ChemE 7770: Advanced Principles of Biomolecular Engineering
Kinetic Monte Carlo simulations of traction force dynamics
Project Deliverable IV

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Abstract

The fate of the cells such as shape and migration patterns are determined by properties of the environment such as stiffness, fluidity. Hence, analysing the traction dynamics between the cell and the extracellular matrix (ECM) is crucial. In this project we focused on the model built by Chan and Odde in their seminal paper published in 2008. Furthermore, we explored extensions to the model by adding components that would simulate in- vivo behaviour. The objective of the project was three fold. (1) Recreate the model built by Chan and Odde. (2) Understand the dynamics in the presence of a viscoelastic substrate. (3) Study the role of Vincullin in clutch stability.

Introduction

Studying the interaction between cells and its environment is crucial since ECM plays a major role in determining the cell fate. Cells use a system of adaptor, trans-membrane cytoskeletal proteins along with myosin to form a motor-clutch system which is used to sense and respond to the environment. The ECM contains different types of polymerized fibers, the most abundant being collagen. The differences in extracellular composition is tied to different cell behaviours[1][2]. The next major component of the system, transmembrane proteins, has a major role in signal transduction from ECM into the cell. Binding of collagen to a transmembrane protein called integrin via fibronectin commences a signal transduction activity in the cytoplasm (Fig 1). This signal results in attachment of cytoplasmic component of integrin to cytoskeleton filaments such as F- actin by recruiting adaptor proteins such as vinculin and talin. The attachment of integrin to F-actin via 'molecular clutches' slows down the retrograde motion of F-actin by 'myosin motors'. This slowed down retrograde motion coupled with the polymerization of G-actin to F- Actin in the plus end of the F-actin creates an intracellular force which aids in the development of protrusions out of the plasma membrane. Chan and Odde developed a model to describe the behaviour of this system by visualizing the intracellular and extracellular molecular linkages to be Hookean springs[3]. Chan and Odde hypothesized that the substrate stiffness in the ECM plays a role in the tension developed in the spring that will affect the motor-clutch system. The various forces that act in the motor clutch system are [a] Characteristic bond rupture force, F_b [b] Single myosin motor stall force, F_m(Appendix I: Fig 6). The molecular clutches can either engage to F-actin at a rate constant k_{on} or disengage with a rate constant k_{off}. Once the clutches are engaged, the retrograde motion of F-actin causes a build up of tension within the spring with a spring constant κ_c. The tensions

that are built up in the intracellular environment have to be matched by the tensions that are built in the extracellular environment and this depends on the compliance of the substrate which has a spring constant of κ_{sub}.

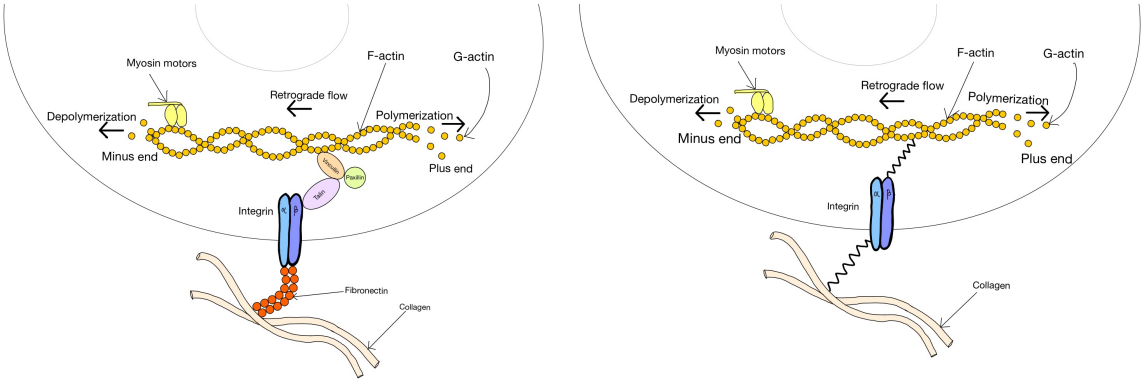

Figure 1: Signal Transduction from ECM to cytoskeleton via intracellular linkage proteins such as talin, vinculin, paxillin (left) Signal Transduction from ECM to cytoskeleton depicted in the form of hookean springs (right)

The key elements in the force transduction system would be the molecular clutches and substrate. Based on the spring constant of each, the force transmission can be regulated. Several other variables are also present in signal transmission: (a) Different modes of attachment of F-actin to integrin[4] (by recruiting different sets of proteins instead of talin and vinculin) thereby affecting the the spring constant and consecutively, the point of breakage[5]; (b) integrin cluster formation[6]. (c) Type of transmembrane protein, for instance LAT transmembrane protein[7] or N-Cadherin[8] (d) substrate viscoelasticity[9].

Methodology

We have built our model using the data we obtained from the published works of Chan and Odde[3] and Bangasser[10]. The motors and the substrate are relatively stiff when compared to the molecular clutches. The molecular clutches are modeled as simple Hookean springs with spring constant κ_c.The tension in the string is given by

$$F_{c(i)} = \kappa_c * (x_i - x_{sub}) \tag{1}$$

where,
x_{sub}: position of the substrate
x_i: position of the molecular clutch.
A Monte Carlo simulation is performed on the modeled system with a time step of 5 ms. For every step of the MC simulation, the molecular clutches are allowed to associate with the F-actin bundle following which a check for any dissociation is done.
The effective dissociation rate of the clutches increases exponentially as tension develops in the

engaged clutches as

$$k^*_{off} = k_{off}.e^{F_c/F_b} \tag{2}$$

where,
F_b: characteristic bond rupture force
k_{off}: unloaded dissociation rate of the molecular clutch from the F-actin bundle.
The compliant substrate is also treated as a simple Hookean spring with stiffness κ_{sub}.
The myosin motors are assumed to move when acting against the elastically loaded substrate according to the formula

$$v_f = v_u \left(1 - \frac{F_{trac}}{F_{stall}}\right) \tag{3}$$

where
v_f: velocity of myosin
v_u: unloaded sliding velocity of myosin
F_{trac}: Traction force of the system which is defined as

$$F_{trac} = \kappa_{sub}.x_{sub} \tag{4}$$

F_{stall}: total myosin stall force of the system, which is defined as

$$F_{stall} = n_m.\hat{F}_m \tag{5}$$

where,
n_m: total number of myosin motors
$\hat{F}_m$: the stall force (force required to stall the activity) of a single myosin motor.
Once the spring network is formed, a force balance analysis gives the position of the substrate at that time step.

$$x_{sub} = \frac{\kappa_c \sum_{i=1}^{n_{eng}} x_i}{\kappa_{sub} + n_{eng}.\kappa_c} \tag{6}$$

where,
n_{eng}: Number of engaged clutches
x_i: Position of molecular clutch
The values of the force acting on the myosin motors and the retrograde flow velocity of the F-actin bundle are calculated for this particular substrate position.
The clutches are then displaced by a distance Δ x, and the simulation proceeds to the next time step. Finally, to get stochasticity, the clutches are displaced by a distance

$$\Delta x = v_f.t$$

where t is the time step. The simulation is run for over 100000 time steps (500 ms) and the plots of average traction force, mean retrograde velocity of the F-actin bundle and the position of the substrates against the clutch stiffness are drawn.

Model Extension to incorporate substrate viscoelasticity

We modified the system to incorporate the effect of myosin motors moving against a visco-elastic loaded substrate. Traction force (F_{trac}) of molecular clutch system in the presence of viscoelastic substrate is given by

$$F_{trac} = \kappa_{sub}.x_{sub} + n_{eng}.\upsilon_f.\mu \tag{7}$$

where,
κ_{sub}: Spring constant of substrate
x_{sub}: Position of substrate
n_{eng}: Number of engaged clutches
υ_f: Retrograde velocity of F-actin
μ: Viscosity of substrate
Total myosin stall force (F_{stall}) of the system is defined as

$$F_{stall} = n_m.\hat{F}_m \tag{8}$$

where,
n_m: total number of myosin motors
$\hat{F}_m$: the stall force (force required to stall the activity) of a single myosin motor.
The equation for velocity of myosin was calculated by putting Eqn (7) in Eqn (3)

$$\upsilon_f = \upsilon_u \left(\frac{1 - \frac{\kappa_{sub}.x_{sub}}{F_{stall}}}{1 + \frac{n_{eng}.\mu.\upsilon_u}{F_{stall}}} \right) \tag{9}$$

where,
υ_f: velocity of myosin
υ_u: unloaded sliding velocity of myosin
The position of the substrate is given by the equation

$$x_{sub} = \frac{\kappa_c \sum_{i=1}^{n_{eng}} x_i - (n_{eng}.\mu.\upsilon_f)}{\kappa_{sub} + n_{eng}.\kappa_c} \tag{10}$$

where,
n_{eng}: Number of engaged clutches
x_i: Position of molecular clutch

Model Extensions to incorporate vinculin

The model for vinculin was built by simulating vinculin attachment to the clutch once a particular traction force was reached. Based on literature, we came to know that binding of vinculin is increased upon increase in the application of force[11]. In order to show this behaviour, we built a simulation where vinculin was allowed to bind only after a force threshold was reached. Following this, the mean traction force and mean retrograde flow was plotted against substrate elasticity.

Results & Discussion

We compared the results that we obtained to the published results by Chan and Odde to validate our model and to show that replication of the published results is possible (Fig 2). Chan and Odde focused on the variation in two parameters with substrate stiffness, namely the retrograde flow velocity and the force acting on F-actin bundle.

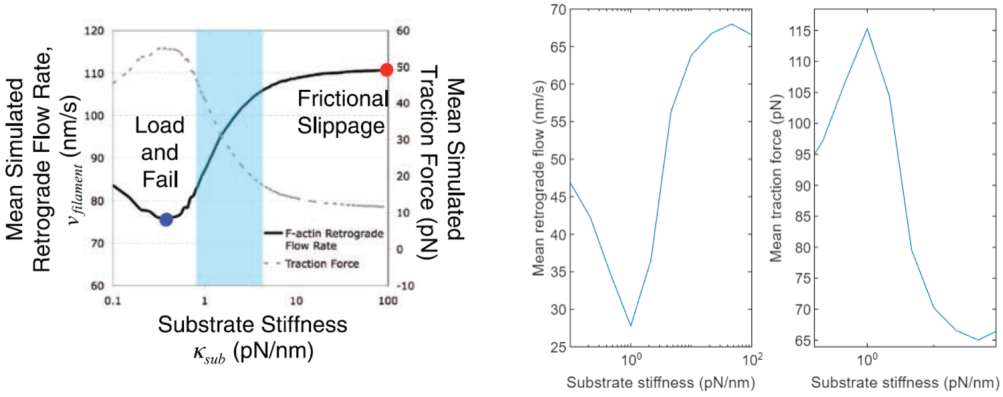

Figure 2: Comparison of the simulation result of Chan Odde [3] (left) with simulation results (right)

As can be seen from the above figure, the trend for the retrograde flow velocity and traction force of our model matches the published results. This validates our model and shows that we have managed to dutifully replicate the concepts put forth by Chan and Odde. Based on the substrate stiffness, two mechanisms of traction force dynamics were noticed: Frictional Slippage in the case of stiff substrate and Load-and-Fail in the case of compliant substrate(Appendix I: Fig 7). The lack of compliance in stiff substrate results in abrupt disengagement of molecular clutches from F-actin (Frictional slippage). However, in the presence of a compliant substrate, the tension slowly builds up in the substrate. In the initial phase, due to the compliance of the substrate, the motors work with unloaded velocity and retrograde motion remains largely unaffected. However, the eventual rise in tension results in the slowing down of the motors and subsequent development of protrusion from the cell membrane. Ultimately, the tension becomes so high that there is simultaneous disengagement of all clutches. Hence, there will be a Load-and-Fail behaviour in the case of compliant substrates.

Viscoelastic Substrates

One interesting extension which we explored is the role of viscosity in these systems. The original idea explored by Chan and Odde assumed a perfectly elastic substrate with no viscosity. Fig 3 shows the stress-strain relationship between a perfectly elastic material and a viscoelastic material.

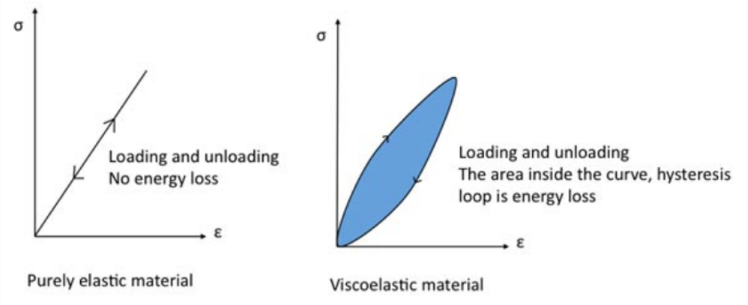

Figure 3: Loading and unloading curves in elastic and viscoelastic substances[12]

Viscous materials, like water, resist shear flow and strain linearly with time when a stress is applied. Elastic materials strain when stretched and immediately return to their original state once the stress is removed. Viscoelastic materials have elements of both these properties and, as such, exhibit time-dependent strain, i.e. show a hysteresis curve. As explained in the model methodology, we developed equations to model viscoelastic behavior of the substrates and the plots for mean retrograde velocity of the substrate and the traction force against the viscosity of the substrate were drawn for a substrate stiffness Ksub=1 pN/nm. These plots are shown in Fig 4.

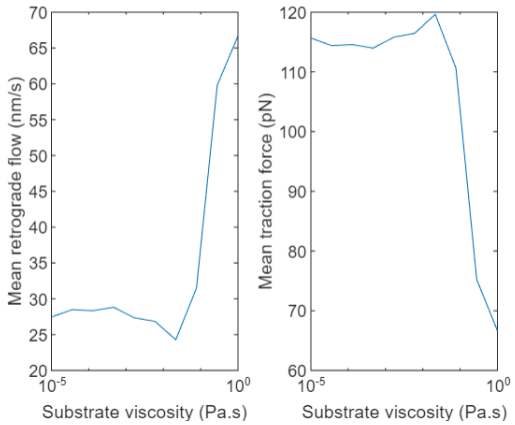

Figure 4: Simulation of molecular clutch behaviour in viscoelastic substrates

The trend followed when substrate viscosity is increased is similar to that observed when substrate stiffness is increased. Initially, we see a slight decrease in mean retrograde flow with increasing substrate viscosity as shown in Fig 4. However, the force on each bond keeps growing up to a point beyond which an increase in viscosity results in frictional slippage behaviour. Due to this, the retrograde flow would increase and traction force would decrease with increasing viscosity of substrate. To understand if our results matched with any of the published literature, we compared our simulation to the data published in the paper by O. Chaudhuri on cell spreading due to substrate relaxation[9], details of which can be found in appendix I.

Effect of Vinculin binding on clutch stability

The Fig 5 shows the plots for mean retrograde flow velocity and the mean traction force as a function of substrate stiffness in the presence of vinculin. We see that while the trend followed is roughly similar in the presence and absence of vinculin, there are small differences where the growth in velocity and the reduction in traction force is smoother and the peaks are at different location. The main issue with the addition of this extension is that not enough is known about the kinetics of vinculin binding. Vinculin binds using a slip clutch method, the figures for which we were not able to find in literature. We thus had to assume a simple model in which the vinculin binding is triggered by the traction force acting on the system in the previous time step. With more experimental and theoretical studies on the kinetics of vinculin binding, we should be able to modify our system easily to more clearly show the role of vinculin in traction force dynamics.

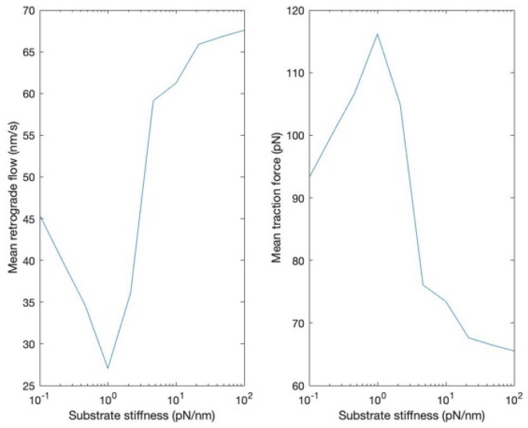

Figure 5: Simulation of molecular clutch behaviour when vinculin in present in the clutch

Conclusions

We have replicated the model proposed by Chan and Odde, and have incorporated some improvements to the model to more closely resemble the biological system, namely the presence of viscoelastic substrates, and increased motor clutch stability in the presence of vinculin. We see that for a given substrate stiffness, an increase in viscosity leads to a decrease in retrograde flow velocity and an increase in traction force. We also see that in the case of vinculin, the motor clutch mechanism is stabilized as the graph becomes slightly smoother and the maximum value of velocity and force is higher.

Acknowledgements

We would like to thank Prof. Matthew Paszek and Prof. Jeffrey Varner for all the support provided throughout the course especially during the trying times of COVID pandemic.

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Appendix I

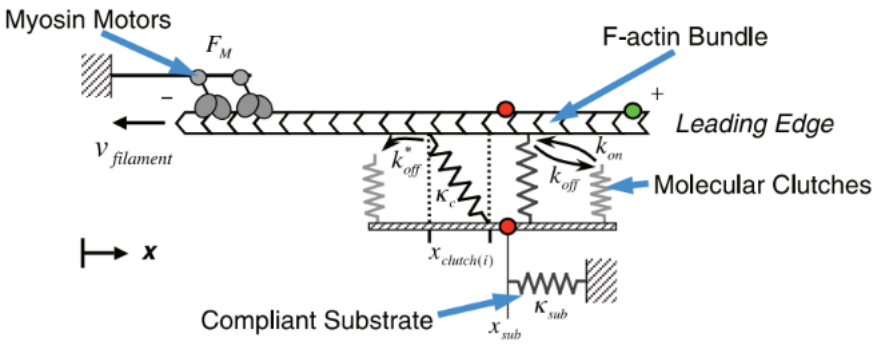


Figure 6: The different forces acting on the motor clutch system: Chan, C. E. Odde, D. J. Traction Dynamics of Filopodia on Compliant Substrates. *Science* **322**, 1687–1691 (2008)

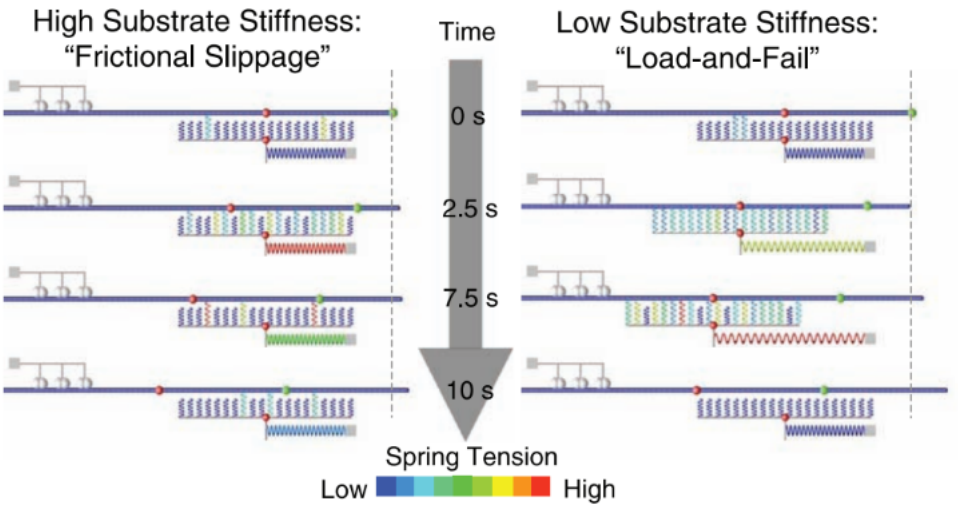


Figure 7: Mechanism of molecular clutch behaviour in different substrates (i) Frictional slippage in stiff substrate (left) vs (ii) Load-and-Fail in complaint substrate (right): Chan, C. E. Odde, D. J. Traction Dynamics of Filopodia on Compliant Substrates. *Science* **322**, 1687–1691 (2008)

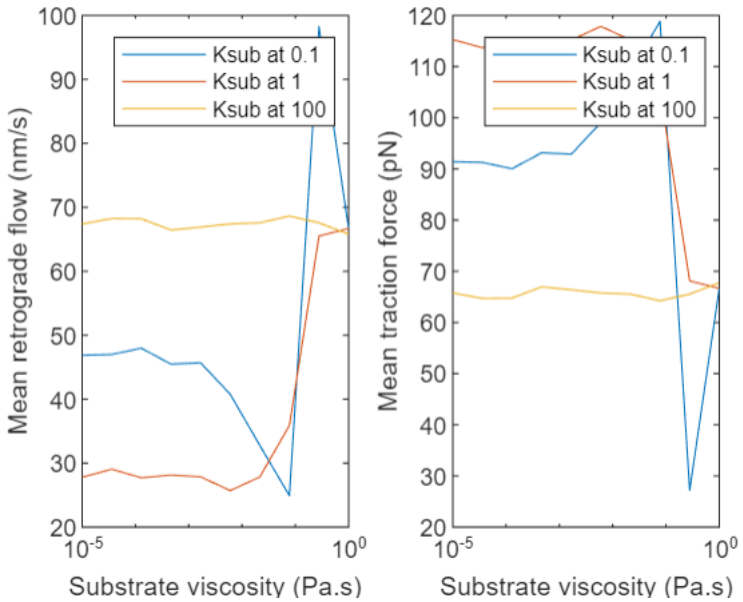


Figure 8: The variation of mean retrograde flow and Force traction w.r.t to substrate viscosity when elasticity component is kept constant

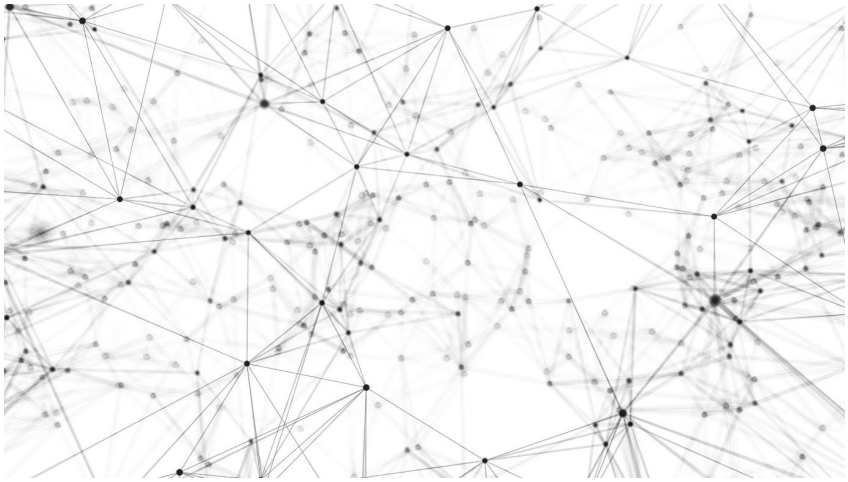
The results in Chaudhuri et al discusses how behaviour of cells vary based on whether they are grown on elastic or viscoelastic substrates. Since the substrate stress relaxation is a function of viscosity, we wanted to explore the behavior of cells when the elastic and viscosity components are varied. The plot above shows the system behaviour by varying viscous component while keeping the elastic component constant. As shown, decreased retrograde flow and increased traction force is observed with increasing viscosity. However, when viscosity is increased beyond a point, the retrograde flow increases. Next, we plotted different trends by modifying the elastic component. It was observed that downward trend in the initial phase is not observed when elastic component is extremely high (stiff substrate). In the case of soft substrates, there seems to be a trend that is followed when viscosity is varied. This may be due to the fact that at low substrate stiffness, the tension on the clutches builds up gradually due to which viscosity affects the system. In the case of stiff substrates where frictional slippage is noticed, tension builds up fast which does not allow for the viscosity to play a role in system behaviour. When the viscosity is high, the cell relaxes slowly and loses it ability to distinguish between substrate elasticity. The behaviour is, hence, the same in soft and stiff substrates.

Appendix II: Presentation Slides

Kinetic MC Simulations of Traction Force Dynamics

CHEME7770: FINAL PROJECT

SIDDHARTH KRISHNAN,
SUTHARA RAMACHANDRAN



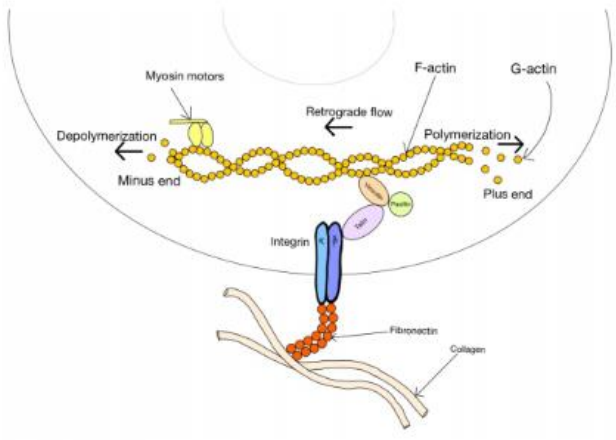
Outline

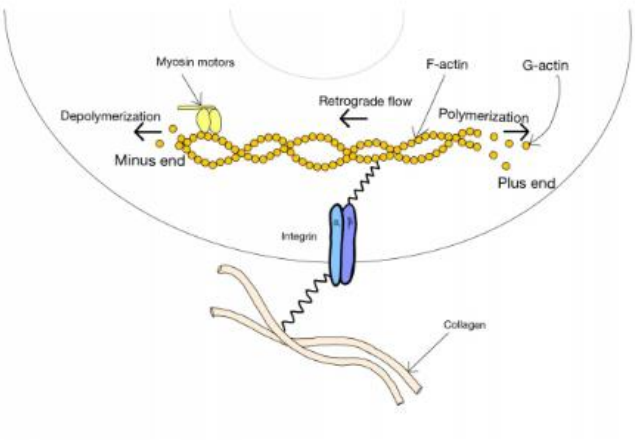
- Introduction
- Goals
- Model Algorithm
- Current model validation and extension
- Results and Inferences

Introduction

- Cells interact with the extracellular environment using several molecular linkages to the extracellular matrix (ECM). This interaction determines many factors such as shape, adhesion, and migration patterns of the cells.
- Motor-clutch transmission system is a model which helps describe the above-mentioned interaction.
- One such model was put forth by Chan and Odde (2008), which uses a kinetic Monte Carlo simulation to model the interaction between the cells and the ECM.

Molecular motor Clutch model



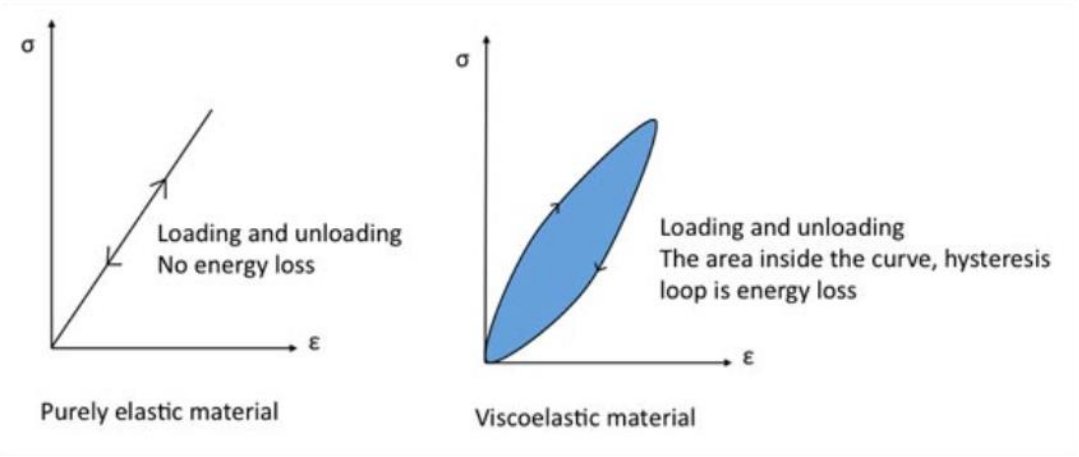


Key Assumptions in Chan & Odde Model

- The clutches and substrate follow Hook's law, that is, they are assumed to be elastic.

- Tension dependent strengthening due to addition of additional adaptor proteins were ignored.

Elastic & Viscoelastic substrates



Source: Xyobalancer.com

Goals

- Understand the mechanism behind molecular clutches.
- Replicate the model put forth by Chan and Odde.
- Model Extension to better understand the role of viscoelasticity in molecular clutch behaviour.
- Model Extension to understand the role of vinculin in clutch stability.

Model Methodology

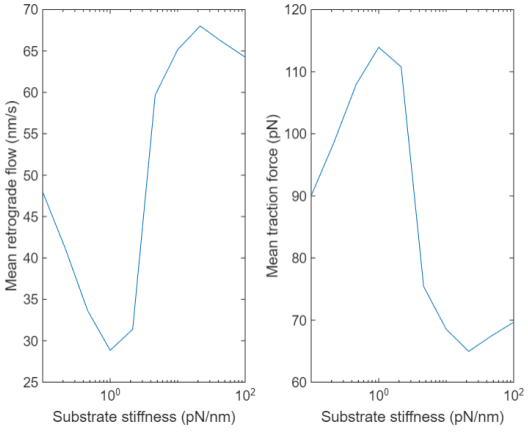
- Chan and Odde hypothesized that the substrate stiffness in the ECM plays a role in the tension developed in the spring that will affect the motor-clutch system.
- The various forces that act in the motor clutch system are [a]Characteristic bond rupture force, Fb, and Single myosin motor stall force, Fm.
- The molecular clutches can either engage to F-actin at a rate constant k_{on} or disengage with a rate constant k_{off} .
- Once the clutches are engaged, the retrograde motion of F-actin causes a build up of tension within the spring with a spring constant κ_{clutch}. The tensions that are built up in the intracellular environment have to be matched by the tensions that are built in the extracellular environment and this depends on the compliance of the substrate which has a spring constant of κ_{sub}.

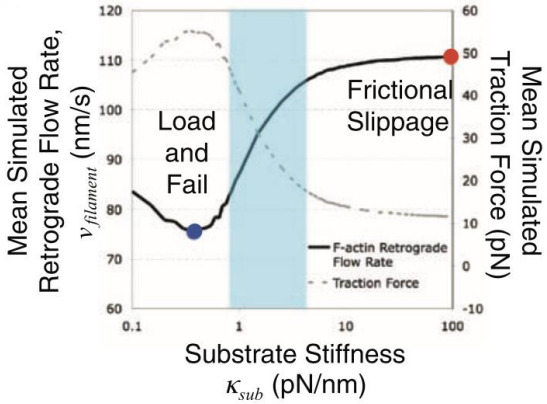
Model Algorithm

- A kinetic Monte Carlo simulation with a varying timestep is performed on the system. The time step for each iteration is chosen as the minimum of the binding or unbinding times of the clutches.
- For every step of the MC simulation, the molecular clutches are allowed to associate with the F-actin bundle following which a check for any dissociations is done.
- Once the spring network is formed, a force balance analysis gives the position of the substrate at that time step. The values of the force acting on the myosin motors and the retrograde flow velocity of the F-actin bundle are calculated for this substrate position.
- The clutches are then displaced by some distance Δ x, and the simulation proceeds to the next time step.
- The simulation is run for over 100000 timesteps and the plots of force, retrograde velocity of the F-actin bundle and the position of the substrates against the clutch stiffness are drawn.

Model Validation and Results

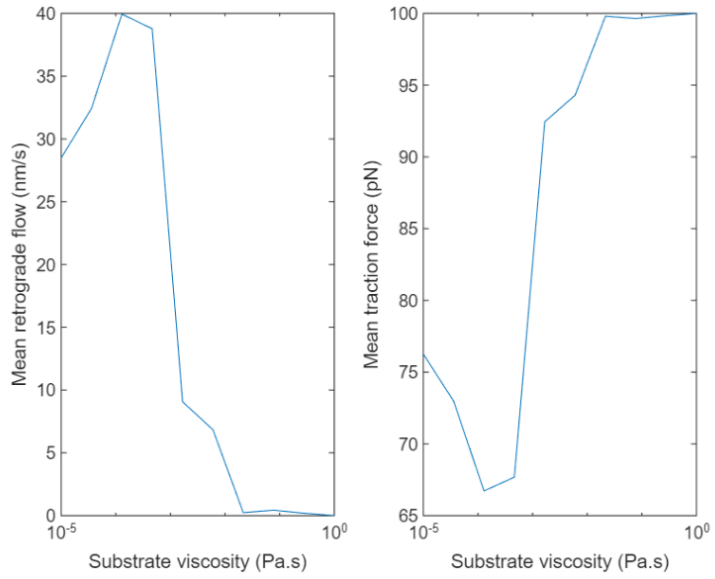
Two parameters were plotted against the substrate stiffness, namely the retrograde flow velocity and force acting on the F-actin bundle.



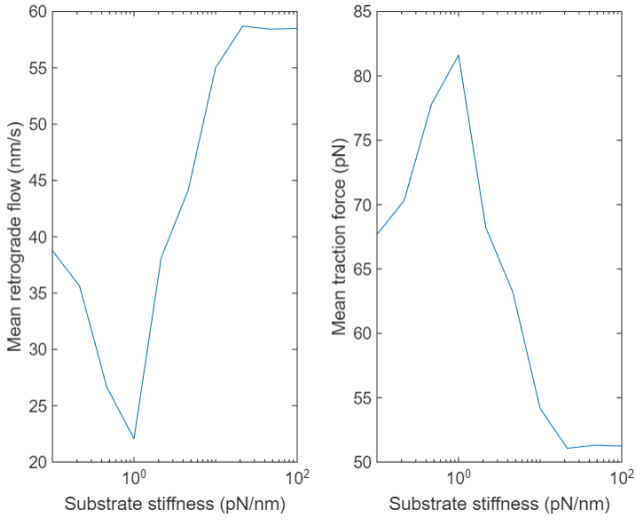


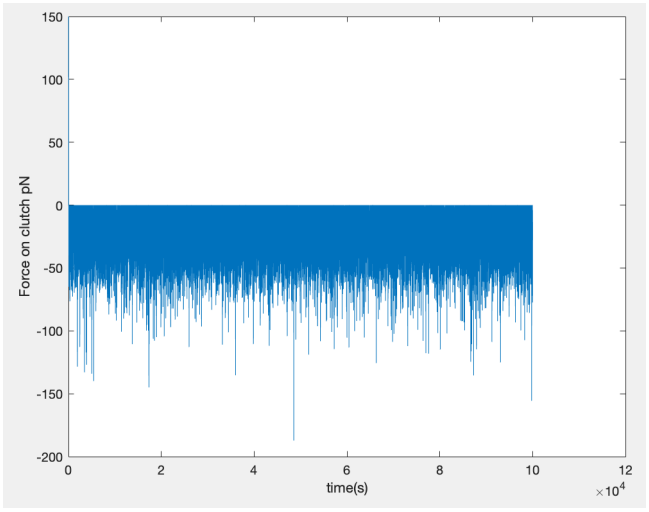
Source:Chan, C. E. & Odde, D. J. *Science* (2008)

Extension: Based on substrate viscoelasticity



Extension: The role of vinculin





Thank You!

Equations of the stochastic model
The molecular clutches are modeled as a Hookean spring with stiffness κ_{clutch}. The tension in the string is given by

$$F_{clutch(i)} = \kappa_{clutch} * (x_i - x_{sub})$$

where x_{sub} is the position of the substrate and x_i is the position of the molecular clutch.

Furthermore, the effective dissociation rate of the clutches increases exponentially as tension develops in the engaged clutches as

$$k^*_{off} = k_{off}.e^{(F_{clutch}/F_b)}$$

where F_b is the characteristic bond rupture force and k_{off} is unloaded dissociation rate of the molecular clutch from the F-actin bundle.

The compliant substrate is also treated as a simple Hookean spring with stiffness κ_{sub}.

The myosin motors are assumed to move when acting against the elastically loaded substrate according to the formula

$$\upsilon_{filament} = \upsilon_u(1 - (\frac{\kappa_{sub}.x_{sub})}{F_{stall}})$$

where $\upsilon_{filament}$ is the velocity of myosin, υ_u is the unloaded sliding velocity of myosin, and F_{stall} is the total myosin stall force of the system, which is defined as

$$F_{stall} = n_m.\hat{F}_m$$

where,
n_m: total number of myosin motors
$\hat{F}_m$: the stall force (force required to stall the activity) of a single myosin motor.
Once the spring network is set up, force balance for the position of the substrate is done as

Equations to account for viscoelasticity

$$F_{trac} = K_S X_S + n_c V f \mu \tag{1}$$

$$V_f = V_u \left(1 - \frac{F_{trac}}{F_s}\right) \tag{2}$$

$$V_f = V_u \left(1 - \frac{F_{trac}}{F_s}\right) \left(1 + \frac{n_c \mu V_u}{F_s}\right) \tag{3}$$

where,
F_{trac}: Traction force
K_S: Substrate spring constant
X_S: Position of substrate
n_c: Number of engaged clutches
n_m: Number of myosin motors
μ: Viscosity
V_u: Unloaded sliding
V_f: Retrograde velocity
F_s: Total Motor stall force

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Model built with the help of code found on: http://oddelab.umn.edu/software.html