FORCE TRANSDUCTION BEHAVIOR OF VISCOELASTIC SUBSTRATE IN F-ACTIN MYOSIN 'MOTOR CLUTCH'

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ABSTRACT

Force transduction is a key cellular process by which cells sense their environment. The response to various physical stimuli is integral towards the process of cell mobility and morphology but is also associated with cancer malignancy. The force transduction network is multifaceted and force singling is achieved via different pathways that elicit a downstream cellular response. Interaction with extracellular matrix (ECM) serves as one of the main connections cells have to their environment. These interactions are facilitated by the actomyosin force transduction network termed a 'motor clutch'. This 'motor clutch' is composed of F-actin fibers and myosin in the cytoskeleton which connect to the ECM via integrin focal adhesions. Herein we utilize Gillespie Monte Carlo simulations methods to elucidate the effects of ECM substrate viscoelasticity and stiffness has upon the 'motor clutch' network. This analysis suggests that viscoelasticity shows a large affect on substrate traction force at low stiffness, but limited modification of 'motor clutch' behavior at high stiffnesses.

INTRODUCTION:

Recent findings have shown that substrate stiffness can play a large role in the mobility, morphology of cells, population growth characteristics, and metastatic capability in cancer. Much of this functionality is associated with the force transduction network which is the method by which cells sense physical characteristics and perturbations of their environment. Force transduction is a process integral to the function of cells, luckily recent work has served to illuminate some of these structures in the transduction network. It is believed that force transduction is mediated through three possible methods: Protein, lipid-initiated protein, and spatial alteration of signal centers.[1]

On the outer membrane and cytoskeleton of the cell, an intricate network of proteins and integrins form the force transduction network. One method by which protein mediated force transduction is achieved is via the change in confirmation of proteins exposing active sites and the response of crucial cytoskeleton crosslinks such as a-actinin and vinculin upon experiencing external forces. Talin which is responsible for linking F-actin to cellular integrins is known have a conformation change response to linear forces, exposing active sites required for vinculin binding.[2] Lipid-initiated mechano-sensing arises when the compressional forces or lack there-of generated by the lipid bilayer on transmembrane proteins during a force event results in a re-conformation of a transmembrane protein and thus activation.[1] Decompression of the plasma membrane has been shown to induce a conformation change in transmembrane ion channels from a close position to an open functional position and is especially relevant to the regulation of cellular osmotic pressure.[3] Spatial alteration mediates force transduction through the change in reaction kinetics dynamics in such as diffusion time due to decreased distance between ligands and proteins in signaling centers during a force event.[1]

'Motor Clutches' describe the actomyosin force transduction network and is a reference to the stop-go mechanisms present in a car transmission that the network resembles.[4] Myosin in the cytoskeleton binds to F-actin fibers and generates a pulling force on them resulting in a constant retrograde flow. Meanwhile, G-actin polymerize to F-Actin and elongates at the leading end and this process is countered by the degradation at the trailing end.[5] Actin is linked via proteins such as Talin to cellular integrins that form focal adhesions (FA's) with the extracellular matrix (ECM).[2] These FA provide a resistive force to the retrograde motion induced by the myosin motor and the degree of resistance to motion serve as an indicator to ECM stiffness. **(FIGURE 1)** Major efforts have been taken to construct models for the force transduction network. Prior work has shown that Gillespie Monte Carlo and original differential equations can simulate actomyosin 'motor clutch' dynamics and is comparable to results observed experimentally.[6,7] However, these models are often simple and ignore key aspects of the force transduction network like viscoelasticity. Herein we

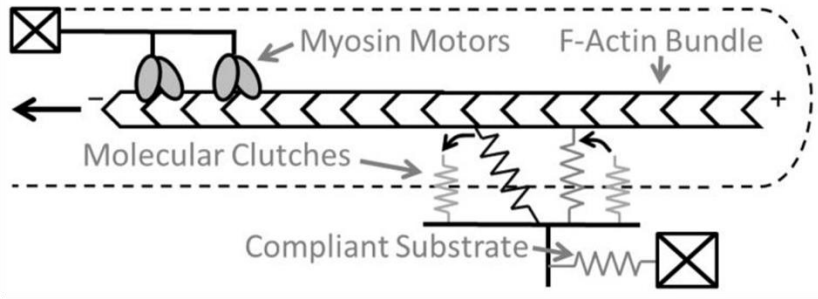

FIGURE 1: Image from Bangasser, et. al (2013) Schematic of actomyosin 'motor clutch' model. Myosin motors retract F-actin and clutches modeled as springs are tethered to the substrate that is modeled as a string.

derive a model for a 'motor clutch' system with a viscoelastic substrate and elucidate the effects viscoelasticity has on the molecular dynamics.

METHODS:

'Motor Clutch' behavior was simulated via a Gillespie Monte Carlo Algorithm. The code was derived from an initial MATLAB script from the Odde Lab. This code was reconstructed in Python to confirm results. All subsequent codes were written in MATLAB due to its faster runtime. Parameter values used were taken from the Odde and Gong papers.[7,8] (**TABLE A1**) A visual simulation of the 'Motor Clutch' was completed utilizing Processing, a Java based program for visual works. **(A2)**

SIMPLE ELASTIC MODEL:

The final Monte Carlo model was composed of 'clutches' which engage or disengage the substrate at a constant rate K_{on} and K_{off}, respectively. For each individual 'clutch' the K_{off} rate was tuned based on the force on the 'clutch', F_c, and the bond rupture force, F_b, via the Bell's Law relation:

$$K_{off,i} = K_{off} \exp\left(\frac{F_{c,i}}{F_b}\right) \quad \text{EQN 1}$$

The force on the 'clutch' was determined via a modified Hookean relation where X_c is the clutch position, X_{sub} is the substrate position and K_c is the characteristic spring constant of the 'clutch'.

$$F_{c,i} = \kappa_c \times \left(X_{c,i} - X_{sub}\right) \quad \text{EQN 2}$$

To determine whether a 'clutch' engages or disengages to the substrate a time variable T_{bind} and T_{unbind} were determined which varies based on a random value, **delta**, from 0 to 1. In the Monte Carlo procedure, the value T_{bind} or T_{unbind} which is lowest was found and the associated 'clutch' unbind/bind event was accepted. The code was run for a set number of events.

$$T_{bind,i} = -\frac{\log(\delta)}{K_{on}} \quad \text{EQN 3}$$

$$T_{unbind,i} = -\frac{\log(\delta)}{K_{off,i}} \quad \text{EQN 4}$$

The traction force, F_{trac}, was calculated from the substrate spring constant, K_{sub} equal to the characteristic substrate stiffness and the substrate position, X_{sub}.

$$F_{trac} = \kappa_{sub} \times X_{sub} \quad \text{EQN 5}$$

The substrate position, $\mathbf{X_{sub}}$, was determined via a force balance between the total 'clutch' Force and substrate traction force. Here 'clutch' position is **Xc**, number of engaged 'clutches', $\mathbf{N_{eng}}$, 'clutch' spring constant, $\mathbf{K_c}$, and the substrate spring constant, $\mathbf{K_{sub}}$.

$$X_{sub} = \kappa_c \times \frac{\sum X_{c,eng}}{\kappa_{sub} + N_{eng}\kappa_c} \quad \text{EQN 6}$$

The engaged 'clutch' position, $\mathbf{X_{c,eng}}$ was calculated based on a time step, **dt**, which is equal to the accepted event time $\mathbf{T_{bind}}$ on $\mathbf{T_{unbind}}$, prior position, and the actin filament velocity, $\mathbf{V_f}$.

$$X_{c,eng,t} = X_{c,eng,t-1} + V_f \times dt \quad \text{EQN 7}$$

The disengaged 'clutch' position, $\mathbf{X_{c,disen}}$ was set equal to the substrate position, $\mathbf{X_{sub}}$.

$$X_{c,disen} = X_{sub} \quad \text{EQN 8}$$

The actin filament velocity, $\mathbf{V_f}$, was determined via the characteristic unloaded filament/motor velocity, $\mathbf{V_u}$, the substrate spring constant, $\mathbf{K_{sub}}$, the substrate position, $\mathbf{X_{sub}}$, the number of myosin motors, $\mathbf{N_m}$, and the motor stall force, $\mathbf{F_m}$.

$$V_f = V_u \times \left(1 - \frac{\kappa_{sub} \times X_{sub}}{N_m \times F_m}\right) \quad \text{EQN 9}$$

VISCOELASTIC MODEL:

In the viscoelasticity model the substrate was treated as a Kelvin–Voigt material introducing a viscosity modification term to the characteristic equations. The Kelvin-Voight model is represented by a spring and dashpot in parallel where the spring constant is the substrate stiffness and the viscosity is the substrate viscosity. The addition of viscosity introduces a time derivative which was approximated.

Here the modification is applied to **EQN 5**, where **eta** is the viscosity

$$F_{trac} = \kappa_{sub} \times X_{sub,t} + \eta \frac{X_{sub,t} - X_{sub,t-1}}{dt} \quad \text{EQN 10}$$

EQN 11 arises as a modification to **EQN 9**

$$V_f = V_u \times \left(1 - \frac{\kappa_{sub} \times X_{sub} + \eta \frac{X_{sub,t} - X_{sub,t-1}}{dt}}{N_m \times F_m}\right) \quad \text{EQN 11}$$

EQN 12 is the viscoelastic modification to **EQN 6**

$$X_{sub} = \kappa_c \times \frac{\sum X_{c,eng} + \eta \frac{X_{sub,t-1}}{dt}}{\kappa_{sub} + N_{eng}\kappa_c + \eta \frac{1}{dt}} \quad \text{EQN 12}$$

RESULTS & DISCUSSION:

ODDE MODEL REPLICATION

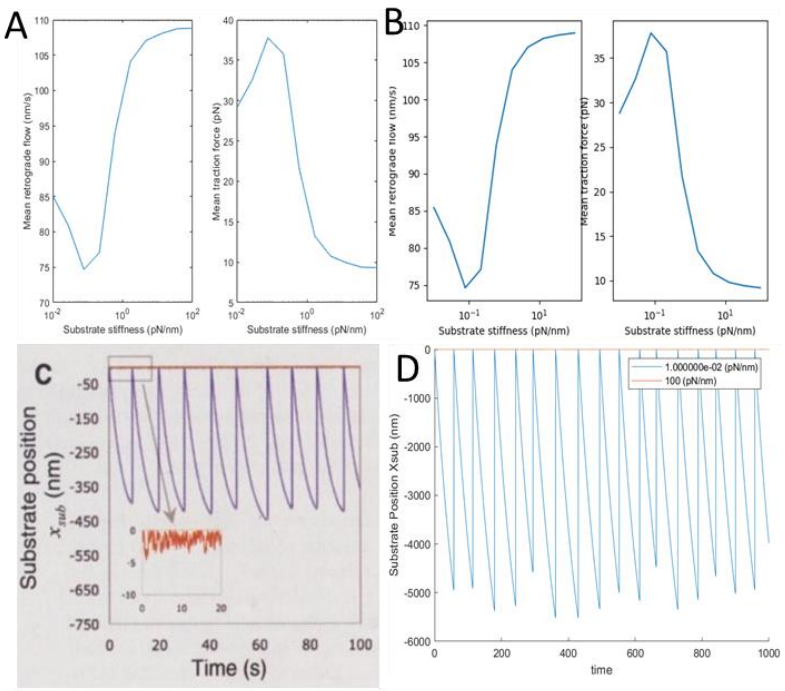

FIGURE 2: Original Odde et al. MATLAB Monte Carlo Model successfully reconstructed in Python (A) Odde et al. MATLAB Monte Carlo Model output (B) Reconstructed Python Monte Carlo Model output (C) Frictional-Slippage/Load and Fail figure from Odde et al. paper.[6] (D) Reconstructed Monte Carlo shows Frictional Slippage and Load-and-Fail behavior described in Odde paper

The Monte Carlo simulation presented by Chan and Odde was a purely elastic model comprised of 'molecular clutches' connected to a substrate, each represented as a spring. Substrate displacement values in the reconstructed Odde Monte Carlo model fluctuated over time. This simulation was reconstructed in Python and similar results were observed. At high stiffness the substrate position remained around zero but at lower stiffness there were multiple peak substrate positions at around 5000 nm. This was observed along with a time delay of ~70 secs before returning to zero. **(FIGURE 2)** This is indicative of the Load-and-Fail behavior described by Chan and Odde where clutches are said to engage before simultaneously failing.[6] Variation in the degree of substrate movement and time to failure is attributed to differences in starting parameters. The higher stiffness substrate exhibits the frictional slippage also reported by Chan and Odde where clutches quickly fail after engaging resulting in little change in substrate position.[6] Retrograde flow and mean traction force behavior in the Python model closely followed that from the Odde simulation. This would indicate that the Python model is a successful reconstruction of the Odde Monte Carlo model.

VISCOELASTIC MODEL

To expand on the basic elastic Monte Carlo model a new model was derived taking into consideration the effects of viscoelasticity of key 'molecular clutch' components. The molecular clutches were still represented by a spring, however viscoelastic behavior was added to the substrate to account for the viscoelastic nature of ECM. At low stiffness, retrograde motion increased with degree of viscoelasticity which we determine as a magnitude of the viscosity term in the constituent equation. **(EQN 10)** The opposite trend was observed at high stiffnesses where retrograde flow decreased towards a minimum of ~90nm/s. **(FIGURE 3)** The traction force behavior reflects the retrograde motion behavior and all trends described with the retrograde flow are vice versa.

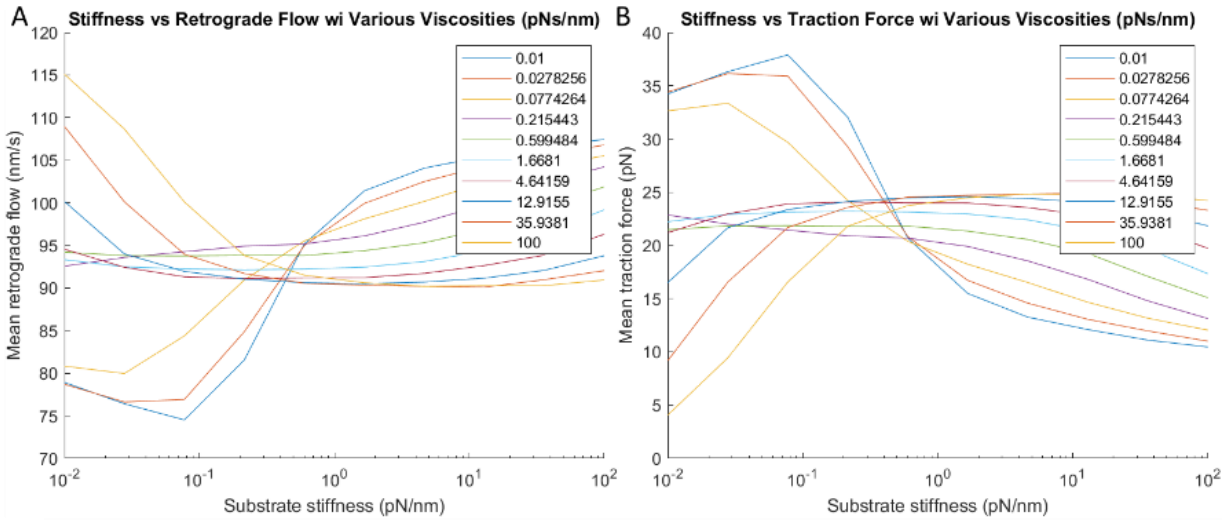

FIGURE 3: Degree of viscoelasticity modifies 'Motor Clutch' Retrograde Flow and Traction Force Behavior (A) Retrograde Flow at various viscosities shows inflection of flow behavior and an increase in retrograde flow with increasing viscosity at low substrate stiffnesses (B) Traction Force at various viscosities shows inflection of force behavior and a decrease in retrograde flow with increasing viscosity at low substrate stiffnesses

Fluctuations in substrate displacement was observed in the viscoelastic substrate much as seen in the elastic model results, however a new behavior was observed that indicates viscoelastic substrates do not display Load-and-Fail clutch dynamics. Four domains of behavior were observed for substrate with low stiffness: Low 0.01pNs/nm, intermediate 0.215pNs/nm, intermediate 0.60pNs/nm, and high viscosity 4.64pNs/nm. **(FIGURE 4)** In the first domain with low viscosity molecular clutches engage and disengage the substrate but there is never a total clutch failure. The second intermediate domain is characterized by chaotic fluctuations and along with the third domain can be considered transitional. The final domain is characterized by minimal fluctuations and is indicative of a growing linearity in substrate position and time. These behaviors indicate a balance between stiffness and viscosity where stiffness results in stable fluctuations while viscosity leads to a time linearity.

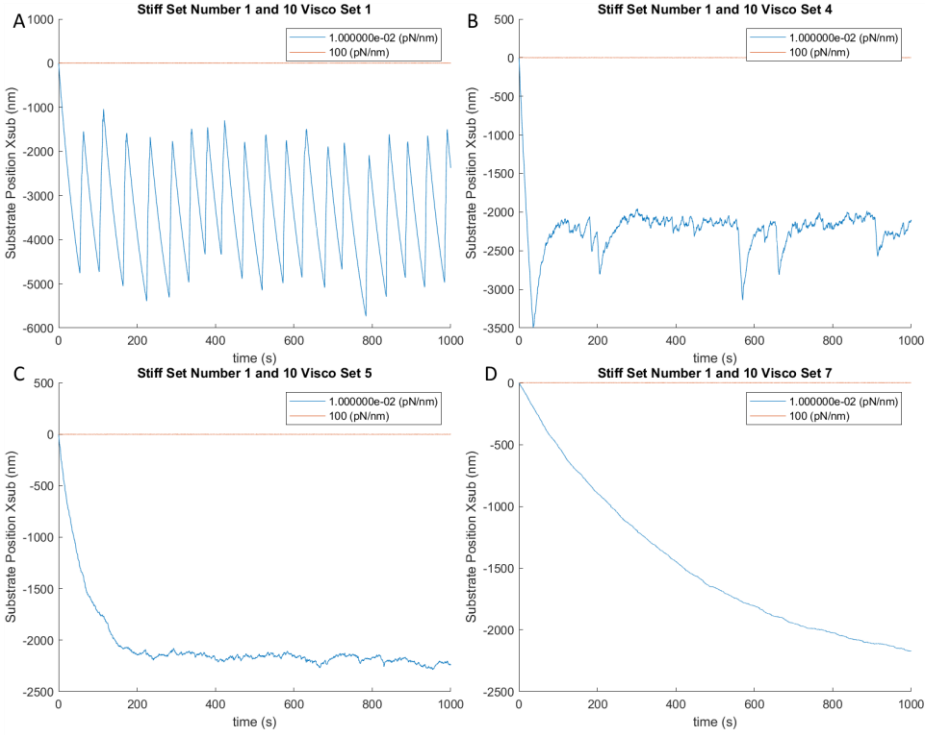

FIGURE 4: Four distinct domains of substrate behavior with increasing substrate viscoelastic nature. (A) Substrate displacement fluctuates around ~3000nm and shows no signs of complete retraction and clutch total failure indicative of Load-and-Fail. (B) Substrate displacement fluctuates around ~2000nm with marked peaks and change in response at viscosity ~0.215 pNs/nm (C) Displacement fluctuation minimizes (D) At high viscosity substrate displacement becomes increasing linear with respect to time

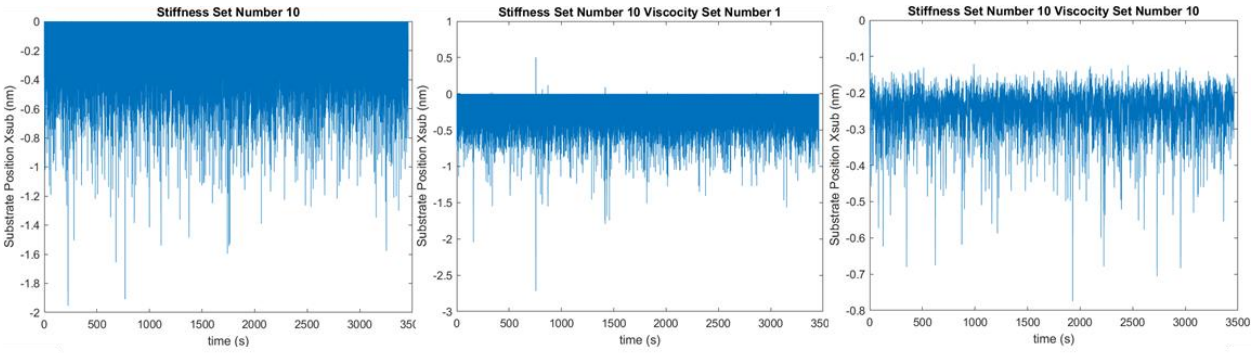

FIGURE 5: As degree of viscoelasticity increases model behavior at high stiffness departs from frictional slippage

Similarly, a departure from frictional-slippage behavior was observed in substrates of high stiffness as viscous nature increased. Low compliance substrates maintained frictional slippage behavior until a viscosity of 1.67pNs/nm. **(FIGURE 5)** This appear to indicate that viscosity serves to decrease clutch disengagement by absorbing force which can be attributed to the endothermic nature of viscoelastic materials during an elongation cycle.

CONCLUSION:

Force transductions allows cells to be highly attuned to their physical environment. The actomyosin 'motor clutch' serves a transducer of force and extracellular matrix properties. While substrate stiffness has been implicated in key cell processes like cell mobility and morphology, the affects of viscoelasticity of these substrate needs further investigation. Through Monte Carlo simulations we observed that viscosity plays a stiffness dependent role on retrograde flow and substrate traction force, both key force transduction indicators. Low stiffness substrates experience an increase in retrograde flow and a decrease in traction force as substrate viscoelastic nature increases. Less compliant substrates experienced a change in 'motor clutch' dynamics albeit to a smaller degree. At high stiffnesses viscosity increased traction force and decreased retrograde flow. As most biological substrate exhibit low stiffness and viscoelasticity this indicates that viscoelastic nature has a larger role in the dynamics of force transduction au naturel and should be marked as an area of further study.

ACKNOWLEDGMENT:

Special Thanks to the Odde Lab from which the original Monte Carlo code was taken.

REFERENCES:

1. Janmey, P. A.; Weitz, D. A. Dealing with Mechanics: Mechanisms of Force Transduction in Cells. *Trends in Biochemical Sciences* **2004**, *29* (7), 364–370.
2. Yan, J.; Yao, M.; Goult, B. T.; Sheetz, M. P. Talin Dependent Mechanosensitivity of Cell Focal Adhesions. *Cellular and Molecular Bioengineering* **2014**, *8* (1), 151–159.
3. Sukharev, S.; Sachs, F. Molecular Force Transduction by Ion Channels – Diversity and Unifying Principles. *Journal of Cell Science* **2012**, *125* (13), 3075–3083.
4. Elosegui-Artola, A.; Trepat, X.; Roca-Cusachs, P. Control of Mechanotransduction by Molecular Clutch Dynamics. *Trends in Cell Biology* **2018**, *28* (5), 356–367.
5. Haase, K.; Al-Rekabi, Z.; Pelling, A. E. Mechanical Cues Direct Focal Adhesion Dynamics. *Progress in Molecular Biology and Translational Science Mechanotransduction* **2014**, 103–134.
6. Chan, C. E.; Odde, D. J. Traction Dynamics of Filopodia on Compliant Substrates. *Science* **2008**, *322* (5908), 1687–1691.
7. Bangasser, B. L.; Odde, D. J. Master Equation-Based Analysis of a Motor-Clutch Model for Cell Traction Force. *Cellular and Molecular Bioengineering* **2013**, *6* (4), 449–459.
8. Gong, Z.; Szczesny, S. E.; Caliari, S. R.; Charrier, E. E.; Chaudhuri, O.; Cao, X.; Lin, Y.; Mauck, R. L.; Janmey, P. A.; Burdick, J. A.; Shenoy, V. B. Matching Material and Cellular Timescales Maximizes Cell Spreading on Viscoelastic Substrates. *Proceedings of the National Academy of Sciences* **2018**, *115* (12).

APPENDIX:

To see the complete set of figures generated refer to the Supplementary Materials folder. The figures in their entirety are not attached here due to space constraints.

TABLE A1: List of Parameter Values used for Monte Carlo Model

Parameter	Value	Description
Nm	50	Number of myosin motors
Fm	-2.0 pN	Motor stall force
Vu	-120 nm/2	Unloaded motor velocity
Nc	50	Number of Molecular Clutches
Kon	0.3 1/s	On rate constant
Koff	0.1 1/s	Off rate constant
Fb	-2.0 pN	Bond rupture force
Kc	0.8 pN/nm	Clutch spring constant
η	10^-2 to 10^2 pNs/nm	Substrate viscosity
Ks	10^-2 to 10^2 pN/nm	Substrate Stiffness
Events	100000	Number of events

Stiffness Set #	1	2	3	4	5	6	7	8	9	10
Value (pN/nm)	0.010	0.028	0.077	0.215	0.599	1.668	4.642	12.915	35.938	100.000

Viscosity Set #	1	2	3	4	5	6	7	8	9	10
Value (pNs/nm)	0.010	0.028	0.077	0.215	0.599	1.668	4.642	12.915	35.938	100.000

A2: Processing Visual 'Molecular Clutch' Simulation

A visual simulation was constructed of the molecular clutch system for the case of a simple elastic substrate. Program and requisite files can be found in Supplementary Materials folder. The visual simulation can be run at any of the modeled stiffness points generated by inputting a value for s (stiffness set number) from 1-10 which refers to a specific stiffness with 1 being lowest 10^-2 pN/nm and 10 the highest, 10^2 pN/nm. Simulation movement is not directly representative of substrate movement because of the inclusion of a scaling term to allow visualization of movement at all stiffnesses.


Motor_Clutch_S1.mov


Motor_Clutch_S4.mov


Motor_Clutch_S7.mov