Mechanics of Cell Migration Using Motor-Clutch Model and Optimality in Cell Migration

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This is to certify that the dissertation titled **"Mechanics of Cell Migration Using Motor-Clutch Model and Optimality in Cell Migration"** submitted by **Pranay Jaiswal (Reg. No. MS16142)** for the partial ful-filment of BS-MS dual degree programe of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Declaration

The work presented in this dissertation has been carried out by me under guidance of Dr. Abhishek Chaudhuri at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of work done by me and all sources listed within have detailed in the bibliography.

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In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Abhishek Chaudhuri

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Finally and most importantly, I thank Simranjit, for everything.

Abstract

As we know that cell migration is a very important process in life, from the development of embryos continuing until death. The extracellular environment impacts the cell to a great extent in migration. Many cells exhibit a stiffness optimum at which the migration is maximum. This optimum stiffness varies over vast ranges depending on the cell. In this thesis, I give an account of a motor-clutch model of cell traction which displays a maximum in traction force with respect to substrate stiffness. I look at this optimality with varying parameter values. Finally I incorporate motor attachment/detachment to see the effect of this variation on the cell migration model.

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CHAPTER 1

Introduction

Optimality is one of the sovereign concepts of the world. May it be in the field of economics, geography or science etc optimality is ubiquitous. The reason behind why we are living on earth can be understood in context of optimality for instance, food is a basic necessity for humans to survive and food can't be grown until and unless we have an optimal environment for that crop to be cultivated.

The main focus of this disquisition is to understand how cells behave according to the environment given and to sense the optimal stiffness. If the adhesivity of the cell is too high then it becomes very difficult for the cell to wander around because it sticks too tightly with its location. Whereas, if the adhesivity is too low then also it becomes difficult for the cell to drift because there is not enough traction force against the environment. Therefore, cell seems to have a particular environmental stiffness at which its migration is maximum[22][26].

1.1 Cell Migration

The cell migration can be explained by a cyclic process consisting of four steps : (1) polarization of the migrating cell (2) extension of the protrusion (3) formation of adhesion and (4) disassembly of adhesion (**Figure 1.1**).

The extension of the cell membrane at the front of the cell is done by polymerization reaction of cytoskeleton polymer actin, due to this a protrusion is created and it is called filopodium if it is narrow and it is called lamellipodium if it is broad. The molecules

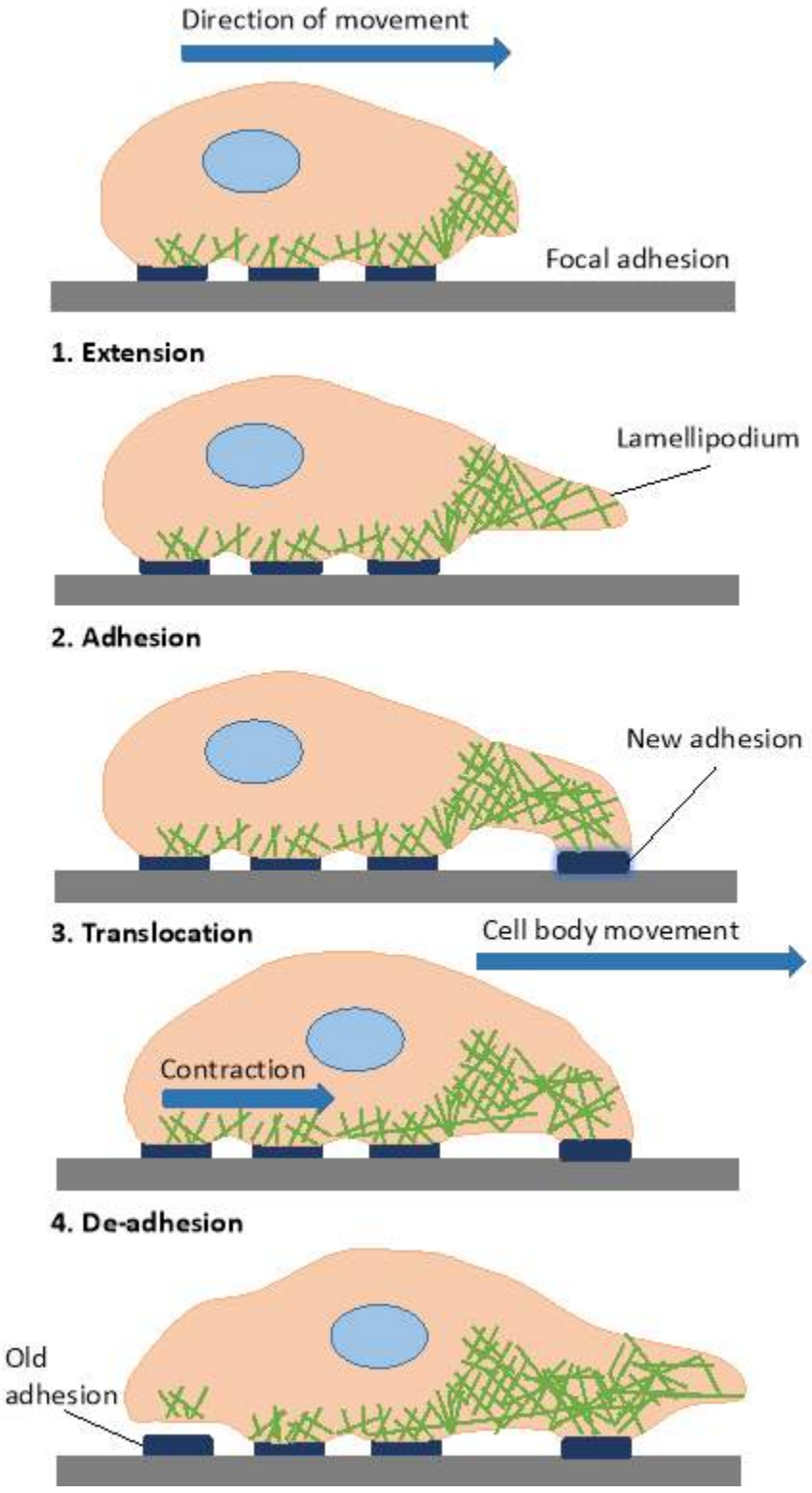


Figure 1.1: Steps of Cell Migration [8]

existing in the cell membrane join to the intracellular protein to form an adhesive complex and this complex binds the protrusion to the extracellular molecules. Lastly the forward advancement of the cell results when the molecular motors contract at the back end of the cell. These are the processes for a cell to migrate [1].

Cell migration is important for us because a lot of biological processes involve cell migration such as wound healing, embryonic development, morphogenetic processes (nervous system development), inflammatory response etc. Many unpleasant consequences like chronic Inflammatory diseases, cancer metastasis and osteoporosis occur due to malfunction of cell in migration[24].

1.2 Cell Migration Model

There are two different categories of cell migration models mentioned above (1) Molecular scale (2) Cellular scale). The first cellular scale models generally followed a random walk or biased walk framework to describe the movement of the cell.

1.2.1 DiMilla et al. [11]

This model describes the stiffness-sensitive cell migration used to describe the relationship between optimality of cell migration and adhesion strength, also called adhesivity. A network of viscoelastic nodes comprising a cell which includes the three processes of the cell migration. The cell cycles through a process of extension, adhesion and retraction after imposing a different value of adhesion strengths for front and back of the cell. By changing the binding affinity of molecules at the front and back of the cell the cell adhesivity is altered. The model output mimicked experimental results obtained by measuring cell speed on differing concentrations of the adsorbed protein. It was observed the cell speed was low at both low and high adhesivity and it was high at intermediate adhesivity. This model comprises adhesion molecular mechanics and intracellular mechanics. It is inefficient to be used to describe cell migration in different mechanical environments as this model fails to care for the extracellular mechanics. Moreover, the model takes a

different polarity to the front and back of the cell without explaining its origin.

1.2.2 Zaman et al. [33]

In this model the adhesion tension grows consistent with the environmental stiffness. The drawback of this model is that it contains no molecular scale information, and as the force on adhesion is constant, it contains no adhesion dynamics. This model predicts both the stiffness and adhesivity optima of the cell migration.

1.2.3 Pathak and Kumar [22]

This model is very much similar to the previous model which engaged two functions which varied stiffness - one was controlling number of cellular sites for adhesion and the other was controlling the cellular contractile force. This model also lacks molecular details and adhesion dynamics.

1.2.4 Paszek et al.[21]

In this model, the integrins are considered to be as Hookean springs with some off-rates which increases along with the tension according to the bell model. Rigid substrates do not easily distort the shape which means that grouping of integrin is required to cooperatively distort the substrate and form stable adhesion and the soft substrates deform easily as there is not enough tension on the integrin. The algorithm used in this model was Kinetic Monte Carlo or Gillespie Stochastic Simulation. The advantage of this algorithm is that it allows one event in every time cycle [14].

1.2.5 Walcott et al. [31]

This model is similar to the previous model for adhesion development and decay. Over here the tension on adhesion molecules are caused due to contraction of actin myosin. We see that adhesion grows faster and is more stable on the stiff substrate. So the results were also similar to the previous model but none of the model explains the reason behind the biphasic relationship of cell migration on substrate stiffness.

1.2.6 Motor-Clutch model of Cellular Force Transmission

This model is the main focus of my research work. This model was explored in great detail by Odde and coworkers.[4]

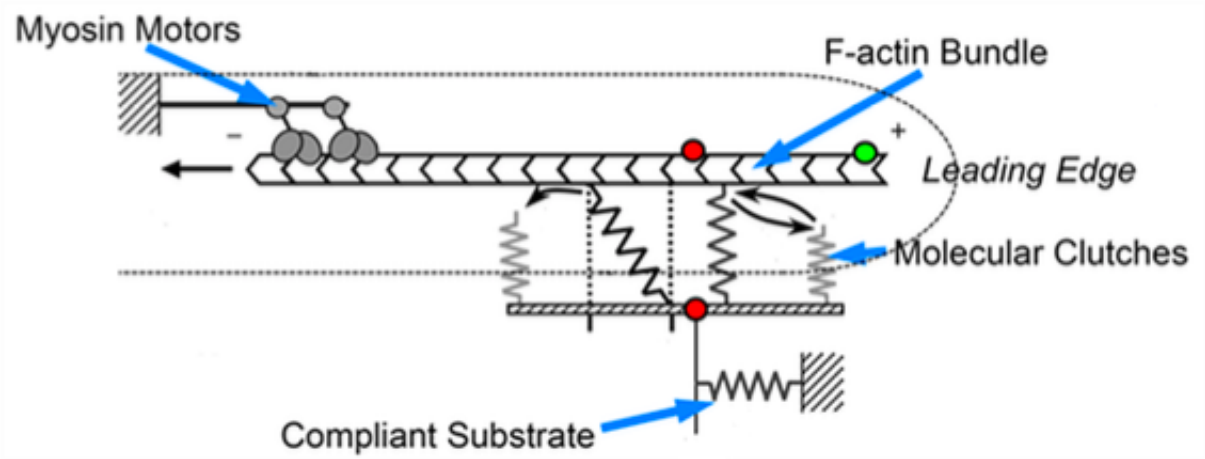


Figure 1.2: Motor-Clutch Model for Cell Traction Force [3]

Here we have n_m is the number of molecular motors, F_m is the stall force which is passed on to the extracellular substrate which is considered to be a spring with rate constant K_s (see Fig. 1.2). The stall force acts on the actin filaments and adhesion molecules or the "clutches". n_c represents the number of clutches. Each clutch is supposed to be a spring with its spring constant K_c. The motor velocity was given by v_m and v_m^* represents the velocity of actin filaments when none of the clutches were fastened to the f-actin or is also known as the unloaded velocity. The motor velocity follows an inverse force-velocity relationship given by the equation (**1.1**)

$$v_m = v_m^* \left(1 - \frac{K_s X_s}{n_m F_m}\right) \tag{1.1}$$

Here in the above equation X_s is the substrate position.

The clutches from the F-actin as described overhead gets attached to the substrate at a constant rate, K_{on}. And the rate of unbinding is given by K_{off} see equation (**1.2**) which increases exponentially with the force on the clutch, F_c, specified by the bell model.[5]

Where K^*_{off} is the unloaded off-rate, and F_b is the bond rupture force.

$$k_{off} = k^*_{off} exp\left(\frac{F_c}{F_b}\right) \tag{1.2}$$

The force on each clutch is given by the hooke's law in the given equation (**1.3**). Where x_c is the position of clutch attachment to F-actin:

$$F_c = K_c\left(x_c - x_s\right) \tag{1.3}$$

The model was carried out by a fixed time step Monte Carlo algorithm, the substrate position was calculated one step at a time by the force balance equation between the clutch ensemble force and the substrate spring force by the equation (**1.4**) given below

$$K_s - K_c \sum_{i=1}^{n_c} \left(x_{c,i} - x_s\right) = 0 \tag{1.4}$$

The motor-clutch model precisely predicts the load fail cycle of cellular force transmission as seen experimentally in migrating cells[10]. The molecular clutch begins to bind from actin to the extracellular substrate this allows the molecular motors to transmit force to the substrate. Initially, the backward movement of the actin filaments is fast because there is not enough force to withstand the motion of the same. As the clutches get attached this builds up a force. As the substrate also starts to move from its position and hence the flow of f-actin slows down. This also increases the force of bounding clutches and this increases its off rate as we can see from the equation above. A few moments later, the clutches begin to unbind due to tension this leads to cascading failure of entire adhesion molecules. The substrate reverts back to its original position and it relaxes. Then the cycle restarts again.

1.3 Motor-Clutch Model Provide a Better Framework Than the Other Models

This model displays an optimum stiffness at which the force transmission is maximal and F-actin retrograde flow is minimal, it is substrate sensitive even though none of the input function straightforwardly depends on the substrate. It carry out an inverse force-velocity relationship to accurately describe the F-actin flow as it was observed in the vitro experiment. It shows the load-fail dynamics which is obligatory for cell migration. If there won't be any adhesion failure then the cell would remain secured at its starting position and will be ineffectual to migrate. These are the few key aspects which were not accommodated together in the previous models [27].

CHAPTER 2

Details of Cell Migration

2.1 Polymerization of Actin and creation of cellular protrusion

Hydrolysis of adenosine triphosphate (ATP) favours the polymerization reaction and the cytoskeleton component filamentous (F)-actin forms from the polymerization of globular (G)-actin monomers. The mostly occupied protein in eukaryotic cells is the Actin[12]. The polymerization of actin conducts to extension of cell membrane for the formation of protrusion [7], this also gives a stability to the protrusion by adding cell membrane at a particular region of the cell see (**Figure 2.1**) [25]. F-actin is polarized with one "hooked" end and one "sharp" end. G-actin gets attached to the hooked end of the polymer, while monomers generally segregates at the pointed end [6].

The type of protrusion formed depends on the organization of F-actin. Lamellipodia results from F-actin networks created by the Arp2/3 complex which allows for nucleation of F-actin branching off of an existing filament [28]. Arp2/3 is activated by the WAVE/Scar complex and N-WASP, which in turn are activated by the Rac small GTPase [16]. Conversely, Filopodial formation is mainly promoted by the small GTPase Cdc42. Filopodia are believed to be extrapolated structures that a cell may use to probe its environment[18]. Filopodia is formed from the unbranched parallel F-actin bundled together by the cross-linking proteins such as facin and α-actinin [9].

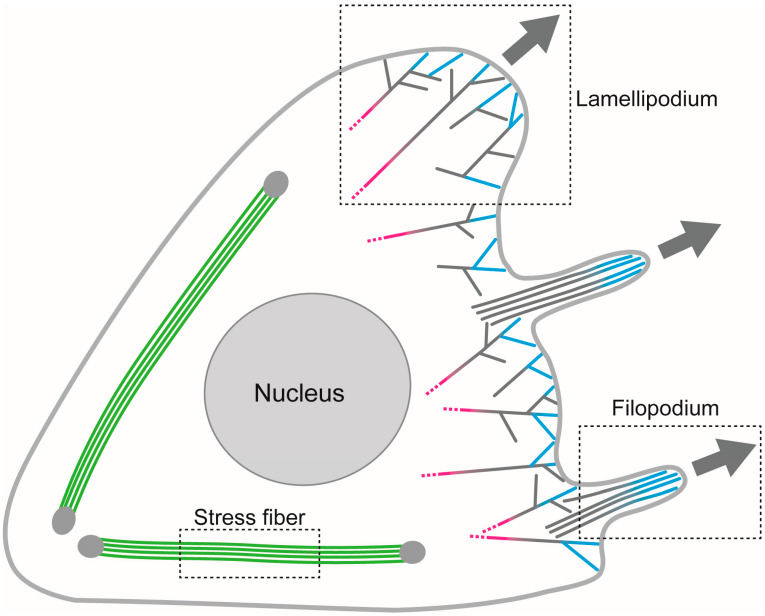


Figure 2.1: Green filaments indicate actin filaments while blue filaments in lamellipodium and filopodia are polymerized by formin bound to the barbed end near the cell membrane.[29]

2.2 Cellular Adhesion

Cellular adhesion permit cells to tie up with their environment and other cells. These adhesion molecules serve in adding stability and signaling capacity; they accommodate transmembrane molecules which bind to the environment exterior to the cell [2]. Cell to cell adhesion is formed by cadherins [15], while transmembrane integrins and other cell adhesion molecules (CAMs) are the reason for the cell to adhere to the extracellular matrix (ECM) fibres [23]. Several different molecules team up to form the intra cellular complex of adhesion complex. Vinculin stabilizes talin in its tension-induced conformation while Talin link integrin to F-actin undergoes a conformational change under tension [17]. The formation maturation, and disassembly of adhesion is regulated by signalling molecules such as focal adhesion kinase (FAK) and paxillin [30, 32].

2.3 Myosin and Cellular Retraction

When myosin attaches to F-actin, the hydrolysis of ATP results in a conformational change in the myosin molecules which give rise to "walk" along the F-actin towards the hooked end. If the tail of myosin remains motionless then this results in the retrograde flow F-actin away from the edge which is leading towards the central part of the cell. Cellular adhesions bound to the F-actin may act as a clutch and withstand this retrograde flow. Retraction occurs when adhesion lets go of the F-actin, allowing myosin to pull it towards the central part of the cell. The membrane retracts with the F-actin as during retraction, the F-actin is typically no longer polymerizing against the membrane in the region of the release. The myosin walking velocity is inversely proportional to the force generated such that at low force, we see a high velocity and at a high force, we see a low velocity [27]. This kind of relation is also seen while the actin polymerization against the cell membrane [20].

The rapid increase in the number of cells, its differentiation, survival and migration all depends a lot on the environment at which the cell is present[13]. It has been seen that the cell migration speed increases with the substrate Young's modulus. (For example: U87 and U373 glioblastoma, vascular smooth muscle cells, MCF10A epithelial cells). Conversely, few other studies say that the cell migration speed decreases with the increases in Young's modulus. (For example: 3T3 fibroblast, T24 carcinoma cells, neutrophils). Therefore, these two opposite results suggest that the cell speed shows a biphasic relationship to the substrate stiffness. Depending on the cell type and environmental conditions the cell speed may rise and then fall, exhibiting some maximal speed in some intermediate stiffness.

2.4 Motor-Clutch Hypothesis

In this hypothesis the F-actin gathers at the plasma membrane to push the membrane forward while myosin motors pull the F-actin rearward to generate a F-actin retrograde

flow [19]. In this the adhesion molecules or the "clutches" it transmit force to the extracellular environment and this results in slowing down the retrograde flow and induces the forward movement of the cell.

This model imposes force velocity relationships on the motors. The formation of the model is such that it looks after the how the cell responds for different substrates and how to different substrate stiffness and it also shows the load and fail cycle. n_m is the number of molecular motors with stall force F_m. It transmits the load to the substrate with "clutches", n_c is the number of clutches with there on and off rate given as k_{on} and k^*_{off} respectively and the velocity of F-actin when none of the clutches were attached also known as the unloaded velocity v^*_m.

As the clutches load with their spring constant K_c, their off-rate increases exponentially which is scaled by a characteristic bond rupture force known as F_b see (**Figure 1.2**). Therefore, on a total we have 5 clutches parameters (n_c, K_{on}, K^*_{off}, F_b and K_c) and 3 motors parameters (n_m, F_m and v^*_m) which defines the motor-clutch model.

2.5 Gillespie Stochastic Algorithm

I used a stochastic algorithm to stimulate the motor-clutch model. The event time of each event (binding/unbinding) was evaluated at each iteration by incorporating a Gillespie Stochastic Simulation.

$$t_{event,i} = \frac{-\ln(URN_i)}{k_i} \tag{2.1}$$

In the above equation (**2.1**) URN_i is the uniformly distributed random number between zero and one, and k_i is the kinetic rate for the clutch (binding or unbinding). This method protects us against the possibilities of two events occurring at the same time. For example, let's assume a situation in which one clutch is bound and using the fixed time approach that clutch will unbind and another clutch will bind. If the unbinding comes first then the system will collapse but did not because another clutch was allowed to bind at the same time step. The Gillespie SSA allows only one event at each time step

therefore it takes care of such situations.

CHAPTER 3

Motor Clutch Model

3.1 Working of the Motor-Clutch Model

Treating molecular clutches and the substrate as simple, Hookean springs (**Fig. 1.2**), we reproduce a stochastic physical model based on the motor-clutch hypothesis[3]. n_m myosin motors drive retrograde flow by exerting force on actin at every time step. Individual molecular motors with a constant rate k_{on} and k_{off} are permitted to attach/detach to/from the F-actin bundle. The clutches which are successfully engaged with the F-actin will build tension with spring constant K_c. Due to retrograde motion the clutches stretch. According to Bell's law, the tension along the engaged clutches increases their off rate k^*_{off} exponentially, with a characteristic breaking force F_b. Traction force is formed due to tension in the engaged clutches; this traction force must be balanced with the deformation of the compliant substrate with spring constant K_{sub}. Myosin motors work against this load force, slowing their motor sliding velocity according to a linear force velocity relation.

	Parameters	Symbols	value
clutch parameters	clutch spring constant	K_c	0.8 pN/nm
	clutch on-rate	$k_o n$	0.3 s^{-1}
	clutch off-rate	k^*_{off}	0.1 s^{-1}
	clutch bond rupture force	F_b	2 pN
	number of clutches	n_m	50
motor parameters	motor unload velocity	v^*_m	120 nm/s
	motor stall force	F_m	2 pN
	number of motors	n_m	50

Table 3.1: Base parameter values for motor-clutch model

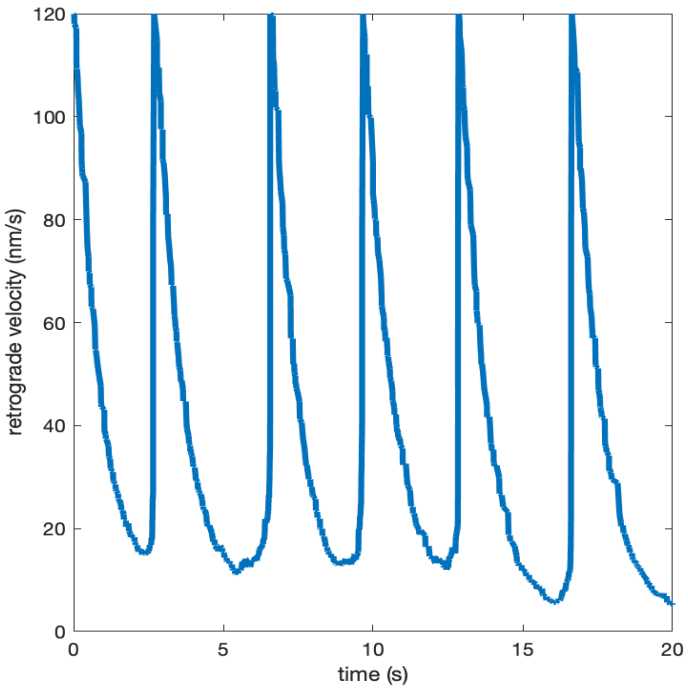


Figure 3.1: Model-predicted retrograde velocity as a function of time highlights load and-fail on compliant substrates

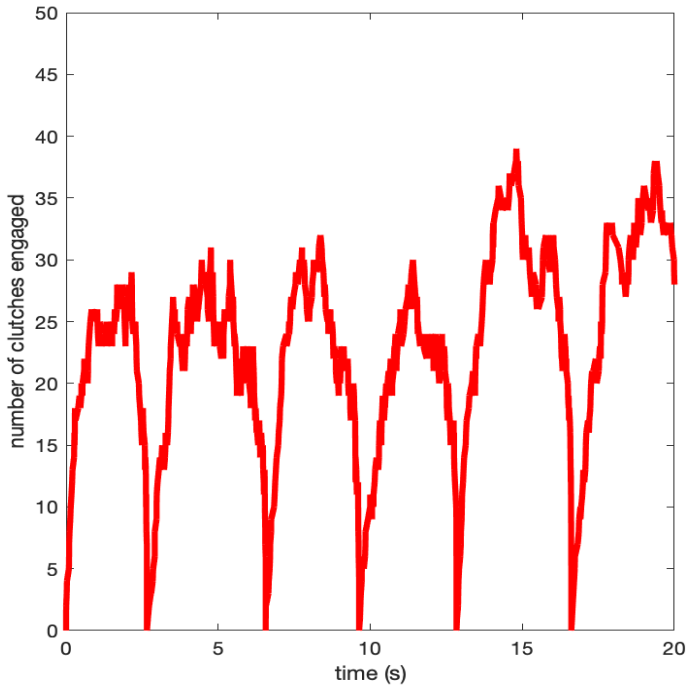


Figure 3.2: Model-predicted attached clutches as a function of time highlights load and-fail on compliant substrates

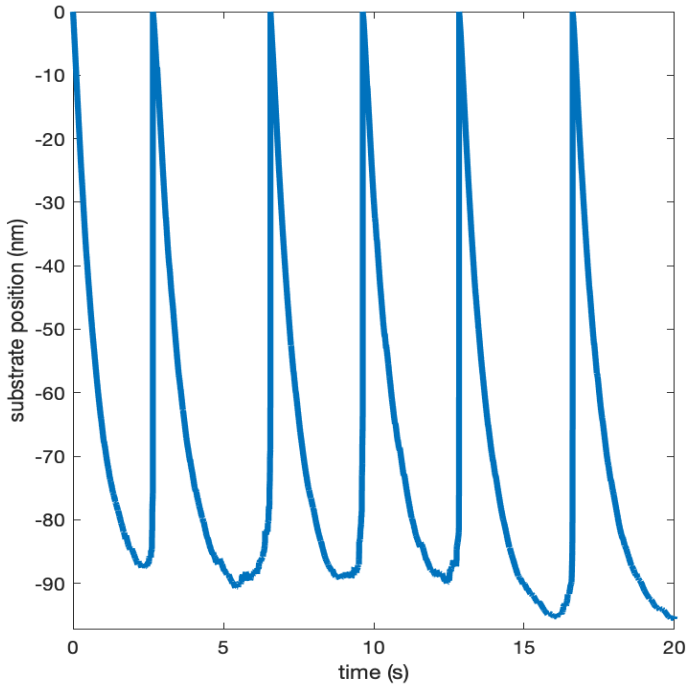


Figure 3.3: Model-predicted substrate position as a function of time highlights load and-fail on compliant substrates

Substrate compliance slows the rate at which tension builds along individually engaged clutches, on soft substrate. This elongates the interaction time of F-actin/clutch. In early time of the cycle, most clutches remain engaged to the F-actin bundle. As tension slowly develops within the substrate so during this time there is little relative motion between the F-actin bundle and the substrate. Myosin motors work near their unloaded sliding velocity, leading to high rates of F-actin retrograde flow and a slight retraction of the leading edge because of this lack of resistance. Clutches largely remain occupied due to sharing of the mechanical load among engaged neighbors, as the substrate strains and greater tension is built. This provides considerable resistance to the motor force, substantially slowing retrograde flow see (**Fig. 3.1**). Afterwards, the load becomes so great that the stochastic loss of one clutch leads to a decent (**Fig. 3.2**), in which the unsupportable load shifts gradually to remaining bonds, further destabilizing the F-actin/clutch interaction. This swiftly leads to an immediate coupling failure where all clutches rapidly disengage, therefore unloading the substrate and causing it to slide back to its initial rest position see (**Fig. 3.3**).

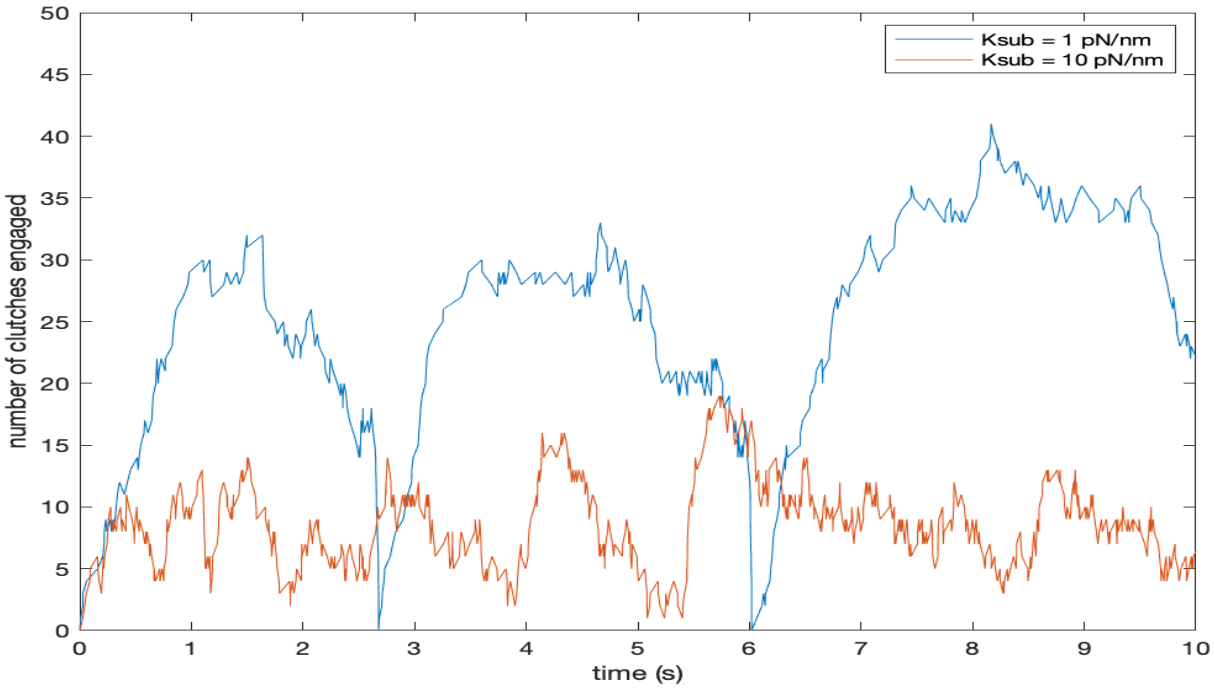


Figure 3.4: Effect on attached clutches by changing substrate stiffness

3.2 Effect on Attached Clutches by Changing Substrate Stiffness:

Here I see that on decreasing the substrate stiffness there is an increase in the number of clutches engaged see (**Fig. 3.4**). It takes more time for the force to be built on the clutch bound, therefore decreasing in the rate at which the clutch bond breaks when the substrate is soft. This is the reason why we see an increase in the number of clutches engaged on substrates which have less stiffness.

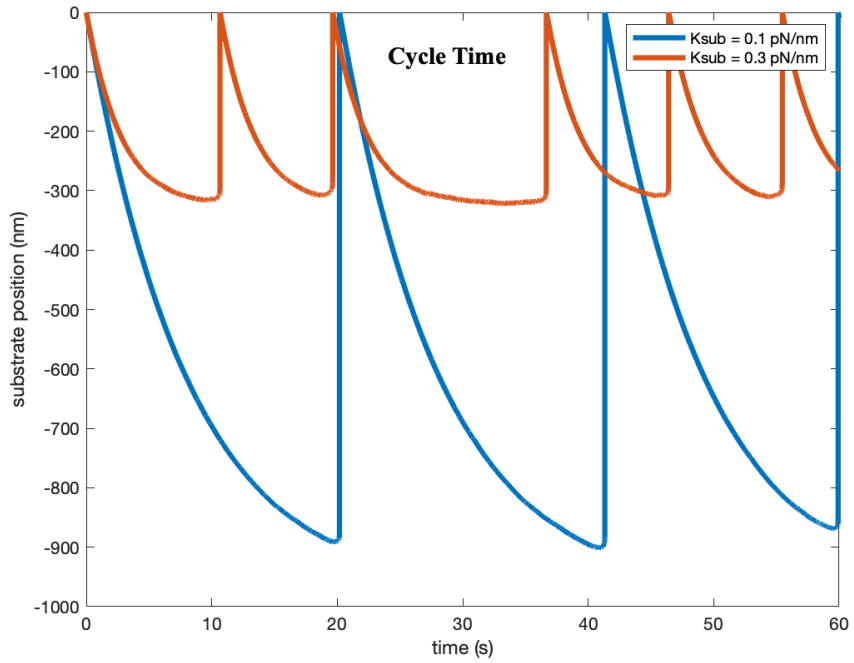


Figure 3.5: Effect on cycle time by changing the substrate stiffness

3.3 Effect on cycle time by changing the substrate stiffness

The load and fail cycle time near optimum stiffness increases with the decrease in substrate stiffness see (**Figure 3.5**). On soft substrates it takes longer for the clutches to reach the load required for the collective failure of the clutches.

CHAPTER 4

Optimality

4.1 Changing Individual Parameter

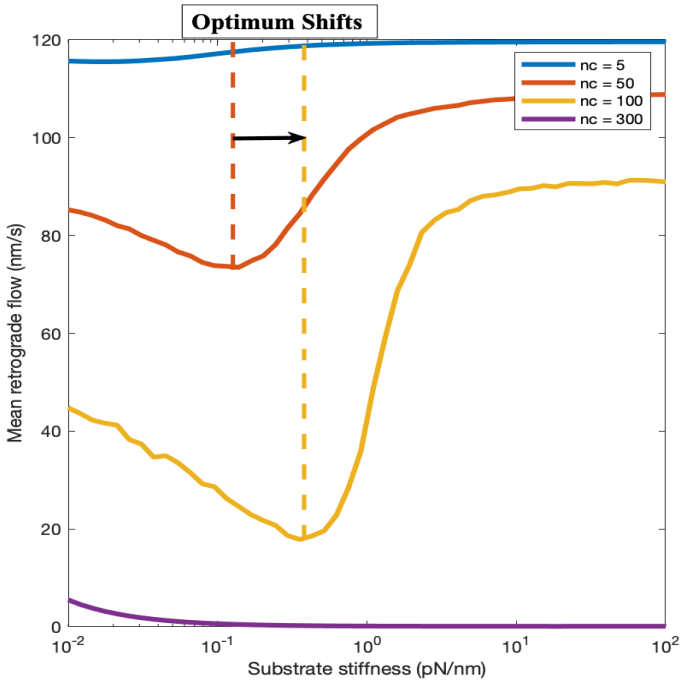


Figure 4.1: Effect of retrograde flow on changing the clutches

We see (**Figure 4.1**) that the minimum F-actin retrograde shifts towards the higher substrate stiffness. Similarly, the maximum traction force see (**Figure 4.2**) shifts to high substrate stiffness by increasing the number of clutches (n_c). The optimal stiffness is defined as the stiffness at which the F-actin retrograde flow is minimum and the stiffness at which the traction force is maximum.

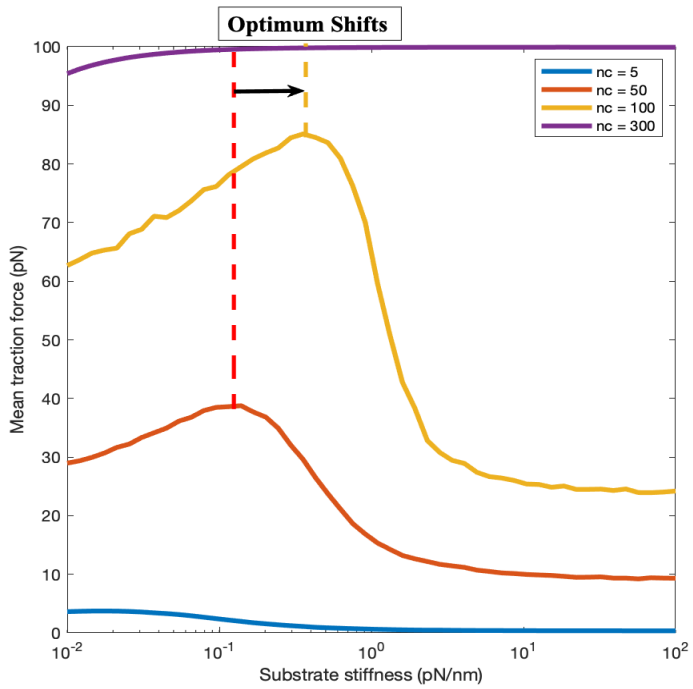


Figure 4.2: Effect of traction force on changing the clutches

As it is seen from the (**Figs. 4.1,4.2**), on increasing the number of clutches the system becomes insensitive to the stiffness of the environment. This is because there are enough clutches resisting the force of the motors so that the clutches never collapse. Therefore, at this limit the traction force is constant and the retrograde velocity remains nearly equal to zero for all stiffness. Conversely, if the number of clutches is very low then the system is free flowing. So here the stall force provided by the motors is too large such that the clutches cannot resist it and the traction force is also minimum and therefore the F-actin flow is nearly equal to the unloaded velocity for all stiffness value. Between these two extremities there is one configuration of number of clutches when the motors and clutches are approximately balanced so that the substrate cyclically loads and fails when the substrate stiffness is near the optimum.

There is one parameter which does not shift the stiffness optimum that is the K_c (clutch stiffness). In general by increasing the clutch parameters $(K_{on}, F_b, n_c, K_c, K^*_{off})$ has a tendency to increase the optimum stiffness whereas increasing the values of motor parameters (F_m, n_c, v^*_m) decreases the optimum stiffness. One exception is the clutch off

rate K^*_{off} but this can be understood as increasing the off-rate weakens the clutches, whereas increasing the other parameters strengthens the clutches. Similarly, increasing the motor parameters increases the strength of the motors.

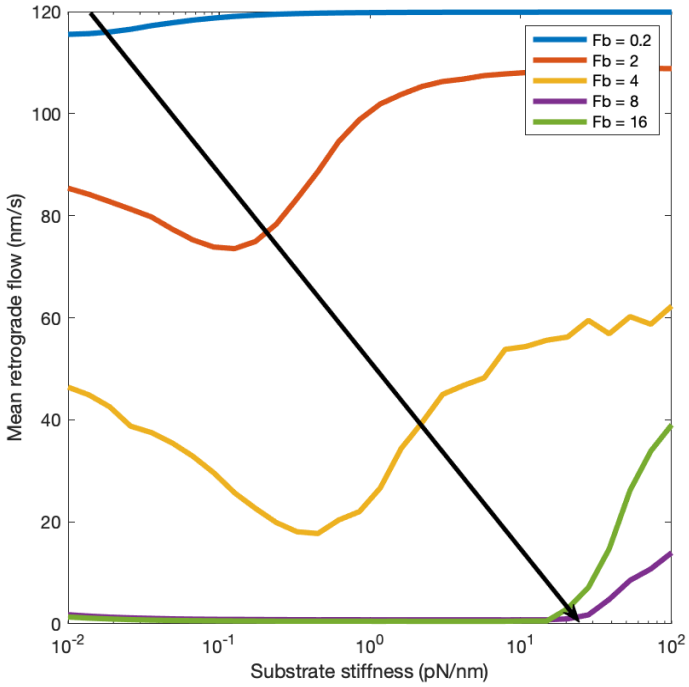


Figure 4.3: Shift in optimality by changing F_b

4.2 Analysis of each Clutch Parameter

4.2.1 Clutch bond rupture force (F_b):

As it is seen from the expression of K_{off} it is clear that on increasing the F_b the off-rate for the clutches will decrease which means that it is causing an increase in the average number of clutches bound. As the number of clutches increases, this increases the ensemble clutch stiffness and this results in the increase in the optimum stiffness (see **Fig. 4.3**). If I increase the F_b too much then it causes the system to be stalled because the clutches bound to the F-actin is so high that it becomes too difficult for the motors to break all the clutch bonds. A decrease in the F_b causes it to decrease the ensemble clutch stiffness and this results in the shifting of optimum to a lower substrate region.

This occurs because a decrease in the number of clutches bond is very less to resist the F-actin retrograde flow which results in a free-flowing system as the motor can break the bonds very quickly.

4.2.2 Clutch on-rate (k_{on}):

When we increase k_{on} this results in the increase in the average number of clutches bound on any stiffness. This causes the ensemble clutch stiffness to increase, therefore increase in the optimum stiffness is observed. If we increase the k_{on} to a very large value this leads to a stalled system as the force provided by the motors are not enough to break the bond of the clutches. A decrease in k_{on} results in the decrease in the ensemble clutch stiffness, therefore decreasing the optimum stiffness. If we decrease the on-rate too much then the system results in free flowing as motors can quickly break the bonds as the number of clutches are too less to resists this force (see **Fig. 4.4**).

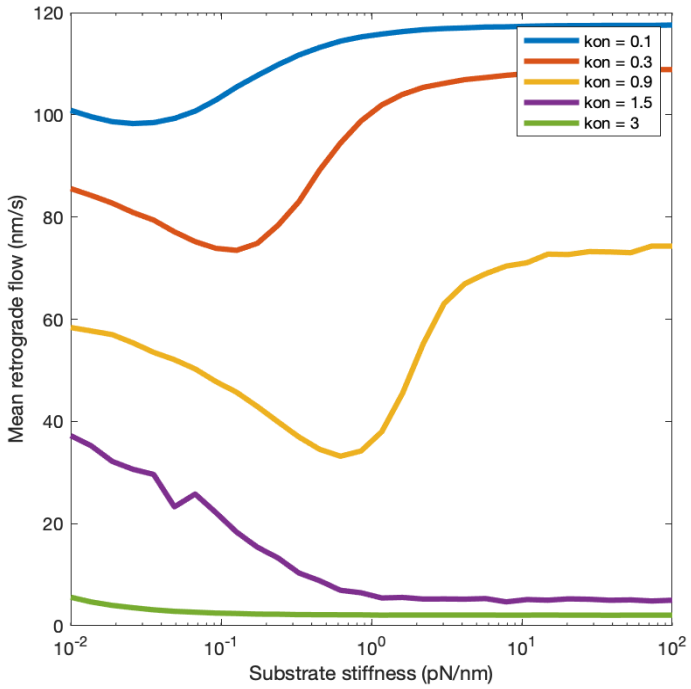


Figure 4.4: Shift in optimality by changing k_{on}

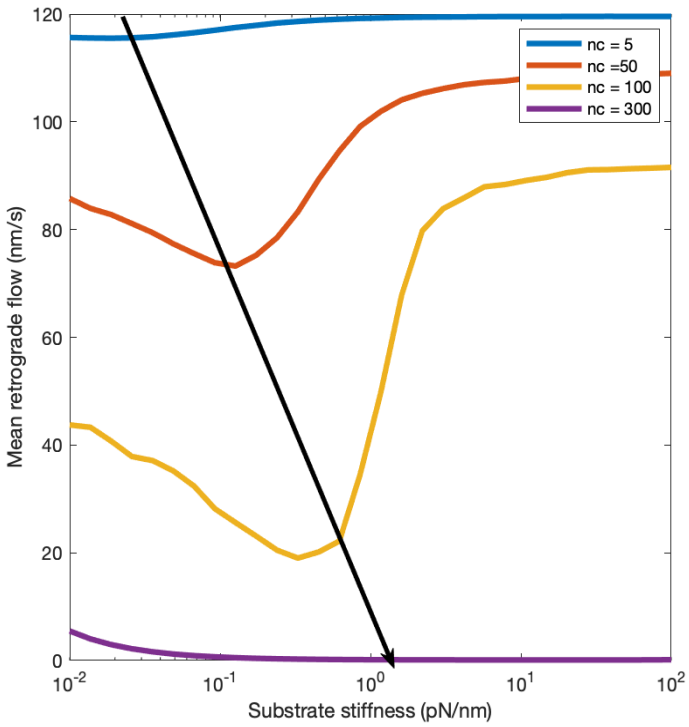


Figure 4.5: Shift in optimality by changing n_c

4.2.3 Number of Clutches (n_c):

An increase in the number of clutches increases the average number of clutches bound. This causes an increase in the ensemble clutch stiffness and it results in the shit of optimum to a higher value. Now if we increase the clutches too much then this will cause the system to be stalled as the motor will not have enough force to break the bonds between the clutch and the F-actin. While decreasing the number of clutches will decrease the ensemble clutch stiffness which results in the decrease in optimum to lower stiffness. As we decrease the number of clutches too much then this results in a free flowing system as we see that the motor can easily break the bond between the clutches and F-actin(see **Fig. 4.5**).

4.2.4 Clutch unloaded off-rate (k^*_{off}):

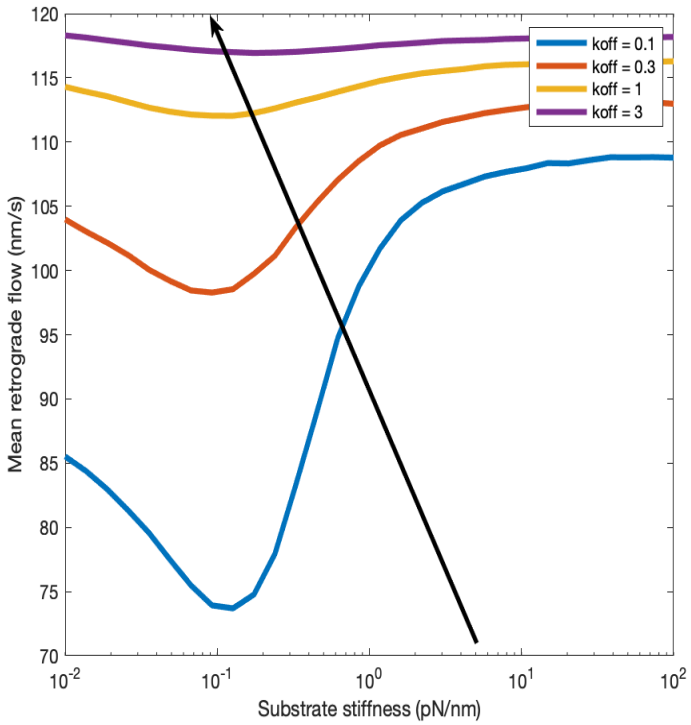


Figure 4.6: Shift in optimality by changing k_{off}

If we increase the unload off-rate k^*_{off} this will decrease the number of clutches bound on any stiffness, due to which we see that the optimum shifts to a lower substrate stiffness. If we increase the off-rate to a very large value then the clutches attached will be very less in numbers and the force provided by the motors can easily break the clutches and therefore the system will be free flowing. If we decrease the off-rate this will increase the average number of clutches bound on any stiffness, due to which we see that the optimum shifts to a higher substrate stiffness. If we decrease the off-rate to a very low value then the clutches will not break their bound easily as the number of clutches attached is large so the force provided by the motors will be not enough to break the clutches therefore the system will stalled (see **Fig. 4.6**).

4.3 Analysis of each Motor Parameter

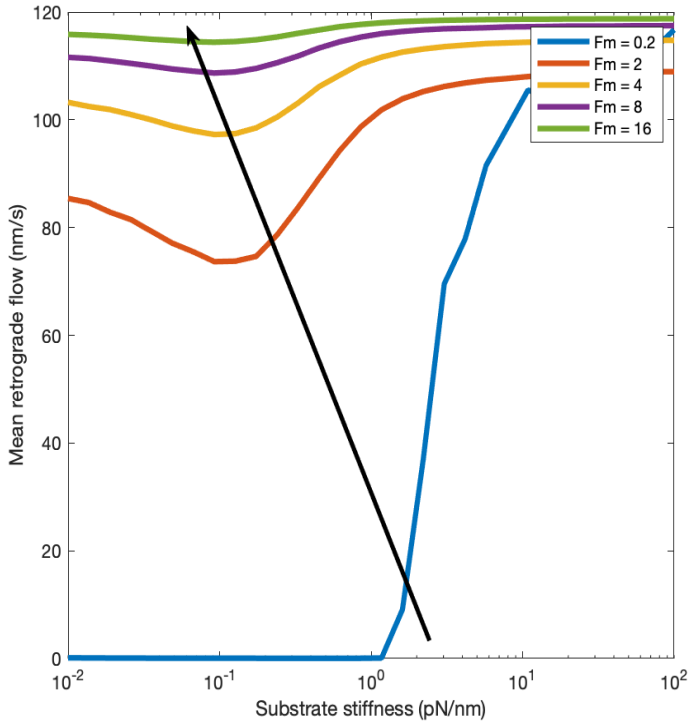


Figure 4.7: Shift in optimality by changing F_m

4.3.1 Motor Stall Force (F_m):

An increase in F_m strengthens the motor and decreases the load and fail cycle time. This results in changing the optimum to a lower substrate stiffness. If we increase the motor stall force too much then the system results in free flowing as clutches can be broken easily. Whereas, decrease in F_m weakens the motor and increases the load and fail cycle time. This results in changing the optimum to a higher substrate stiffness. If we decrease the motor stall force too much then the system will be stalled as the force provided by the motors will not be enough to break the clutches easily (see **Fig. 4.7**).

4.3.2 Number of motors (n_m):

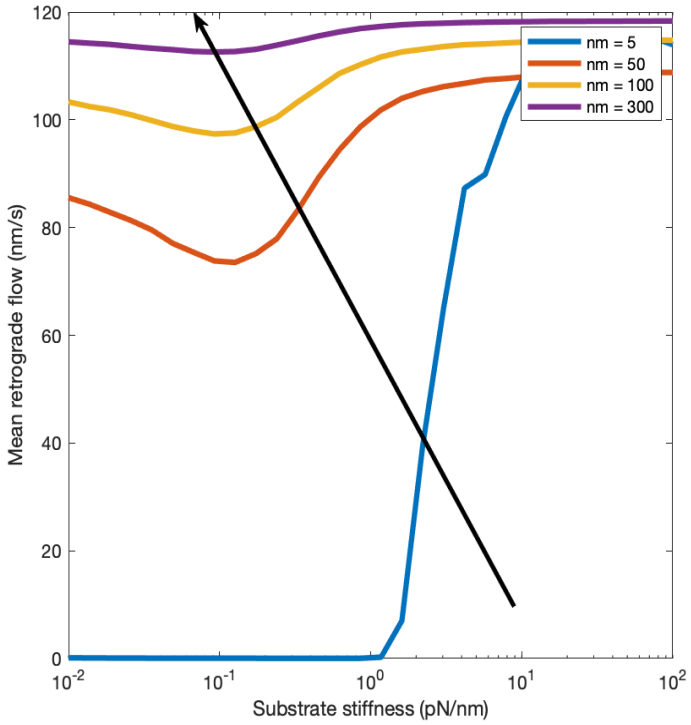


Figure 4.8: Shift in optimality by changing n_m

An increase in the number of motors will strengthen the motors and decrease load and fail cycle time. This decreases the optimum stiffness to lower substrate stiffness. If we increase the number of motors to a very large number then the system will result in free flowing as the force provided by the motors will be large enough to break the clutches. Whereas a decrease in the number of motors will increase the load and fail cycle which increases the optimum to a higher substrate stiffness. If we decrease the number of motors to a very large value then this results in the stalled system as force provided by the motors will not be large enough to break the bounds of the clutches (see **Fig. 4.8**).

4.3.3 Motor-unload velocity (v_m^*):

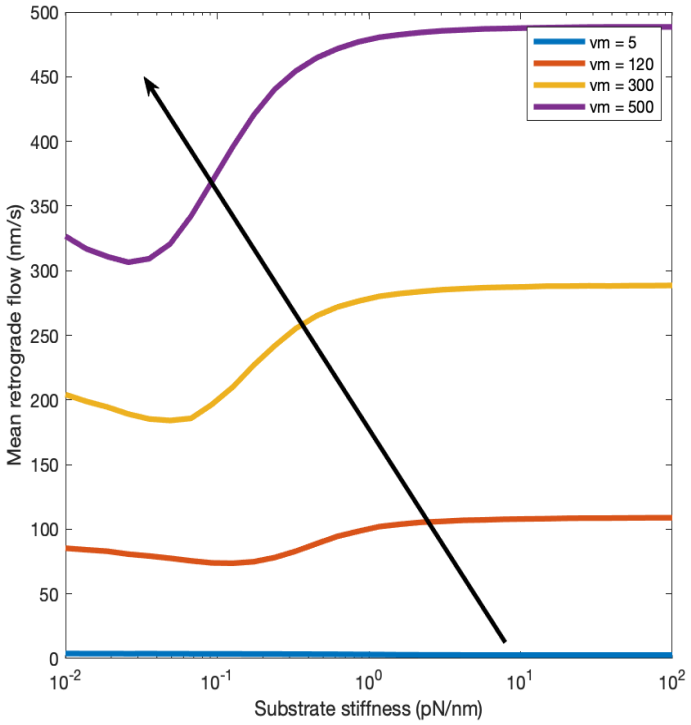


Figure 4.9: Shift in optimality by changing v_m^*

As we increase the motor-unload velocity this strengthens the motors which decreases the load and fail cycle. Therefore we see that the optimum shifts towards the lower stiffness. If we increase the unload velocity then the system will not be freely flowing but such things will not be possible. Whereas, if we decrease the unloading velocity this will weaken the motors and will increase the load and fail cycle time and the optimum will shift towards the higher substrate stiffness. If we decrease the unloading speed too much then this will result in a stalled system because this retrograde velocity will be zero (see **Fig. 4.9**).

4.4 Clutch Stiffness (K_c):

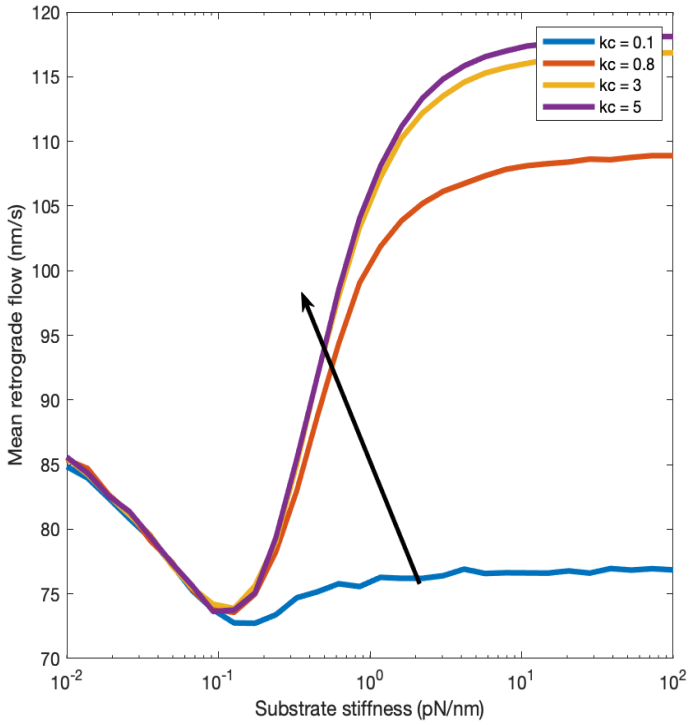


Figure 4.10: Shift in optimality by changing K_c

The increase in the clutch stiffness means that the clutch bonds are getting stiffer and hence this tends to increase the ensemble clutch stiffness and shifts the optimum to higher substrate stiffness. On the other hand, stiffer clutch bounds also tend to load and fail quicker which means that this decreases the average number of clutches attached and results in decreasing the ensemble clutch stiffness. As it is observed in my simulation that the optimum does not change therefore, we may say that these two effects are cancelling out each other. If we increase the clutch stiffness it is seen that it increases the retrograde F-actin velocity on high substrate stiffness. The system is tending towards free flowing because the high stiffness frictional slippage regime means decreasing the number of engaged clutches. In the low stiffness frictional slippage regime in this regime the clutches tend to fail spontaneously prior to reaching appreciable loads because the substrate stiffness is softer than the ensemble clutch stiffness. This is why the model

behaviour in the low substrate stiffness regime is insensitive to the mechanical property of the clutch itself. This is the most surprising result to single parameter changes: the optimum stiffness is insensitive to clutch stiffness (see **Fig. 4.10**)

CHAPTER 5

Incorporating motor attachment/detachment

In this chapter, I would like to incorporate attachment-detachment dynamics to myosin motors with the actin filaments. How does the variation of these motors affect the stability of the Motor-Clutch system? Until now we saw that the response to cell traction force with substrate stiffness shows a biphasic relationship i.e. force first increases and then it decreases as we increase the substrate stiffness. Now I would like to observe the difference in results by modifying my earlier system by attachment/detachment of myosin motors.

5.1 Modified Model Description

The myosin motors are rigidly fixed at one end whereas the other end gets attached to the F-actin bundle and causes a retrograde flow by applying a force on the bundle. The molecular clutches get attached to this f-actin bundle to resist the retrograde flow. The force which is built up to the attached molecular clutches leads to traction force which is balance by the tension and deformation in the substrate (**Fig. 1.2**).

Now the myosin motors are modeled as stretchable springs, which due to energy consumption via hydrolysis of ATP, undergo attachment-detachment dynamics from the F-actin bundle. Here, one end of the spring is fixed whereas the other end attaches or deattaches from the F-actin with rates $k_{on,m}$ and $k_{off,m}$ respectively. The detachment rate can be considered to increase exponentially with the load force of the motor F_m as given in the

equation (**5.1**).

$$k_{off,m} = k^*_{off,m} \exp\left(\frac{F_m}{F_{b,m}}\right) \tag{5.1}$$

Where $F_{b,m}$ is the bond rupture force for the motors and $K^*_{off,m}$ is the loaded off-rate for motors, i.e. the off-rate at initial configuration. And F_m is calculated by the equation (**5.2**)

$$F_m = K_m(x_m) \tag{5.2}$$

K_m is the motor spring constant. x_m is the length of extension of the motors. The motor velocity, v_m remains the same as it was earlier, but now instead of a fixed number of motors the n_c is now changing and it is given by the equation (**5.3**)

$$v_m = v^*_m \left(1 - \frac{K_{sub} x_{sub}}{n_m F_m}\right) \tag{5.3}$$

V^*_u is the velocity when the clutches were unloaded, i.e. the initial velocity given to the f-actin filaments when the system starts at $t = 0$. Here K_sub is the substrate stiffness x_sub is the length of the substrate. The clutches and motors will bind with the F-actin with a constant rate $k_{on,c}$ and $k_{on,m}$ respectively. The rate of detachment of clutches is given by $k_{off,c}$ the equation (**5.4**)

$$k_{off,c} = k^*_{off,c} \exp\left(\frac{F_c}{F_{b,c}}\right) \tag{5.4}$$

Here F_c is the force on the clutches given by the equation (**5.5**)

$$F_c = K_c \left(x_c - x_{sub}\right) \tag{5.5}$$

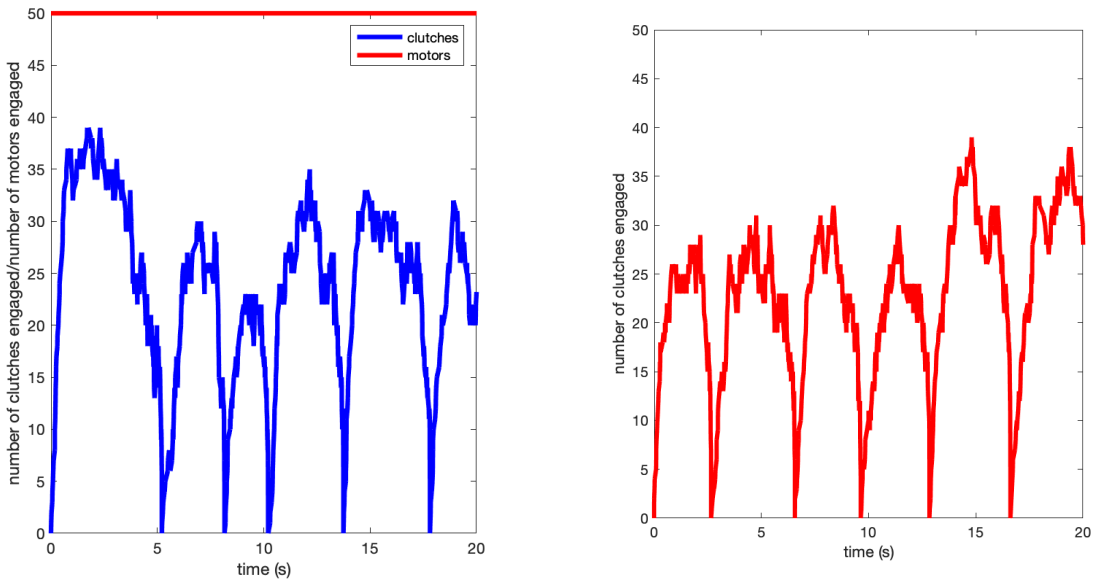
Here, K_c is the spring constant for the clutches and x_c is the position of clutch attached to the F-actin filaments. The model was implemented through a fixed time step Monte Carlo simulation.

5.2 Results and Discussion

I will first check by setting my off-rate for motors to zero and setting my on-rate to be one i.e. $k^*_{off,m} = 0$ and $K_{on,m} = 1$ respectively. Now my system should behave the way it was behaving earlier in the case of motor-clutch model as by setting up these parameter values I am ensuring that my motors are always attached and never get detached from the F-actin.

Comparison with the Previous Results

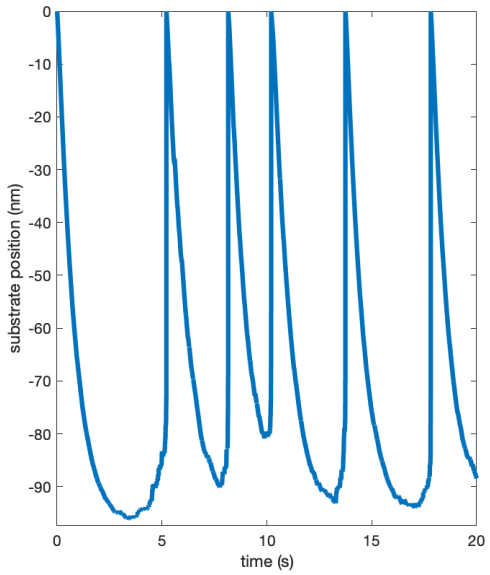
In the **Figs. 5.1, 5.2, 5.3**), on the left hand side we have the results obtained after modification and on the right hand side I have the result of the previous motor-clutch model



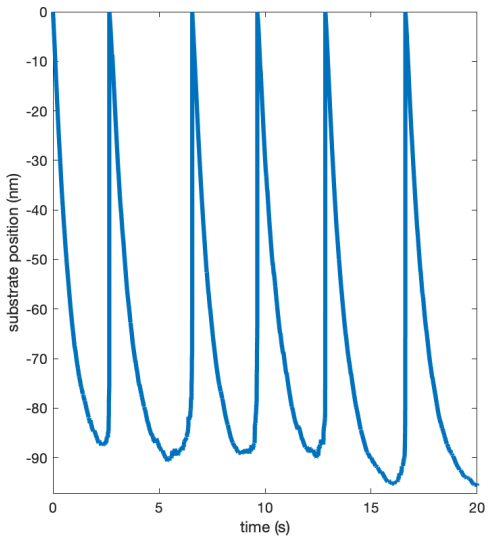
(a) n_c, n_m attched

(b) n_c attached

Figure 5.1: Configuration of the Attached Motors and Clutches

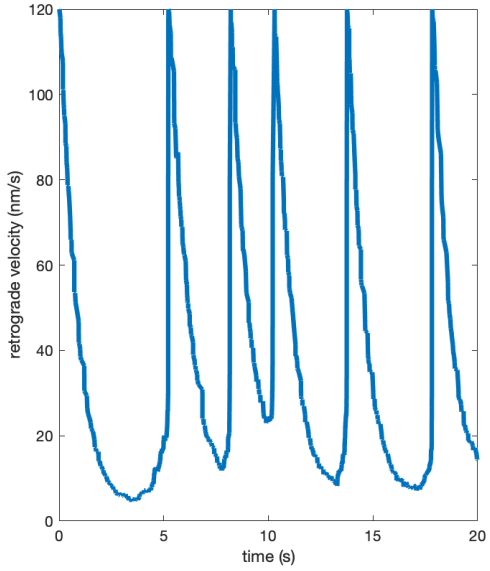


(a) x_{sub} in new model

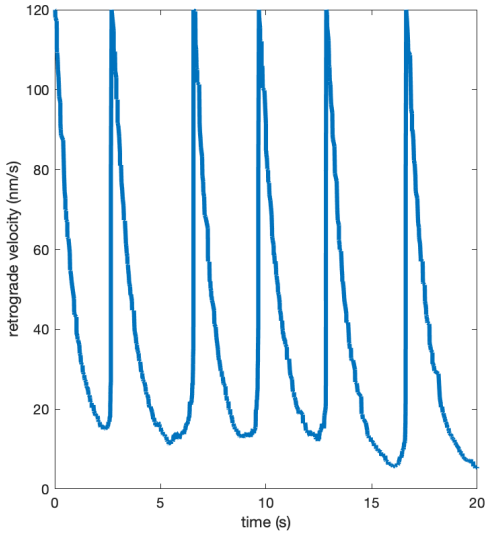


(b) x_{sub} in old model

Figure 5.2: Substrate Position as a Function of Time



(a) v_m in new model



(b) v_m in old model

Figure 5.3: Retrograde Velocity as a Function of Time

I observe that my behaviour of new model is same as the old model in the specified condition. Therefore, now I will look for the biphasic relationship of force and velocity (see **Fig. 5.4, 5.5**).

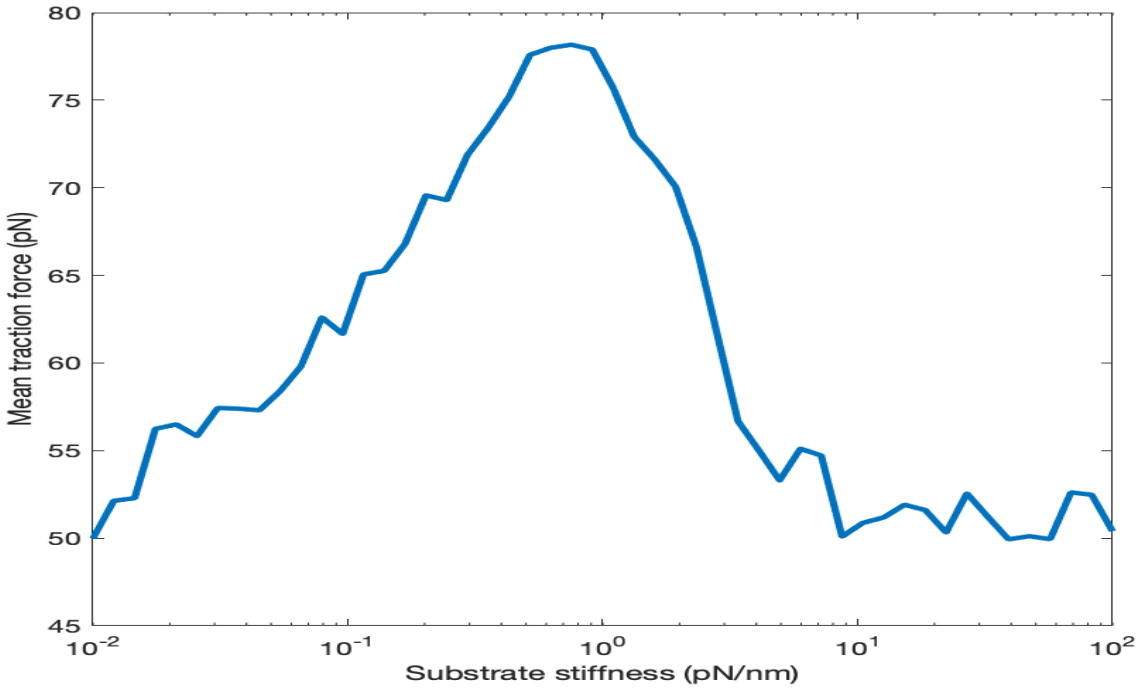


Figure 5.4: Biphasic Relation for Traction force

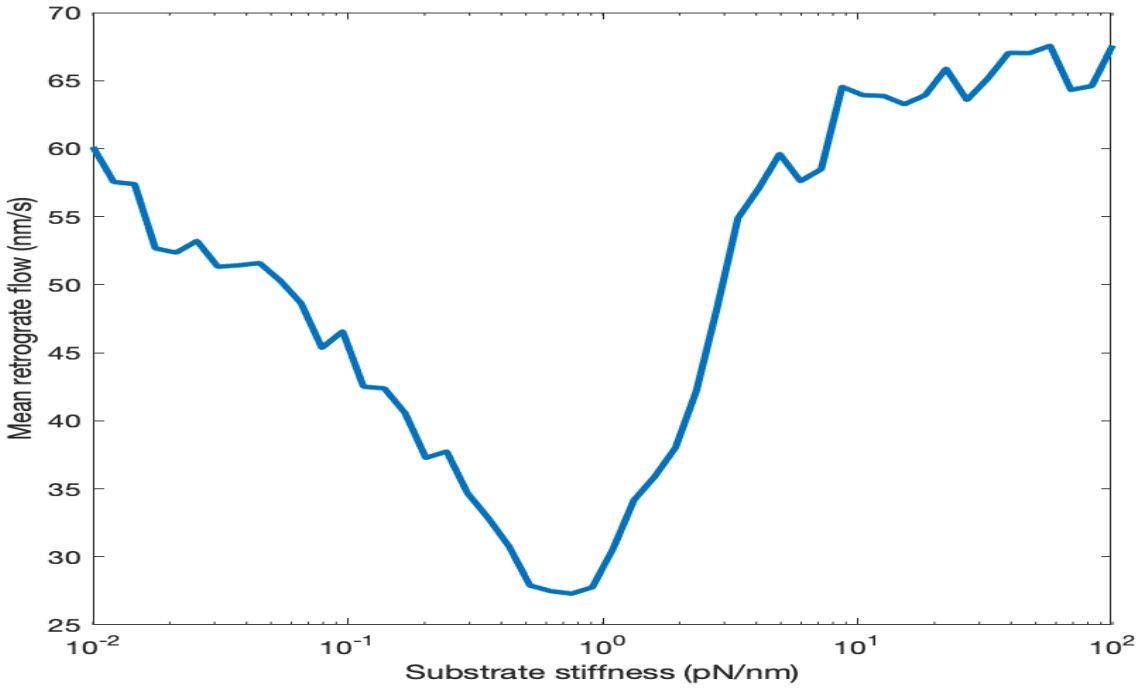


Figure 5.5: Biphasic Relation for Cell Migration

The result obtained over here is shows a biphasic relationship but with a lot of fluctu-

ation, we observe a global maxima and a global minima for the case of traction force and cell migration respectively. Now I will give a positive off-rate to my molecular motors to check my results.

5.3 Behaviour of the System with a positive $K_{off,m}$

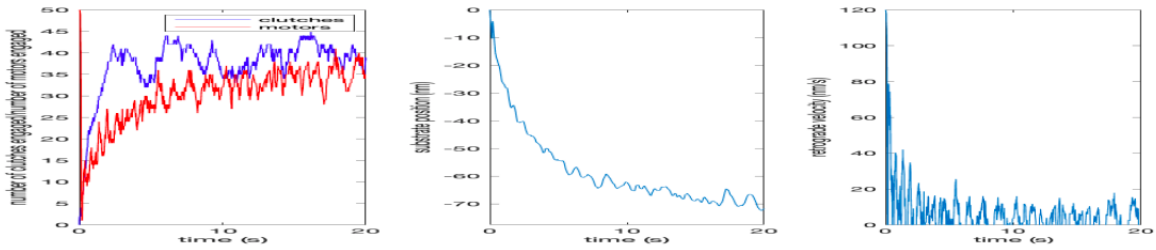


Figure 5.6: $k_{off,m} = 0.1s^{-1}$

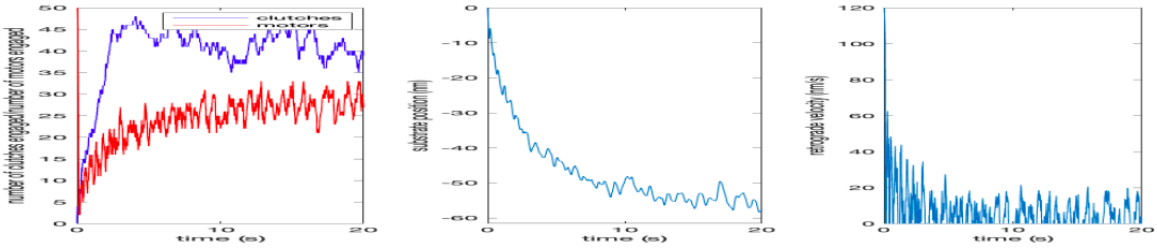


Figure 5.7: $k_{off,m} = 0.5s^{-1}$

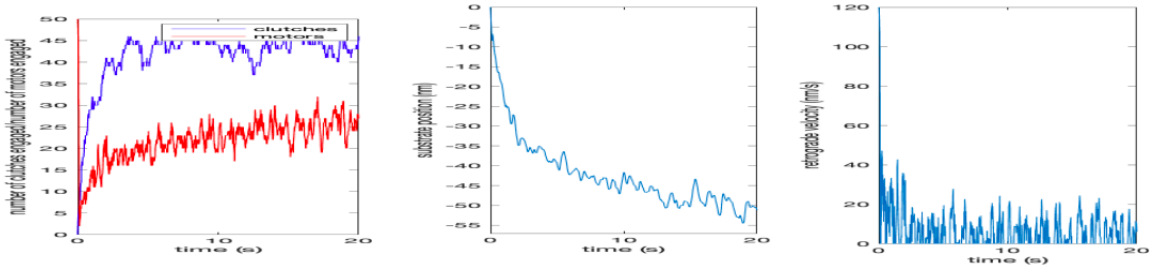


Figure 5.8: $k_{off,m} = 0.7s^{-1}$

As I change the off-rate of the molecular motors, the load-fail cycle is lost. We are trying to figure out the reasons for such a result. We expect that at very low detachment rates, the oscillations should be seen again.

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