

Determinants of Maximal Force Transmission in a Motor-Clutch Model of Cell Traction in a Compliant Microenvironment

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Force Transmission in a Motor-Clutch Model

Submitted January 10, 2013, and accepted for publication June 18, 2013.

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Supplementary Information

Model Simulation Algorithm

The model simulation algorithm from Chan and Odde(1) was updated from a fixed time step Monte Carlo simulation to a variable time step Gillespie Stochastic Simulation Algorithm (SSA), also known as a Kinetic Monte Carlo simulation(2). Additionally, the order of events in the simulation was updated to:

1. Calculate the off-rate for each engaged clutch based on the clutch deformations
2. Determine event times based on clutch on- and off-rates (see **Eq. 2**)
3. Advance time by the minimum calculated event time
4. Execute the reaction corresponding to the minimum calculated event time (binding or unbinding of the particular clutch)
5. Calculate F-actin retrograde flow rate based on the current substrate deformation using as linear force-velocity relationship(1).
6. Advance engaged clutch positions by the product of the F-actin retrograde flow rate and time step.
7. Calculate substrate position through a force balance on the substrate and clutch springs
8. Return to step 1

This ordering ensures elastic equilibrium is achieved at the end of every time step, leading to more accurate calculation of clutch off-rate at the beginning of the next step. As shown in **Figure S1A**, using the SSA algorithm resulted in behavior qualitatively similar to that obtained from our previous fixed time step algorithm. In particular, the biphasic dependence of F-actin retrograde flow rate (and equivalently traction force) remains a central and robust aspect of the model. By modest modification of a subset of model parameters, the SSA algorithm produces simulated model behavior consistent with our previous published data (**Fig. S1B** and **C**).

Analysis of each clutch parameter

The effect of each clutch parameter on the shape of F-actin retrograde flow rate as a function of substrate stiffness, and the position of the optimum is examined below.

Clutch on-rate (k_{on}): An increase in k_{on} causes an increase in the average number of clutches bound on any stiffness. This causes the ensemble clutch stiffness (see **Fig. 6A** and **B**) to increase, which results in an increase in the optimum stiffness. A large increase in k_{on} eventually causes a stalled system because more clutches are bound at any given time, providing too much resistance for the motors to break all of the clutch bonds. A decrease in k_{on} decreases the ensemble clutch stiffness, which shifts the optimum to lower stiffnesses. A large decrease causes few clutches to be bound at any given time resulting in a free flowing system because the motors quickly break the clutch bonds.

Clutch bond rupture force (F_b): An increase in F_b causes an increase in the average number of clutches bound on any stiffness. This causes an increase in the ensemble

clutch stiffness, which results in an increase in the optimum stiffness. A large increase in F_b causes a stalled system because more clutches are bound at any given time, providing too much resistance for the motors to break all of the clutch bonds. A decrease in F_b decreases the ensemble clutch stiffness, which shifts the optimum stiffness to lower values. A large decrease causes few clutches to be bound at any given time resulting in a free flowing system because the motors quickly break the clutch bonds.

Number of clutches (n_c): An increase in n_c causes an increase in the average number of clutches bound on any stiffness. This causes the ensemble clutch stiffness to increase, which increases the optimum stiffness. A large increase in n_c causes a stalled system because more clutches are bound at any given time, providing too much resistance for the motors to break all of the clutch bonds. A decrease in n_c decreases the ensemble clutch stiffness, in turn decreasing the optimum stiffness. A large decrease causes few clutches to be bound at any given time resulting in a free flowing system because the motors quickly break the clutch bonds.

Clutch stiffness (κ_c): An increase in κ_c means that clutch bonds are stiffer, which, by itself, would tend to increase the ensemble clutch stiffness and shift the optimum to higher substrate stiffness. However, stiffer clutch bonds also tend to load quickly and therefore fail quickly, which decreases the average number of engaged clutches. By itself, the decreased number of engaged clutches would decrease the ensemble clutch stiffness. Combined, these two opposing effects might effectively cancel each other, and in fact this is observed in our simulations. The only obvious effect of increasing clutch stiffness is that the retrograde flow rate on high substrate stiffnesses (i.e. well above the optimum) increases. In this high stiffness frictional slippage regime (see **Fig. 6E**), the decreasing number of engaged clutches as the clutch stiffness increases means that the system is tending toward free flowing (i.e. higher F-actin retrograde flow rate). In the low stiffness frictional slippage regime (i.e. below the optimum, see **Fig. 6E**), the substrate stiffness is softer than the ensemble clutch stiffness, and in this regime clutches tend to fail spontaneously prior to reaching appreciable loads. For this reason, the model behavior in the low substrate stiffness regime is insensitive to the mechanical properties of the clutch itself. This is perhaps one of the most surprising findings of the single parameter changes: the optimum stiffness is insensitive to the clutch stiffness, the only parameter that describes a cellular mechanical property.

Motor stall force (F_m): An increase in F_m strengthens the motors and decreases the load-and-fail cycle time, which shifts the optimum stiffness to lower values. A large increase in F_m causes clutch bonds to quickly break and results in a free flowing system. A decrease in F_m weakens the motors and increases the load-and-fail cycle time, which shifts the optimum stiffness to higher values. A large decrease weakens the motors to the point where they cannot break the clutch bonds and the system stalls.

Number of motors (n_m): An increase in n_m strengthens the motors and decreases the load-and-fail cycle time, which decreases the optimum stiffness. A large increase in n_m causes clutch bonds to quickly break and results in a free flowing system. A decrease in n_m weakens the motors and increases the load-and-fail cycle time, which increases the optimum stiffness. A large decrease weakens the motors to the point where they cannot break the clutch bonds and the system stalls.

Clutch unloaded off-rate (k_{off}): An increase in k_{off} is similar to an increase in k_{on} in that it causes a decrease in the average number of clutches bound on any stiffness, which results in a decrease in the optimum stiffness. A large increase in k_{off} causes few clutches to be bound at any given time resulting in a free flowing system because the motors quickly break the clutch bonds. A decrease in k_{off} increases the ensemble clutch stiffness, in turn increasing the optimum stiffness. A large decrease causes a stalled system because more clutches are bound at any given time, providing too much resistance for the motors to break all of the clutch bonds.

Motor unloaded velocity (v_u): An increase in v_u strengthens the motors and decreases the load-and-fail cycle time, which shifts the optimum stiffness to lower values. A large increase in v_u does not cause a free flowing system, but it does cause F-actin retrograde flow rates above the physiologically relevant limit. A decrease in v_u weakens the motors and increases the load-and-fail cycle time, which shifts the optimum stiffness to higher values. A large decrease essentially stalls the system because all retrograde flow is near zero.

Analysis of select dual parameter changes

Dual parameter changes can shift the optimum stiffness over a wide range of values, as long as the changes compensate for each other by avoiding the transitions to either free flowing or stalled states. Below, two examples are explained in further detail.

Number of clutches/number of motors (n_c/n_m): An increase in n_c by itself shifts the optimum higher and eventually will stall the system. A compensatory increase in n_m rescues the stalled system but shifts the optimum to lower stiffnesses. However, the optimum has a higher positive sensitivity to the clutches than it has a negative sensitivity to the motors (see **Fig. 3B**). The result is a shift of the optimum stiffness to higher values, but with a lower sensitivity value (S). Although the sensitivity of the optimum to the dual parameter change is less, the range is much larger resulting in a high sensitivity-range (SR , see **Fig. 5A-C**).

Clutch on-rate/Clutch off-rate (k_{on}/k_{off}): An increase in k_{on} by itself shifts the optimum higher and eventually will stall the system. A compensatory increase in k_{off} rescues the stalled system but shifts the optimum to the left. However, the optimum has a higher positive sensitivity to the on-rate than it has a negative sensitivity to the unloaded off-rate (see **Fig. 3B**). The result is a shift of the optimum stiffness to higher values, but with a lower sensitivity value (S). Although the sensitivity of the optimum to the dual parameter change is less, the range is much larger resulting in a high sensitivity-range (SR , see **Fig. 5A-C**).

Parameter	Symbol	Fig. 7A	Fig. 7A	Fig. 7A	Fig. 7B	Fig. 7B	Fig. 7C
		Fig. 7A	(-)clutch	(-)motor	Fig. 7B	(-)motor	Fig. 7C
Number of motors	n_m	4000	4000	1000	75	40	-
Motor stall force (pN)	F_m	2	2	2	2	2	2
Motor unloaded velocity (nm/s)	v_u	120	120	120	120	120	120
Number of clutches	n_c	2000	500	2000	75	75	-
Clutch bond rupture force (pN)	F_b	2	2	2	2	2	8
Clutch on-rate (s^{-1})	k_{on}	0.3	0.3	0.3	1	1	0.1
Clutch unloaded off-rate (s^{-1})	k_{off}	0.1	0.1	0.1	0.03	0.03	0.004
Clutch spring constant (pN/nm)	k_c	0.8	0.8	0.8	0.02	0.02	5
Substrate spring constant (pN/nm)	k_{sub}	-	-	-	50×10^9	50×10^9	-
Clutch catch force (pN)	F_c	-	-	-	-	-	8
Clutch unloaded catch off-rate (s^{-1})	$k_{off,c}$	-	-	-	-	-	10

Supplementary Table S1: Model parameters for experimental validation.

Supplementary Legends

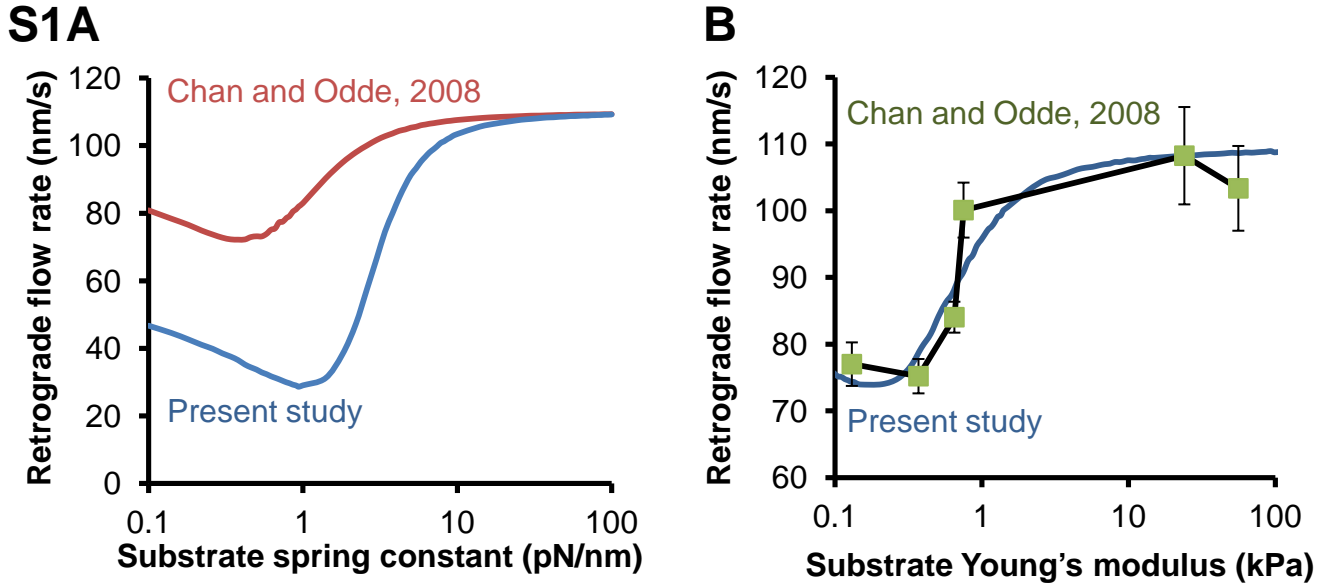
Supplementary Movie S1: The movie demonstrates motor-clutch behavior with different numbers of clutches (n_c). Myosin motors appear in blue on the left side. The F-actin filament is purple with dots placed on it to visualize F-actin retrograde flow. Clutches are the vertical blue lines, and the red is the substrate. When a clutch binds it will extend from the red substrate to the purple F-actin. In the top case, $n_c=6$ and the system is free flowing. The F-actin flows at near its unloaded velocity because the clutches provide little resistance to the motors. For $n_c=50$, the system loads and fails. F-actin retrograde flow is fast at the beginning of the cycle and slows toward the end of the cycle. In the bottom case, $n_c=150$, and the system stalls. In this case, there are too many clutches for the force of the motors to break all the bonds.

Supplementary Figure S1: Modified model algorithm. A) The changes in the model algorithm caused a change in the quantitative shape of the output using the same parameters. B and C) Using the new algorithm the model was fit to the embryonic chick forebrain neuron data from Chan and Odde(1) to obtain a new set of base parameters. The new value of κ_c was assigned based on experimental data for the spring constant of integrins(5)(6). The values of n_m , n_c , k_{on} , and k_{off} were allowed to change subject to the constraint $n_m=n_c$. The fitted value of k_{off} turned out to be the same as the original value, so only n_m , n_c , k_{on} , and κ_c were altered in the new parameter set. Clutch parameters are highlighted in gray.

Supplementary Figure S2: Pairwise parameter limits. A and B) For the dual parameter sensitivity tests, upper and lower limits were reached before resulting in stalled or free flowing system. The upper and lower limits of the multiplier c are shown. Cases where a physiological limit was imposed are highlighted in orange. These tables use the same above and below the diagonal convention used in **Figure 5**. Clutch parameters are highlighted in gray.

Supplementary References

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C

Parameter	Symbol	Chan and Odde, 2008	Present Study
Number of motors	n_m	75	50
Motor stall force	F_m	2 pN	2 pN
Motor unloaded velocity	v_u	120 nm/s	120 nm/s
Number of clutches	n_c	75	50
Clutch bond rupture force	F_b	2 pN	2 pN
Clutch on-rate	k_{on}	1 s ⁻¹	0.3 s ⁻¹
Clutch unloaded off-rate	k_{off}	0.1 s ⁻¹	0.1 s ⁻¹
Clutch spring constant	k_c	5 pN/nm	0.8 pN/nm

S2A

	k_{on}	F_b	n_c	κ_c	F_m	n_m	k_{off}	v_u
k_{on}	5.3	1.7	1.7	5.1	10	32	1000	6.1
F_b	27	2.3	1.5	2.3	10	1000	200	2.4
n_c	17	1000	2.2	2.2	10	1000	1000	2.3
κ_c	14	7.4	7.2	13	7.5	7.5	14	1.6
F_m	1.7	1.5	2.7	2.3	7.5	2.8	3.4	1.4
n_m	1.7	1.5	1.5	2.3	1000	7.48	3.4	1.4
k_{off}	3.8	3.3	3.4	5.1	1000	50	14	1.4
v_u	1.4	1.4	1.4	13	2.4	2.4	5.8	1.6

B

	k_{on}	F_b	n_c	κ_c	F_m	n_m	k_{off}	v_u
k_{on}	0.073	0.29	0.29	0.071	0.0083	0.02	0.005	0.05
F_b	0.001	0.13	0.37	0.14	0.005	0.02	0.038	0.05
n_c	0.001	0.001	0.12	0.14	0.02	0.02	0.059	0.05
κ_c	0.2	0.45	0.45	0.013	0.43	0.43	0.21	0.077
F_m	0.30	0.36	0.67	0.13	0.45	0.67	0.59	0.48
n_m	0.30	0.36	0.37	0.13	0.02	0.44	0.59	0.48
k_{off}	0.43	0.59	0.59	0.059	0.037	0.037	0.19	0.27
v_u	0.30	0.48	0.48	0.63	0.05	0.05	0.05	0.083