverses during reinforcement (Ji et al., 2008; Mendoza et al., 2015).

The formation of small, transient adhesions in the lamellipodium, termed nascent adhesions, also promotes and is required for protrusion velocity (Choi et al., 2008; Shemesh et al., 2009; Welf et al., 2013). In response to actin polymerization, counterforce from the membrane induces actin retrograde flow away from the membrane and towards the cell center (Pollard, 2007). Nascent adhesions work as molecular clutches (Gardel et al., 2010) that physically anchor the actin retrograde flow to the substrate (Giannone et al., 2004; Hu et al., 2007). They form when integrin receptors at the cell surface bind extracellular matrix and connect to actin filaments through actin binding proteins (Gardel et al., 2010). In this way,

adhesions transmit the actin flow into traction on the substrate (Giannone et al., 2004; Hu et al., 2007) and increase the protrusion velocity (Prass et al., 2006; Alexandrova et al., 2008; Ji et al., 2008). Adhesion traction increases along with actin flow, but then decreases as actin flow increases further, as high flow breaks the connection with adhesions (Gardel et al., 2008; Ji et al., 2008). Consistent with this, traction force peaks before actin flow reaches its maximum in the reinforcement phase of edge protrusion (Lee et al., 2015; Mendoza et al., 2015). Filament anchoring also has the secondary effect of decreasing the motion or retrograde velocity of actin filaments (Alexandrova et al., 2008; Ji et al., 2008). Because anchoring actin filaments onto adhesions also maintains the actin barbed ends against the membrane, the assembly rate of nascent adhesions correlates with and promotes edge velocity (Giannone et al., 2004; Choi et al., 2008). Indeed, when nascent adhesions uniformly disassemble, the cell edge retracts (Oakes et al., 2018). Thus, engaged adhesions build a link between actin and edge motion.

While nascent adhesions promote protrusion velocity, how nascent adhesions control protrusion period is unclear. A study in fibrosarcoma cells found that longer-lived adhesions, generated by substrate alignment, were associated with longer protrusion periods (Kubow et al., 2017). However, experiments manipulating intracellular signals that inhibit actin assembly (Mendoza et al., 2015), but prolong adhesion lifetime (Webb et al., 2004) reported a decrease in lamellipodium protrusion velocity and period (Mendoza et al., 2015). Computational models can dissect the role of actin and adhesions, but many past computational models of the lamellipodium focused on edge velocity. Models of the Brownian ratchet mechanism for actin polymerization found that actin polymerization governs velocity (Grimm et al., 2003; Schaus et al., 2007; Weiner et al., 2007; Craig et al., 2012; Demoulin et al., 2014; Mueller et al., 2017; Garner and Theriot, 2020). Models that probed the relationship between adhesions and edge

velocity have also confirmed a positive correlation between adhesion assembly and velocity (Rubinstein et al., 2005; Yam et al., 2007; Choi et al., 2008; Rutkowski and Vavylonis, 2021). A model of adhesion clutches found that a low density of adhesions that functioned as nascent adhesions promoted protrusion duration while a high density of adhesions that functioned as mature adhesions present behind the lamellipodium limited duration (Welf et al., 2013).

To elucidate how nascent adhesions control protrusion period, we developed and experimentally tested a computational model of lamellipodium protrusion. Different from previous approaches, our model simultaneously incorporated: individual actin filaments that follow the Brownian ratchet mechanism (BR), variable adhesion dynamics through adhesions that assemble and disassemble following the molecular clutch mechanism (MC), as well as deformation of an elastic membrane that responds to and exerts forces on the filaments (Oakes et al., 2018; Bidone et al., 2019). The new model revealed the mechanism behind nascent adhesions' control of protrusion period. Long-lived adhesions signaled through the BR by maintaining actin filaments against the leading edge, thereby reducing the distance between the This increased the filament pushing force but reduced filament actin and membrane. polymerization, which increased protrusion velocity but reduced the protrusion period, respectively. These results support the idea that lamellipodium adhesion strength and protrusion velocity and period are interdependent. By mechanically linking actin near the cell membrane, adhesions control actin filament assembly, the power behind lamellipodium protrusion.

#### Results

#### Model parameter optimization

We developed a novel computational model of lamellipodium protrusion based on Brownian dynamics. The model incorporated: explicit actin filaments represented as polar rods of interconnected units; nascent adhesions represented as dynamic point particles that link filaments to a fixed substrate; and a flexible membrane represented as a series of rigid rods connected by springs (Figure 1A). Actin filaments fluctuated under thermal motion, polymerized, branched, depolymerized, and linked to adhesions. Branching occurred at ~70° angles within a narrow membrane contact region, at a rate  $r_{branch}$ . Actin polymerization produced a force against the membrane ( $F_{Pol}$ ), which pushed the membrane forward. The displacement of the membrane produced an increase in membrane tension ( $F_M$ ), which pushed the filaments away from the membrane and resulted in actin retrograde flow. Adhesions formed at adhesion activation rate  $r_{on}$  and linked to proximal actin filaments. The adhesion-actin linkage converted actin retrograde flow into traction on the substrate ( $F_A$ ), until the adhesions became unbound.

We simultaneously implemented the Brownian ratchet mechanism of actin filament polymerization (BR) and the molecular clutch mechanism for adhesion disassembly (MC) (Figure 1B). The BR mechanism incorporated force-dependent actin filament polymerization against the membrane load. Actin filaments' probability of polymerization depended on a polymerization coefficient ( $C_p$ ), which decreased as the filaments approached the membrane, according to the decreasing exponential force-velocity relation (Mogilner and Oster, 1996; Carlsson, 2001, 2003; Mogilner and Oster, 2003; Ryan et al., 2017). Polymerization induced  $F_{Pol}$ , which increased as actin filaments approached the membrane (Mogilner and Oster, 1996; Carlsson, 2001, 2003; Mogilner and Oster, 2003; Ryan et al., 2017). With each model iteration,  $F_{Pol}$  and  $F_M$  were summed for each membrane segment, which repositioned the membrane and controlled the next iteration of polymerization. The MC mechanism incorporated force-dependent adhesion unbinding and reproduced a biphasic force-dependent lifetime ( $\tau$ ) for adhesions (Figure 1C) (Chan and Odde, 2008; Elosegui-Artola et al., 2016). The peak in adhesion lifetime ( $\tau_{max}$ ) corresponded to a force of 30 pN to mimic integrin unbinding from fibronectin under load (Kong et al., 2009; Yao et al., 2014; Yeoman et al., 2021). The adhesion lifetimes exemplified the nascent adhesions that form and disassemble along a protruding edge in migrating cells (Han et al., 2021).

The model reproduced continuous filament polymerization, filament branching against the membrane, and nascent adhesion assembly and disassembly. Rates for actin polymerization and adhesion activation and inactivation were obtained from the literature (Table 1). Actin monomers were discretized as 2.7 nm units with default  $r_{pol} = 11 \text{ s}^{-1} \mu \text{M}^{-1}$ , which generated actin polymerization rates of the order of physiological 0.4 µm/s (Pollard, 1986; Theriot et al., 1992; Marchand et al., 1995; Pollard and Borisy, 2003). Membrane tension was set to  $k_m = 0.3$  pN/nm, consistent with tension measurements in epithelial cells (Lieber et al., 2013; Shi and Baumgart, A constant rate of actin filament branching  $(r_{branch} = 1.0 \text{ s}^{-1})$  corresponded to 2015). experimentally measured ARP2/3 activation (Beltzner and Pollard, 2008). Adhesion density was maintained within the physiological range ~1000 integrins/ $\mu$ m<sup>2</sup> (Wiseman et al., 2004). Adhesion tension before bond breakage was set to  $k_a = 10$  pN/nm, consistent with measured tension across early adhesions (Wang et al., 2015). When the actin filaments interacted with the membrane, they graphically appeared as having passed the membrane but were interpreted as bent and exerting force against the membrane. Membrane displacement, membrane tension, actin retrograde flow, and adhesion-mediated anchoring of the flow emerged from the relative

force interactions between filaments, adhesions, and membrane (Figure 2A, Supplemental movie 1).

#### The BR-MC Model reproduces physiological lamellipodium motion

We tested that our modeling strategy reproduced experimentally measured properties of the lamellipodium. We tested the model's emergent protrusion velocity, retrograde flow velocity and adhesion traction force. In the BR-MC model, individual membrane segments exhibited velocity peaks of ~30 nm/s and slowed to 15 nm/s as the protrusion progressed (Figure 2B). We quantified protrusion period as the time between the large oscillations in membrane velocity, which we identified by Empirical Mode Decomposition (EMD) as in previous experimental studies (Mendoza et al., 2015; Han et al., 2021). We first applied a low pass filter to remove large spikes in the velocity changes due to the small time step (Fig. 2C). Low pass filtering followed by EMD signal processing generated velocity traces with protrusion peaks that lasted ~30 s, similar to the protrusion period observed Cos7 epithelial cells (Samson et al., 2019) (Figure 2D). Actin retrograde flow exhibited peaks of 70 nm/s and mean flow of 15.6 nm/s (Figure 2C, D). Traction forces exhibited peaks of 7 pN (Figure 2E), consistent with experimental measurements of individual adhesions in the lamellipodium (Gardel et al., 2008).

We tested the stability of the model by varying time step, lamellipodium length, and membrane rod width to identify conditions in which the emergent membrane displacement is stable and unaffected by discretization artifacts. With time steps of 0.01 ms to 1 ms, protrusion velocity was maintained within the physiological range of ~25 nm/s measured in PtK1 and Cos7 epithelial cells (Supplemental figure 1A, (Lee et al., 2015; Mendoza et al., 2015; Samson et al., 2019)). In contrast, increasing the time step from 1 to 5 s ms caused a drop in velocity to 20

nm/s. Since longer time steps enable faster computation of lamellipodium dynamics, we captured physiological velocity with 0.2 ms. Changing the length of the 2D lamellipodium domain had no effect on edge velocity (Supplement figure 1B). We therefore used lamellipodium length 2  $\mu$ m, twice the segmentation dimension used in experimental studies of leading edge motion (Machacek et al., 2009; Tkachenko et al., 2011; Lee et al., 2015; Mendoza et al., 2015). Increasing membrane rod width did not affect edge velocity (Supplemental figure 1C).

# Nascent adhesion lifetime promotes lamellipodium protrusion velocity but limits protrusion duration.

We used the BR-MC Model to understand how adhesion lifetime controls protrusion velocity and period, when force feedbacks control actin polymerization, membrane tension, and adhesions. We systematically varied the peak in adhesion lifetime ( $\tau_{max}$ ) from 3 s to 7.5 s and 12 s (Figure 1C). When adhesion lifetime was increased from 3 to 12 s, membrane velocity increased 20% (from 24.3 nm/s to 29.5 nm/s). In contrast, adhesion lifetime decreased protrusion period 5.3% (from 35.7 s to 33.9 s, Figures 3A and B). We tested this result by examining the higher frequencies in edge motion, in which fewer intrinsic mode functions (IMFs) were subtracted during EMD processing to extract the protrusion peristence. In all cases, increasing adhesion lifetime decreased protrusion period (Supplemental figure 2A-D). We also tested if altering membrane tension affected the relationship between adhesion lifetime and protrusion velocity and period. Low membrane tension (0.03 pN/nm) represented the scenario of protrusion initiation and high membrane tension (3 pN/nm) represented the protrusion reinforcement phase (Parekh et al., 2005; Prass et al., 2006; Zimmermann et al., 2012)). Under

both membrane tensions, as well as the standard 0.3 pN/nm used in other simulations, adhesion lifetime promoted edge velocity 21-24% and reduced protrusion period 5-9% (Supplemental figure 3).

We also manipulated adhesion lifetime by changing the adhesion spring constant from its default value of  $k_a = 10$  pN/nm. Increasing  $k_a$  stiffens the link between adhesions and actin, so that the  $\tau_{max}$  breakpoint tension is reached more quickly upon actin binding. We confirmed this effect with  $\tau_{max} = 3$  s and 12 s. Changing  $k_a$  to 5 pN/nm or 15 pN/nm had little effect on adhesion lifetime, but increasing  $k_a$  beyond 15 pN/nm dramatically decreased adhesion lifetime (Supplementary figure 4). Accordingly, with  $\tau_{max} = 3$  s or 12 s,  $k_a > 20$  pN/m slowed protrusion velocity and prolonged the period, consistent with  $k_a$ 's control of adhesion lifetime (Figure 3C and D). Since signaling pathways that control adhesion disassembly also control actin polymerization by activating ARP2/3, we tested how actin polymerization and adhesions together control edge velocity in the presence of feedback to adhesion disassembly. Varying  $r_{branch}$  and  $\tau$  in the BR-MC Model showed that both actin filament polymerization and adhesion lifetime increased membrane velocity (Figure 3D). However, actin filament polymerization had no effect on protrusion period (Figure 3E). Thus, in cells with regulation of both actin and adhesion dynamics, adhesion lifetime is the main regulator of protrusion period.

#### Integrin activation promotes lamellipodium velocity and decreases its period.

We experimentally tested the finding that adhesion lifetime promotes lamellipodia protrusion velocity but limits the period in COS7 epithelial cells. We labeled adhesions in COS7 cells using transient expression of Paxillin-mApple and imaged adhesion and edge dynamics during 5 min of steady-state migration. Paxillin recruitment to adhesions is detectable ~4 s

before the onset of force (Han et al., 2021). We segmented the adhesions using focal adhesion analysis software for quantification of the adhesions' lifetime (Han et al., 2021) and used morphodynamics software to track the edge motion (Machacek and Danuser, 2006) (Figure 4A and B). Negative edge motion was observed as contours of the cell edge that receded in time. Such retractions are the result of myosin II activity in mature adhesions, present in a structure behind the lamellipodium termed the lamella, which contracts the actin fibers (Ponti et al., 2004; Choi et al., 2008). We noted that protrusions exhibited adhesions with heterogeneous lifetimes, in which clusters of short-living adhesions co-resided with a few longer-lifetime adhesions. The range of long lifetimes varied per movie, which appeared to be related with edge protrusion. For example, a cell in which the longest lifetimes are  $\sim 4.7$  min (orange-colored adhesions in Figure 6A) showed slow, persistent progression of the cell edge. On the contrary, a cell in which the longest lifetimes are ~10.6 min (yellow-colored adhesions in Figure 4B) showed more fluctuating protrusion behavior. We sampled the lifetimes of the top 1 percentile of long-living adhesions per movie and obtained the corresponding protrusion velocities and persistent times of the closest edge segments. Plotting edge velocity and persistence as a function of adhesion lifetime showed that cell protrusions with longer mean adhesion lifetimes were associated with faster protrusion velocity but shortened protrusion period (Figure 4C and D).

We also treated COS7 cells with  $Mn^{+2}$ , which increases adhesion lifetime and density (Gailit and Ruoslahti, 1988; Smith et al., 1994; Mould et al., 1995; Kong et al., 2009).  $Mn^{+2}$  stabilizes nascent adhesions by promoting integrins' structural shift to high-affinity conformations for binding to extracellular matrix (Kamata et al., 2005; Lin et al., 2013; Oakes et al., 2018). The cells transiently expressed Emerald-Lifeact to label the cell edge. We imaged the cells' steady-state protrusion-retraction cycles and quantified protrusion velocity and persistence

with morphodynamics software. Integrin activation with  $Mn^{+2}$  increased mean protrusion velocity but decreased period when compared to untreated cells (Figure 4E and F). These results supported our model that longer adhesion lifetimes are associated with faster protrusion velocity but reduced protrusion period.

# Adhesion lifetime shortens the protrusion period through the Brownian ratchet force feedback to actin.

We isolated the BR and MC mechanisms to study their relative effects on protrusive velocity and period. The BR Model incorporated force-dependent actin filament polymerization by implementing the Brownian ratchet mechanism (Figure 5A). We applied a constant rate of adhesion binding ( $r_{off} = 0.2 \text{ s}^{-1}$ ) to obtain adhesion density ~1000 adhesions/ $\mu$ m<sup>2</sup>, as in the BR-MC Model (Table 1). The effect of adhesion lifetime on protrusion velocity and period in the BR Model resembled that of the BR-MC Model. Increasing adhesion lifetime from 3 to 12 s resulted in a 22% increase in membrane velocity (from 23.8 nm/s to 29.1 nm/s, Figure 5B) and a 4.5% decrease protrusion period (from 35.7 s to 34.1 s, Figure 5C and Supplemental figure 5).

The MC Model incorporated force-dependent adhesion unbinding, but actin force and polymerization were binary, with constant rates of 1.0 or 0 depending on the distance. If the filament tip was less than the size on an actin monomer (< 2.7 nm) from the membrane,  $r_{pol} = 0$  and  $F_{pol} = 1.0$ . If the filament tip was  $\geq 2.7$  nm from the membrane,  $r_{pol} = 1.0$  and  $F_{pol} = 0$  (Figure 5D). In the MC Model, velocity was slower and the period was shorter than in the BR-MC or BR Models (Figure 5E). In this case, increasing adhesion lifetime increased velocity by only 4%, compared to the 20% in the BR-MC Model (Figure 5E and Supplemental figure 5). Furthermore, increasing adhesion lifetime in the MC Model reduced the protrusion period by

only 2.5%, compared to the 5.3% decrease in the BR-MC Model (from 32.1 s to 30.3 s, Figures 5F and Supplemental figure 5). Thus, the Brownian ratchet mechanism is required for adhesion lifetime to control edge velocity.

We hypothesized that adhesion lifetime controls the protrusion period through the attached actin filaments' Brownian ratchet behavior. To test this, we quantified the number of attached versus free filaments in the BR-MC Model with different  $\tau_{\text{max}}.$  We included an additional  $\tau_{max} = 5$  s so that we could better detect trends in the response (Supplemental figure 6A). Increasing adhesion lifetime increased the percentage of actin filaments bound or attached to adhesions (Figure 6A). 55% of the filaments were attached with  $\tau_{max} = 3$  s (391 attached filaments out of 702 total filaments), while 78% of the filaments were attached with  $\tau_{max}$  = 12 s (571 attached filaments out of 730 total filaments, Figure 6A). We then tested each of the factors that control actin filament pushing force and polymerization in the Brownian ratchet mechanism. The angle of interaction between the attached filaments and the membrane was unaffected by adhesion lifetime (1% decrease in angle with  $\tau_{max} = 12$  s versus  $\tau_{max} = 3$  s, Figure 6B). However, filament length and distance from the membrane were decreased 4% and 10%, respectively, as adhesion lifetime was increased to 12 s (Figure 6C and D). The small decrease in filament length likely resulted from an increased percentage of adhesion-attached actin filaments within the model's branching window (Figure 6A), where polymerization events occurred on new branches with an initial length of 0 nm, in addition to new free filaments with initialization length of ~89 nm. We further probed how adhesion binding to actin filaments controlled filament distance. As adhesion lifetime was increased to 12 s, the free filaments underwent a 5% increase in distance while the attached filaments underwent a 5% decrease in distance (Supplemental figure 6B). The free filaments' increased distance occurred alongside an increase

in actin retrograde flow (Supplemental figure 6C). The attached filaments were retained near the membrane, so did not exhibit retrograde flow (Supplemental figure 6D). Thus, as the longer adhesion lifetime shifted the filament mass to more attached, the distance between the overall filament population and the membrane was shortened (Figure 6B).

We next quantified actin pushing force and polymerization, the drivers of protrusion velocity and period. In the BR-MC Model, the attached filaments exhibited higher pushing force than free filaments, calculated as the y-component of membrane tension (Figure 6E). As adhesion lifetime and the percentage of attached filaments increased, the overall filament force increased (Figure 6E), consistent with the increase in protrusion velocity (Figure 3A). This was associated with increased membrane tension (Figure 6F), the cause of the observed increase retrograde flow (Supplemental figure 6C). The attached filaments exhibited lower probability of polymerization than the free filaments, whose distance increased due to retrograde flow (Figure 6G). As longer adhesion lifetime increased the percentage of attached filaments, the overall probability of polymerization was reduced (Figures 6G), thereby limiting the protrusion duration (Figure 3B). Thus, adhesion lifetime promoted protrusion velocity and limited the period through force feedbacks between the membrane and actin encoded in the BR mechanism.

#### Overall adhesion strength limits protrusion period.

Because the percentage of actin filaments attached to adhesions controlled actin force and polymerization, we tested whether overall adhesion strength is the key mediating factor of protrusion period. In the BR-MC Model, increasing adhesion lifetime from  $\tau_{max} = 3$  s to 12 s doubled adhesion density (Figure 7A). Increasing the adhesion spring constant reduced adhesion density, as the increased tension increased the probability of adhesion breakage (Figure 7B). We

independently controlled adhesion density by decreasing the number of allowed adhesions, while holding lifetime at a constant  $\tau_{max} = 3$  s (Figure 7C). Indeed, decreasing adhesion density from ~800/µm<sup>2</sup> to 100/µm<sup>2</sup> reduced protrusion velocity and increased the protrusion period (Figure 7D-E). Thus, protrusion period is controlled by overall adhesion strength, which increases the percentage of attached actin filaments – shortening their distance to the membrane and increasing their pushing force and decreases their continued polymerization.

### Discussion

Cell migration is driven by the sustained protrusion of a leading edge (Harms et al., 2005; Riaz et al., 2016; Heck et al., 2020). Nascent adhesions promote fast edge movement, but how they control protrusion duration is unknown. Our computational model of lamellipodium protrusion based on Brownian dynamics allowed us to test and mechanistically dissect adhesionmediated control of protrusion period. We found that adhesion lifetime promoted protrusion velocity and limited the protrusion period, and this result was substantiated by experiments tracking adhesions and edge motion in COS7 cells. The mechanism of control was resolved through model simulations that either incorporated or lacked the BR mechanism for actin response to membrane tension. The BR-MC Model showed that adhesion lifetime limits protrusion period by controlling actin filament polymerization against the membrane. Adhesions' differential regulation of protrusion velocity and period suggest that mechanisms to build and quickly turn over adhesions would most effectively drive the sustained protrusion of the leading edge which is needed for persistent directional migration.

The BR-MC Model presents several elements of novelty with respect to the many existing models of cytoskeletal dynamics. First, we simultaneously incorporated individual actin

filaments following the Brownian ratchet mechanism, variable adhesion dynamics, and a deformable membrane. By using discrete elements for filaments, adhesions, and membrane and their relative interactions, our model allowed for the precise characterization of how forcedependent kinetics of adhesions and filaments govern lamellipodium motion. Much of the previous lamellipodium modeling work was devoted to investigating the importance of actin filament polymerization and actin spatial organization in edge motion, without incorporating adhesions (Grimm et al., 2003; Atilgan et al., 2005; Schaus et al., 2007; Huber et al., 2008; Weichsel and Schwarz, 2010; Zimmermann et al., 2012; Demoulin et al., 2014; Garner and Theriot, 2020). These models included filament response to force through the adaptation of network geometry or polymerization or disassembly rates to variable membrane tension (Craig et al., 2012; Letort et al., 2015; Mueller et al., 2017). Other models of the lamellipodium included adhesion clutches, but typically employed predetermined relations between membrane motion and actin filament concentration or actomyosin contractility (Atilgan et al., 2005; Rubinstein et al., 2005; Huber et al., 2008; Craig et al., 2012; Welf et al., 2013; MacKay et al., 2021). Some models that incorporated both BR and MC represented actin filaments with a mean-field approximation of actin as a gel (Shemesh et al., 2009; Zimmermann et al., 2012; Welf et al., 2013). Models that incorporated individual filament assembly and variable adhesion dynamics did not incorporate a deformable membrane (Mogilner and Oster, 2003; Rutkowski and Vavylonis, 2021; Chandra et al., 2022). The recently-developed model of adhesion and actin signaling showed the mechanical feedbacks between nascent adhesions and polymerized actin promotes protrusion (Chandra et al., 2022). However, edge protrusion was treated as a continuum with steady-state velocity, rather than deformable with periodic fluctuations. In our

BR-MC Model, the filaments, membrane, and adhesions adapt to one another and the contribution of individual filaments to edge motion dynamics are isolated.

The BR-MC Model showed that nascent adhesions' regulation of protrusion velocity and period required the BR mechanism. Adhesion lifetime's inhibition of protrusion period emerged from the force-velocity relation of actin filaments polymerizing against the plasma membrane. By tethering actin filaments near the plasma membrane, longer adhesion lifetimes increased the force on the membrane but decreased the probability of further polymerization. The conclusion that lamellipodium adhesions positively regulate protrusion velocity but negatively regulate the period implies that the protrusion velocity and period are mechanistically linked. Indeed, actin polymerization is needed for nascent adhesion formation in edge protrusion (Alexandrova et al., 2008; Choi et al., 2008). Our findings indicate that adhesions additionally control actin assembly. Thus, signals that simultaneously promote actin assembly and reduce adhesion lifetime would optimally adjust protrusion velocity and period for productive migration. Experiments analyzing the edge motion frequency spectrum have shown that Rho/myosin II signals control the frequency distribution, but not the magnitude, suggesting that some signals can control protrusion velocity and period as orthogonal variables (Ma et al., 2018). However, many pro-migratory signaling pathways, such as PI(3)K/AKT, RAS/ERK and FAK pathways, promote protrusion velocity and period and control both actin assembly and adhesion dynamics (Devreotes and Horwitz, 2015; Lavoie et al., 2020; Samson et al., 2022).

Overall adhesion strength appears to be the key mediating factor in determining the protrusion period. Adhesions' control of edge velocity and period was independent of membrane stiffness and the mode of adhesion disassembly, as it occurred in the BR model in which adhesion unbinding was a first-order reaction. However, increasing the strength of adhesions, by

either enhancing adhesion lifetime or increasing adhesion density or stiffness, reduced the protrusion period. This conclusion is consistent with cell migration models that incorporated myosin activity and found that intermediate adhesion strength best promoted protrusion duration (Rubinstein et al., 2005; Shemesh et al., 2009; Welf et al., 2013). Thus, protrusion period could be controlled by intracellular signals or extracellular signals that control the extracellular matrix environment to reduce adhesion density.

In summary, we establish an unexpected role for adhesion strength in the differential regulation of protrusion velocity and period in cell migration. We expect adhesion's differential regulation of protrusion velocity and period to be maintained in rapidly moving cells with sufficient adhesion strength. On soft substrates, it is unlikely that sufficient traction force will be generated to control edge motion. The BR-MC lamellipodium model is a new framework for testing the signals and mechanics that control protrusion velocity and period in heterogenous environments.

#### Materials and Methods

#### Computational model of lamellipodium protrusion

Our model of lamellipodium protrusion combines the Brownian ratchet mechanism (BR) for actin filament polymerization against a deformable membrane (Carlsson, 2001; Mogilner and Oster, 2003; Schaus and Borisy, 2008; Brangbour et al., 2011) with the molecular clutch mechanism (MC) for adhesion disassembly (Chan and Odde, 2008; Elosegui-Artola et al., 2016). The model is developed using MATLAB R2022b. The domain is 2D, with size 2  $\mu$ m x 0.2  $\mu$ m. In this domain, every actin filament is modeled with rigidly connected actin monomers, each 2.7 nm long. Integrin adhesions are represented as particles transitioning between inactive and

active states, at rates  $r_{on}$  and  $r_{off}$  (Figure 1A). The rectangular domain has a moving boundary at the top that mimics a deformable membrane, formed by 101 rods of 18 nm length and elastically interconnected by springs with stiffness  $k_m$  and equilibrium separation of 2 nm (Figure 1A).

Actin filaments fluctuate in Brownian motion, polymerize at their barbed ends with the addition of units at rate  $r_{pol}$ , and depolymerize at their pointed ends with removal of units at rate  $r_{depolym}$  (rates in Table 1). Filaments can branch from existing filaments if the tip of the existing filament is within 50 nm from the membrane, at rate r<sub>branch</sub>. Filament branches are at least 50 nm apart and directed towards the membrane through the mechanical addition of monomers at an angle randomly selected from a normal distribution with mean angle 70° and standard deviation Filaments additionally become capped at rate  $r_{cap}$  (Table 1), which stops filament 10°. polymerization and branching. The addition of actin monomers to filaments generates pushing force for membrane motion,  $F_{Pol}$  (Figure 1A). As the membrane moves, the springs connecting the rods stretch, which increases membrane stiffness and causes a proportional increase in membrane tension,  $F_M$ . This limits membrane motion, which increases the load on the polymerizing filaments,  $-F_{Pol}$ , and induces actin filament retrograde flow. When a filament is within a distance  $d_{thresh}$  from an active integrin, it forms a harmonic interaction with the integrin. This interaction converts actin filament motion into traction force,  $F_A$ . As integrins switch their state from active to inactive, the connections with the actin filaments are lost and the integrins disappear.

In the BR-MC model, the rates of both actin polymerization and adhesion deactivation are force-dependent (Figure 1B, C). In the BR model, the rate of actin polymerization is dependent on membrane tension, but adhesion deactivation is force-independent (Figure 5A). In the MC model, actin filament polymerization is fixed and independent of membrane tension, while adhesion inactivation is dependent on traction force (Figure 5D).

<u>Model initialization.</u> Adhesion dynamics are pre-run for 2 s before initiation of actin dynamics. The adhesion concentration emerges from the relative magnitudes of  $r_{on}$  and  $r_{off}$ . The maximum number of adhesions allowed to activate is 2000  $\mu$ m<sup>-2</sup>, but the actual adhesion density is 750 – 1600  $\mu$ m<sup>-2</sup> (Table 1, (Wiseman et al., 2004)).

At model time 0 s, actin filament addition begins and filaments are added in a random distribution across the domain in the x direction and 20 nm below the nearest membrane segment in the y direction. Initial filaments are *n* monomers long, with *n* randomly selected from a normal distribution with mean 33 monomers, and standard deviation 2 monomers. Initial filaments are assigned random orientation, sampled from a flat distribution having a mean of  $0^{\circ}$  and range +/- 45°. This results in an average filament length and orientation of 89 nm and  $0^{\circ}$ . During model time 0 - 5 s, an actin filament is added every model iteration until the filament mass threshold is reached, defined as the sum of actin monomers at a given iteration and 12,500 monomers per 1 µm of lamellipodium length. After ~5 s, filament polymerization, depolymerization, and capping bring the model into a steady-state in which depolymerization reduces filament mass.

<u>Model iterations.</u> The model is developed using the explicit Euler implementation scheme for performing each model iteration, which consists of kinetics, force balance, and position updates. At each timestep of the simulations, all elements in the model move. The displacement of filaments and membrane depend on the forces acting on them, while adhesions appear and disappear as they switch between active and inactive states. A periodic boundary is implemented such that the left side of the leftmost membrane segment and the right side of the rightmost membrane segment behave as if they were neighboring and connected by the default 2 nm wide spring for neighboring membrane segments. At every iteration, the filament mass is computed. If the filament mass falls below the threshold due to actin depolymerization, an actin filament of ~33 monomers is added in random distribution across the width of the membrane. Adhesions activate based on  $r_{on}$  and reappear in random positions. If adhesions are within distance  $d_{thresh}$  of actin filaments, they connect to the filament.

Displacements of actin and membrane rods are calculated from the overdamped Langevin Equation, as:

$$d\boldsymbol{x} = \frac{\boldsymbol{F}_{TOT}}{\gamma} dt \qquad \text{eq. 1}$$

where x is a position vector of each element, dt is the time step,  $F_{TOT}$  is the sum of deterministic and stochastic forces acting on the element, and  $\gamma$  is a drag coefficient.

Each membrane segment experiences two forces: filaments pushing against it with a force  $F_{Pol}$  and pulling from neighboring membrane rods,  $F_M$ . Therefore, the total force on each membrane rod is computed as:

$$\boldsymbol{F}_{TOT.MEM} = \boldsymbol{F}_{Pol} + \boldsymbol{F}_{M} \qquad \text{eq. 2}$$

Actin filament pushing force,  $F_{Pol}$ , is the vertical component of force exerted by actin filaments on the membrane rod. For the BR-MC and BR Models,  $F_{Pol}$  is derived from the Brownian ratchet as described by Mogilner & Oster (Mogilner and Oster, 1996) as:

$$\boldsymbol{F}_{Pol}(y_0,\theta,L) = \sqrt{\frac{2k_BTK}{\pi}} \cdot \frac{e^{\frac{-Ky_0^2}{2k_BT}}}{1 + erf\left(y_0\sqrt{\frac{K}{2k_BT}}\right)}\,\hat{\boldsymbol{y}} \qquad \text{eq. 3}$$

where,  $y_0$  is the distance from the filament tip to the membrane segment above it,  $\theta$  is the orientation angle of the filament relative to the y-axis, and *L* is the length of the filament.

 $K = \frac{4\lambda k_B T}{L^3 \sin^2 \theta}$  and  $k_B$  is the Boltzmann constant 0.138 pN nm/K. In the MC model, when filaments are < 2.7 nm from the membrane,  $F_{Pol} = (1pN) \cos \theta$ . When filaments are > 2.7 nm from the membrane,  $F_{Pol} = 0 pN$ .

Membrane tension,  $F_M$ , is the elastic force contribution from the connection with neighboring membrane rods.  $F_M$  is calculated as the y component of the tension between membrane rods using the adjacent stretched spring as:

$$\boldsymbol{F}_{M} = -k_{m} (\boldsymbol{x}_{L} - \boldsymbol{x}_{0,M}) \boldsymbol{y}_{L} \boldsymbol{\hat{y}} - k_{m} (\boldsymbol{x}_{R} - \boldsymbol{x}_{0,M}) \boldsymbol{y}_{R} \boldsymbol{\hat{y}} \qquad \text{eq. 4}$$

where  $x_L$  and  $x_R$  are the left and right spring lengths bordering the rod and  $x_{0,M}$  is the equilibrium separation (Table 1).  $y_L$  and  $y_R$  are the magnitudes of the **y**-component of the force in the left and right springs.  $\mathbf{\hat{y}}$  is the unit vector in the y-axis direction. If  $x \le x_{0,M}$ ,  $\mathbf{F}_M = 0$ . The friction coefficient for membrane rods,  $\gamma_M$ , is the Stokes-Einstein drag coefficient for small spherical particles in a fluid of high viscosity (Edward, 1970):

$$\gamma_M = 6\pi\eta_m r \qquad \text{eq.5}$$

where *r* is the length of the membrane segment, and  $\eta_m$  is the fluid viscosity.

The total force on each actin filament is computed as:

$$\boldsymbol{F}_{TOT.ACT} = \boldsymbol{F}_T + (-\boldsymbol{F}_{Pol}) + \boldsymbol{F}_A \qquad \text{eq. 6}$$

where  $F_T$  is thermal force,  $-F_{Pol}$  is the instantaneous load the membrane exerts on the actin filament and computed from  $F_{Pol}$ , and  $F_A$  is the force from interaction with adhesions. Thermal forces in  $\mathbf{x}$  and  $\mathbf{y}$  directions are calculated as a Brownian stochastic force following the fluctuation dissipation theorem (Underhill and Doyle, 2004). For both  $\mathbf{x}$  and  $\mathbf{y}$  directions,

$$\boldsymbol{F}_T = rnd * \sqrt{\frac{2 * k_B T \, \gamma_F}{dt}} \qquad \text{eq. 7}$$

where *rnd* is a random number from a normal distribution with mean of zero and standard deviation of one. The friction coefficient for actin filaments ( $\gamma_F$ ) depends on the sum length of a branched actin filament structure,  $L_s$ , and the cytoplasmic viscosity,  $\eta_c$ .  $\gamma_F$  is expressed as:

$$\gamma_F = \frac{4\pi\eta_c L_s}{0.84 + \ln\left(\frac{L_s}{d}\right)}$$
 eq. 8

where *d* is the diameter of the filament and = 3.5 nm, on the order measured filament diameters (Oda et al., 2009).

Adhesion force, or traction force  $F_A$ , is calculated as:

$$\mathbf{F}_A = -\sum_i k_a (x - x_{0,A}) \hat{\mathbf{r}}$$
 eq. 9

where *i* is the number of adhesions attached to the filament,  $k_a$  is adhesion spring constant, *x* is the distance between the adhesion and attached actin filament, and  $x_{0,A}$  is the equilibrium separation between them (Table 1).  $F_A = 0$  for  $x \le x_{0,A}$ .

Actin filament polymerization. Actin filaments undergo polymerization at the barbed end. The probability of polymerization is calculated as:  $P_{polym} = C_p r_{pol} dt$ , where  $r_{pol}$  is the polymerization rate,  $C_p$  is a polymerization coefficient that ranges between 0 – 1. In the BR-MC and BR models,  $C_p$  is the product of two different polymerization coefficients:

$$C_p = C_1 C_2 \qquad \qquad \text{eq. 10}$$

 $C_1$  is derived from the Brownian ratchet as described by Mogilner & Oster (Mogilner and Oster, 1996):

$$C_{1}(y_{0}, \theta, L) = \frac{1 + erf\left((y_{0} - \Delta)\sqrt{\frac{K}{2k_{B}T}}\right)}{1 + erf\left(y_{0}\sqrt{\frac{K}{2k_{B}T}}\right)}$$
eq. 11

where  $\Delta = \delta cos\theta$ , and is the actin monomer length 2.7 nm, and  $k_B$  is the Boltzmann constant 0.138 pN nm/K.  $C_2$  introduces the decreasing double exponential for filaments that span the entire 0.2 µm away from the membrane to filaments juxtaposed to the membrane, from Ryan, et al. 2017 (Ryan et al., 2017):

$$C_2(y_0) = A_1 e^{-y_0/\lambda_1} + A_2 e^{-y_0/\lambda_2}$$
eq. 12

where  $A_1 = 0.84$ ,  $A_2 = 0.16$ ,  $\lambda_1 = 0.5 \mu m$ , and  $\lambda_1 = 4.0 \mu m$ . Filaments above the membrane are interpreted as bent, with polymerization approaching zero. Thus, filament polymerization depends on the membrane load and angle to the membrane. In the MC model, if the filament tip is within 1 monomer distance from the membrane (< 2.7 nm), polymerization does not occur, with polymerization coefficient PC = 0. When filaments are > than 2.7 nm from the membrane, polymerization occurs at a fixed rate, with PC = 1.0.

<u>Adhesion kinetics.</u> Adhesions undergo cycles of activation and deactivation. Adhesion activation is governed by  $r_{on}$ . Adhesion de-activation is governed by  $r_{off}$ . In the BR-MC and MC models,  $r_{off}$  is force-dependent. It depends on  $F_A$ , following catch-bond kinetics (Figure 1C), as:

$$r_{off} = Ae^{as_1F_A} + Be^{bs_2F_A}$$
eq. 13

In this way,  $r_{off}$  depends biphasically on  $F_A$ . Lifetimes versus force relations used in the BR-MC and MC models (lifetime  $\tau = 1/r_{off}$ ) are referenced according to their maximum  $\tau$  values ( $\tau_{max}$ ). A, B, a, b, s<sub>1</sub>, and s<sub>2</sub> were empirically adjusted to generate  $\tau_{max} = 3$ , 5, 7.5, and 12 pN (Table 2). In the force-independent BR model,  $r_{off}$  has a constant value of 1/3, 1/7.5, or 1/12 s<sup>-1</sup> (Table 1).

<u>Parameters and parameter fitting</u>. When possible, parameters were obtained from the literature (Table 1). The remaining parameters were either estimated or fitted as follows:

*Branching window* - estimated 50 nm width directly below the membrane, to ensure that branching occurs only on filaments that abut the membrane. If a filament is perpendicular to the membrane, with the barbed end pushing the membrane, it can still branch given that it will only branch at the end of a filament tip. Constraining branching within a fixed distance from the membrane maintains filament density and stable protrusions (Carlsson, 2001; Weichsel and Schwarz, 2010) and is consistent with the membrane-associated activators of ARP2/3 (Suetsugu, 2013).

*Membrane spring equilibrium length* – estimated 2 nm, one order of magnitude smaller than the membrane and filament segment units.

Model assumptions. Key model assumptions:

2D domain. The model represents the lamellipodium as effectively 2D.

*Actin.* The model represents actin as rigid actin filaments that do not bend or rotate, and slide only on two axes (x, y). The filaments exert at most a 1 pN force on the membrane, dependent on the angle of interaction.

*Membrane.* The membrane at the cell edge is represented as an elastic material, while weakening of cortical actin at protrusion onset (Mogilner and Oster, 1996; Bisaria et al., 2020; Welf et al., 2020) suggests that the membrane might have some viscous character at protrusion onset. Despite this, weakening the elastic force by reducing  $k_m$  did not change adhesions' regulation of protrusion velocity or period (Supplemental figure 3).

Adhesions. The model assumes that nascent adhesions function as single point particles (integrins) that do not slide. The adhesion lifetime includes the expected duration of adhesion engaging, un-engaging, and re-engaging actin during slippage. Final disengagement is modeled as disassembly of the integrin-ligand linkage. Despite this limitation, the model produces nascent adhesion traction forces that span the range of negligible and measurable traction forces associated with experimentally-measured paxillin-labeled nascent adhesions (Figure 2E, (Han et al., 2021)). The adhesions do not include myosin II, which is needed for significant negative velocity in edge motion (Giannone et al., 2007), consistent with the absence of retractions in the model.

*Protrusion-retraction cycle.* Model interpretations assume that oscillations in protrusion velocity are representative of the protrusion-retraction cycle. Short-lived negative velocities in edge motion are observed with our model dt = 0.2 ms. When velocity is averaged over 1-10 s, the average is always positive. Fluctuations in the positive protrusion velocity are observed as peaks and drops, which are representative of the oscillations in protrusion velocity driven solely by the actin waves in the lamellipodium (Giannone et al., 2007).

Instantaneous membrane-filament interaction. The model assumes that within a single time step dt, the membrane is functionally static. Thus, the force of the membrane pushing on a filament (- $F_{Pol}$ ) is approximated to be equal and opposite to the force the filament exerts against the membrane ( $F_{Pol}$ ).

Temperature. The model assumes a temperature of 310 K, ~37 °C.

#### Key resources table

Reagent type (species or resource)	Designation	Source or reference	Identifiers	Additional Information
Cell line (Chlorocebus sabaeus)	COS7	ATCC	CCL-81.2	
Transient expression vector	pcDNA3-mApple Paxillin	Smith et al. 2013 PMID: 23990882		Dr. Mary Beckerle (University of Utah, Salt Lake City, UT)
Transient expression vector	mEmerald-Lifeact	Addgene	#54148	Emerald-Lifeact-7

# Cell culture

COS7 cells were obtained from ATCC, cultured in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L D-glucose, L-glutamine, and sodium pyruvate (Gibco 11965092) containing 5% Fetal Bovine Serums (FBS, Avantor Seradigm 97068-085), and tested for mycoplasma every 3-6 months. Mycoplasma-negative COS7 cells were plated on acid-treated 1.5 coverslips within 35 mm glass-bottom dishes (MatTek P35G-1.5-14-C), and transfected the following day with pcDNA3.1/Paxillin-mApple or mEmerald-Lifeact at 20% confluency using TransIT-LT1 (Mirus MIR 2304) following the manufacturer's instructions. Two days post-transfection, medium was replaced with FluoroBrite DMEM (Gibco) supplemented with 10% FBS and 20 mM HEPES.

#### Live cell TIRF imaging of adhesions

For adhesion imaging, cells were imaged on an automated Nikon Ti inverted microscope with motorized total internal reflection fluorescence (TIRF), Perfect Focus 3 to maintain laserbased identification of the bottom of the substrate during acquisition, a CFI Apo TIRF 100x oil Apo 1.49 NA objective, 561 solid-state laser (Vortran), ET620/60m emission filter (Chroma), and Photometrics Prime 95B camera configured at a 100 MHz readout speed to decrease readout noise with Metamorph. Images were taken every 3 s for 5 min, with sequential images at every time point with the TIRF angle set to optimal TIRF and with the TIRF angle set as vertical for effective widefield imaging. The acquired images had an effective pixel size of 45 nm. Imaging was performed at 37°C, 5% carbon dioxide, and 70% humidity. Laser powers were decreased as much as possible and the exposure time set at 200-400 ms to avoid phototoxicity.

#### Adhesion segmentation, detection, and tracking

Nascent adhesions were detected and segmented using point source detection as previously described in (Han et al., 2015; Han et al., 2021). Briefly, fluorescence images were filtered using the Laplacian of Gaussian filter and then local maxima were detected. Each local maximum was then fitted with an isotropic Gaussian function (standard deviation: 2.1 pixels, i.e. ~180 nm) and outliers were removed using a goodness of fit test (p=0.05). The point sources detected for nascent adhesions were tracked over the entire frames of the time-lapse images using uTrack (Jaqaman et al., 2008). Lifetimes of adhesions were calculated from lifetimes of individual tracked trajectories. A GUI-based MATLAB software for the edge analysis is from Danuser lab (Machacek and Danuser, 2006). Due to the noise in edge motion from the 3 s framerate, IMFs were removed in the edge motion data.

#### Live cell confocal imaging of cell edge and analysis

For cell edge imaging, cells expressing mEmerald-Lifeact were imaged on a Nikon Ti inverted microscope with a CFI Apo TIRF 60x oil, 1.45 NA objective employing Perfect Focus, a Yokagawa CSU-10 spinning disk confocal with Spectral Applied Research Borealis modification, a 488 solid-state laser, Chroma ET525/50m filter, and Photometrics Myo CCD camera with Metamorph. Images were taken every 10 s for 5 min, with 400-700 ms exposures. For experiments with prolonged adhesion lifetime, cells were treated with 1 mM MnCl<sub>2</sub> 2 h prior to imaging. Time-lapse images were analyzed for cell edge protrusion dynamics in MATLAB software as described previously (Samson et al., 2019). A GUI-based MATLAB software for the edge analysis is from Danuser lab (<u>https://github.com/DanuserLab/Windowing-Protrusion</u>) (Lee et al., 2015). A two-sample nonparametric Kolmogorov–Smirnov test at 5% significance tested for population distribution equality. IMFs were not removed from this edge motion data.

#### Data representation and statistics

Models were run until additional iterations no longer changed the result output, 5 model runs per simulation, except for tests of time step, lamellipodium length, and membrane unit width which applied 3 model simulations (Supplementary figure 2). Box plots show the  $25^{th}$ - $75^{th}$  percentile of the 5 simulation means and notches are 95% confidence interval (CI). Central lines are the medians. Values outside of 1.5 and 3 box-lengths from the 75th or 25th percentile are depicted as outliers with a + sign. Output calculations do not include the first 10 s of model time, when the actin network is populating the initial lamellipodium domain. The non-parametric Mann-Whitney U test was used to test for difference in the means for all modeling data and adhesion-edge analyses. Positive values in the retrograde flow calculations were due to thermal motion and were removed from the calculations. Adhesion lifetimes were compared by the nonparametric Kolmgorov-Smirnov (K-S) test, to test for differences in the distributions caused driven by  $\tau_{max}$ .

Experiment sample size was chosen based on a minimum of three independent biological replicates and hundreds to thousands of adhesions and protrusion events analyzed, respectively, within each replicate. The Kolmogorov-Smirnov test was used to test for difference in the distribution of edge motion upon  $Mn^{+2}$  treatment. Unless otherwise mentioned in each figure caption, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001.

# Software availability

MATLAB software for the computational model is shared via GitHub at <u>https://github.com/MendozaLabHCI/ActinModel\_01</u>.

A GUI-based MATLAB software for the adhesion analysis is shared via GitHub at <u>https://github.com/HanLab-BME-MTU/focalAdhesionPackage.git; Han, 2021;</u> copy archived at <u>swh:1:rev:6aeb3593a5fd3ace9b0663d1bf0334decfb99835</u>.

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## **Competing Interests**

The authors declare no competing interests.

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#### Figure



Figure 1. Computational model of lamellipodium protrusion. (A) Elements and interactions in the 2D computational model. Actin filaments are represented as polarized rigid rods with each unit representing a single actin monomer. Actin filaments can form branches within the membrane contact region ( $r_{branch}$ ). Polymerization with rate  $r_{pol}$  against the membrane pushes the membrane forward ( $F_{Pol}$ , see inset). The membrane is represented as rods interconnected by harmonic potential energies with defined stiffness  $k_m$ , which create tension upon displacement. The resulting membrane tension ( $F_M$ ) limits membrane motion, which increases the load and thereby restrains actin polymerization and induces retrograde flow. Adhesions are represented as integrins that undergo cycles of activation and deactivation, with  $r_{on}$  and  $r_{off}$ , respectively. When

active (black star), the adhesions attach to actin filaments if within a proximity of 15 nm (red star). Adhesion loading is represented as a spring that builds tension and slows the filament's retrograde flow (traction force,  $F_A$ , see inset). A periodic boundary condition wraps the model edges and creates an infinite domain in which all the membrane segments experience spring tension on both sides. For each time step, the new position for each membrane segment is computed from the sum of  $F_{Pol}$  and  $F_M$ . The new position for each actin filament is computed from the sum of thermal forces, the instantaneous load, and  $F_A$ . (B) The BR-MC model. The rate of actin polymerization is dependent on the load ( $-F_{Pol}$ ), which changes as the membrane is repositioned. The rate of adhesion deactivation is dependent on traction force. (C) Force-dependent relationship of the molecular clutch: adhesion lifetime ( $\tau$ ) versus adhesion-filament bond tension where  $\tau = 1/r_{off}$ . Under high tension (50 pN),  $r_{off}$  increases exponentially with increased tension.



**Figure 2. BR-MC Model reproduces physiological rates of edge protrusion and retrograde flow.** Simulations of protrusion with  $k_m = 0.3$  pN/nm,  $r_{branch} = 1.0$  s<sup>-1</sup>,  $\tau_{max} = 3.0$  s. (A) Simulation snapshots. Individual filaments are depicted in different colors. Parameters listed in Table 1. Membrane movement in **y** from the sum of forces ( $F_{Pol}$  and  $F_M$ ). (B) Heatmap of mean edge velocity. (C) Raw velocity trace with low pass filtering. (D) Velocity time series after period extraction by Empirical Mode Decomposition, in which the first 8 intrinsic mode functions (IMFs) were subtracted. The period was defined as the time between local minimums in membrane segment velocities. (D) Mean retrograde flow at each membrane segment and (E) averaged over entire 2 µm membrane width. (F) Mean traction force at each membrane segment. Heatmaps show emergent properties at each 20 nm membrane position (18 nm segment + 1 nm of spring on each side) as 1 sec moving averages during 2 min of 1 model run, representative of n = 5. Colorbars show 5<sup>th</sup> – 95<sup>th</sup> percentiles.



Figure 3. Increasing adhesion lifetime promotes protrusion velocity but shortens the period. BR-MC Model simulations of 5 min of protrusion with  $k_m = 0.3$  pN/nm, with  $r_{branch} = 1.0$  s<sup>-1</sup>,  $r_{on} = 1.0$  s<sup>-1</sup> and  $r_{off} = 1/\tau$  s<sup>-1</sup>. (A, B) Plots of protrusion velocity and period as a function of adhesion lifetime,  $k_a = 10$  pN/nm. (C, D) Plots of protrusion velocity and period as a function of adhesion spring constant, which shortens lifetime. (E, F) Colormaps of mean protrusion velocity and period when  $r_{branch}$  and adhesion lifetime are varied together. Values between tickmarks are interpolated.



Figure 4. Longer adhesion lifetime is associated with faster edge velocity and shorter period. (A, B) Tracked adhesions and segmented cell edges of COS7 cells with shorter lifetime (A) and longer lifetime (B). Adhesions and cell edges are color-coded for lifetime and frame with  $\Delta t = 3$  s. White arrowheads depict longer-living adhesions per each cell movie. (C, D) Error bar plots of velocity and protrusion period of edge protrusion in cells with different overall lifetime. Error bars are standard error of mean. \* p<0.05, \*\*\* p < 10<sup>-15</sup>. *p* value from Man-Whitney's U test. (E, F) Distribution of protrusion velocity and period, from *m* protrusion events in *n* = 7 cells treated with DMSO and *n* = 8 cells with Mn<sup>+2</sup>. Boxes span the 25<sup>th</sup> to 75<sup>th</sup> percentile. The central horizontal line marks median and notches are 95% CI. *p* value from Kolmogorov–Smirnov test.



Figure 5. Adhesion lifetime controls protrusion period through the Brownian ratchet mechanism. (A) The BR model, in which the rate of actin polymerization is dependent on membrane load and adhesion deactivation is force-independent. (B, C) Plots of lamellipodium velocity and protrusion period as a function of adhesion lifetime for BR Model, 5 min of protrusion simulation with  $r_{on} = 0.2$  s<sup>-1</sup>. (D) The MC model, in which actin filament polymerization is independent of membrane tension and adhesion deactivation is dependent on traction force. (E, F) Plots of lamellipodium velocity and protrusion period as a function of adhesion adhesion deactivation is dependent of adhesion deactivation is dependent on traction force. (E, F) Plots of lamellipodium velocity and protrusion period as a function of adhesion lifetime for MC Model, 5 min of protrusion simulation with  $r_{on} = 1.0$  s<sup>-1</sup>.



Figure 6. Adhesion lifetime acts through the attached filaments to control actin filament force and polymerization. BR-MC Model simulations of 5 min of protrusion with  $k_m = 0.3$ pN/nm, with  $r_{branch} = 1.0 \text{ s}^{-1}$ ,  $r_{on} = 1.0 \text{ s}^{-1}$  and  $r_{off} = 1/\tau \text{ s}^{-1}$ . (A) Plot of number of free versus attached filaments as a function of adhesion lifetime. (B-D) Plots of Brownian ratchet factors that control filament force and actin polymerization as a function of adhesion lifetime: filament angle of interaction with the membrane, filament distance from the membrane, and filament length. (E) Plots of actin filament force, (F) membrane tension, and (G) polymerization coefficient, C<sub>p</sub>. Simulations with  $r_{branch} = 0.5 \text{ s}^{-1}$ .



Figure 7. Adhesion density shortens the protrusion period.

BR-MC Model simulations of 5 min protrusion time,  $k_m = 0.3$  pN/nm,  $r_{branch} = 0.5$  s<sup>-1</sup>,  $r_{on} = 1.0$  s<sup>-1</sup>. (A) Plot of adhesion density with increasing  $\tau_{max}$  and (B) increasing  $k_a$ . (C) Changing adhesion density by decreasing the number of allowed adhesions with  $\tau_{max} = 3$  s. (D, E) Protrusion velocity and period as a function of adhesion density.

# Table 1. Model parameters

Parameter name	Symbol	Default value	Reference
Time step	dt	0.2 ms	estimated
Temperature	Т	310 K	
Boltzmann constant	$k_B$	0.138 pN nm/K	
Actin Filaments	-	•	
Monomer concentration		15 μM	(Pollard, 1986)
Monomer length		2.7 nm	(Pollard, 2016)
-			(Mogilner and Oster, 1996;
		33 monomers,	Svitkina et al., 1997; Svitkina
Average filament starting length		89.1 nm	and Borisy, 1999)
Persistence length	λ	1 µm	(Mogilner and Oster, 1996)
Polymerization rate (barbed end)	$r_{pol}$	$11 \ s^{-1} \ \mu M^{-1}$	(Pollard, 1986)
Depolymerization rate (pointed end)	$r_{depolym}$	$12 \ s^{-1}$	(Pollard, 1986)
Branching rate (barbed end)	<i>r</i> <sub>branch</sub>	$1.0 \ s^{-1}$	(Beltzner and Pollard, 2008)
Branch window height		50 nm	estimated
			(Volkmann et al., 2001; Welch
			and Mullins, 2002; Rouiller et
Mean filament branch angle		70 degrees	al., 2008)
		10.1	(Mullins et al., 1998; Cai et al., $2008$ ; Caledana et al., $2017$ )
Filament branch angle normal distribution SD		10 degrees	2008; Sokolova et al., $2017$ )
Capping rate (barbed end)	$r_{cap}$	35	(Schafer et al., 1996)
Filament mass threshold/ $\mu m$ lamellipodium		12,500 monomers	(Abraham et al., 1999)
Gamma (drag coefficient)	$\gamma_F$	~0.01 pN s/nm	<i>eq.</i> 8
Cytoplasmic viscosity	$\eta_c$	$10 \ Pa \cdot s$	(Wirtz, 2009)
Filament force vector magnitude (MC model)		l  nN	(Kovar and Pollard, $2004$ ; Ecoter et al. $2007$ )
Mombrono		1 piv	100ter et al., 2007)
Mombrong longth (at initialization)		2 1 100	astimated
Segment width		2 µm	estimated
Membrone envire equilibrium length		10 mm	estimated
Memorane spring equilibrium length	$\chi_{0,M}$	2 nm	(Lieber et al. 2013: Shi and
Membrane spring constant	<i>k</i>	0 3 nN/nm	Baumgart 2015)
Gamma (drag coefficient)	Nm VM	0.0 p10/1010	$e_a$ 5 (Edward 1970)
Membrane viscosity	7м <b>п</b>	100 Pa·s	(Wang et al 2019)
Adhesions	'Im	100145	(() ang et al., 2017)
			(Choi et al., 2008: Han et al.,
Activation rate (BR-MC and MC models)	ron	$1.0  s^{-1}$	2021)
	011		(Choi et al., 2008; Han et al.,
Activation rate (BR model)	<i>r</i> <sub>on</sub>	$0.2 \ s^{-1}$	2021)
			(Changede et al., 2015; Han et
Deactivation rate (BR-MC and MC models)	$r_{off}$	$1/\tau s^{-1}$	al., 2021)
		1	(Changede et al., 2015; Han et
Deactivation rate (BR model)	$r_{off}$	$1/3, 1/7.5, 1/12 s^{-1}$	al., 2021)
	7		(Han et al., 2015; Wang et al.,
Adhesion-filament spring constant	$k_a$	10 pN/nm	2015)
Adhesion-filament spring equilibrium length	$x_{0,A}$	2 nm	(Plotnikov et al., 2012)
Filament connection threshold	$d_{thresh}$	15 nm	(Kanchanawong et al., 2010)

Adhesion region depth	0.2 µm	
Symbols		
Length of actin filament (nm)	L	
Length of branched filament structure (nm)	$L_s$	
Filament angle relative to membrane	$\theta$ (perpendicular = 0)	
Distance from filament tip to membrane, in y	y <sub>0</sub>	
Adhesion lifetime	τ	

	$\tau_{max} = 3$	$\tau_{max} = 5$	$\tau_{max} = 7.5$	$\tau_{max} = 12$
А	2.0	0.93	0.5	0.25
В	5E-6	5E-6	5E-6	5E-6
a	-9.727E-3	-8.40E-03	-7.286E-3	-6.12E-3
b	0.3	0.3	0.3	0.3
<b>S</b> <sub>1</sub>	6.8	6.8	6.8	6.8
<b>s</b> <sub>2</sub>	1.0405	0.975	0.9228	0.8606

Table 2. Parameters for  $\tau_{max}$  (eq. 10)