SUPPLEMENTARY MATERIALS

Rigidity Sensing : A Single Cell Acts as a Muscle

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I. LINK BETWEEN INCREASE OF dF/dt WITH k AND RIGIDITY SENSING

dF/dt increasing with k (Fig. 2b) means that after a given time t, cells will apply higher forces on stiffer substrates, as directly shown on Fig. 1. This response to stiffness has many implications for rigidity sensing and associated cellular features such as migration along rigidity gradients for instance.

Indeed, cell migration is a cyclic process. In each step, new cell-substrate contacts are formed at the front of the cell, traction forces are then applied to these contacts, the rear end eventually detaches from the substrate and the cell is propelled forward. Let us consider a cell migrating on an anisotropic substrate (Fig. S3 - A1 to A5). In a given step of its migration, new adhesions are formed and submitted to increasing traction forces. Our findings indicate that, after a time Δt (the duration of a migration step), the cell will apply higher forces on the adhesion complexes situated along the directions of high rigidity (Fig. S2). As a consequence, the net force applied to the cell will be more oriented along the stiffer direction of the substrate than the initial direction of migration (Fig. S2). This is a purely mechanical effect : the total force applied to the center of mass of the cell (which indicate the direction of displacement at each step) will rotate towards the stiffer axis of the substrate (Fig. S3-B). It is noteworthy that migration along the stiffer direction of the substrate would then be "stable" (any deviation from this direction is automatically corrected in the following steps), while migration along the softer direction would be "unstable" (deviation is amplified until cell aligns along the stiffer direction).

The previous purely mechanical scenario illustrates how the increase of dF/dt with k (Fig. 2b) is necessarily important for mechano-sensing and, in particular, to migration. Of course, the effect described above can be amplified by the specific mechano-sensitivity of adhesion complexes. For instance, on can imagine that a threshold force F_{min} could be necessary to stabilize newly formed adhesion complexes. Forces applied on complexes situated along the softest directions may then fail to reach F_{min}, and the corresponding adhesions may thus detach before any cell displacement has occurred. Only forces applied along the directions of high rigidity would then be effective, and this would lead, at a given step, to a more pronounced rotation of the migration path.

Note that the scenario presented here is supported by recent measurements using multiple optical tweezers of different stiffness [1]. It has indeed been shown that traction forces generated within a single cell can vary over distances of less than one micrometer, depending on the local stiffness of the laser traps.

II. HILL BEHAVIOR AND PROTEIN RECRUITMENT

Our results suggest that rigidity sensing is due to force-dependent binding kinetics of contractile acto-myosin units. Then, one important issue to address is that of a possible recruitment of myosin or of focal adhesions, and its potential contribution to the observed cell response to rigidity (or, equivalently, its potential effect on the measured F-V relationship). In our report we show that force increase was correlated with cell shape evolution (*i.e.* with cell spreading between the plates). Thus, in the time course of a given experiment (one cell pulling on a plate of a given stiffness), the cell structure must be remodeled and proteins are certainly recruited. However, as illustrated in Fig. S4, experiments at fixed stiffness values can not give any information about rigidity sensing. To test the effect of the stiffness on the traction force generated by the cell, we measured the force and speed values (respectively $F(\delta_0)$ and $V(\delta_0)$) observed **at a given plate deflection δ_0 for different cells pulling on plates of different stiffness** (Fig. 6). The fact that $F(\delta_0)$ and $V(\delta_0)$ were measured at a same deflection δ_0 ensured that all tested cells where at the same stage of their spreading process (same overall shape). The question of a possible recruitment must then be formulated as follows. When cells pulling on plates of different stiffness are observed at the same stage of their spreading, do they have the same amount of contractile acto-myosin units and the same number of focal contacts? We argue below that the number of active contractile units should be the same for all k values in order to ensure a Hill F-V relationship as observed experimentally. Conversely, there is no incompatibility between Hill F-V behavior and focal adhesion recruitment.

A. Myosin recruitment

Recently, Debold *et al.* [2] measured the speed of an actin filament trapped in optical tweezers and sliding on a mini-ensemble (~ 8 heads) of myosin motors. Applying forces of different magnitudes (in the pN range), they showed that the F-V relationship at the molecular level conformed to the Hill equation. Thus, on the one hand, each contractile acto-myosin unit involved in force generation by the cell is characterized by a Hill force-velocity relationship. On the other hand, our measurements show that the F-V relationship at the scale of the whole cell also conforms to the Hill equation. The question of myosin recruitment is then equivalent to the following one : would the F-V relationship be conserved from the scale of one contractile unit to that of the whole cell if the total number of units N_{tot} was not the same for different plate stiffness values? We show below that the answer is no. In other words, N_{tot} must be independent of the load applied to the cell and, thus, independent of the plate stiffness k.

The fact that the force-velocity data conformed to the Hill equation (Fig 3 and 4) means that:

$$V = \frac{c}{F+a} - b = \frac{c}{k\delta_0 + a} - b \tag{1}$$

where *a, b, and c are Hill fitting* ***constants*** and $\delta_0 = 1\mu m$ is the deflection value at which V and F data were measured. Following Debold *et al.* [2], individual contractile units (*i.e.* a mini-ensemble of myosin heads interacting with one actin filament or bundle) also conform to the Hill equation:

$$v = \frac{c_u}{f + a_u} - b_u \tag{2}$$

where a_u, b_u, and c_u are the Hill constants characteristic of one contractile unit and f the force applied to it. One further finds that $a_u = \frac{f_{max}}{4}$, $b_u = \frac{v_{max}}{4}$, and $c_u = \frac{5 f_{max} v_{max}}{16}$. Thus, a_u, b_u, and c_u are representative, respectively, of the maximum force f_{max} a contractile unit can generate, of its maximum speed of shortening v_{max} and of the corresponding characteristic mechanical power $f_{max} v_{max}$. V and F at the scale of the whole cell are also related to the characteristic v and f of one contractile unit. In particular, $\{a, b, c\}$ must be functions of $\{a_u, b_u, c_u\}$ and also functions of the number N_{tot} of contractile units. Thus a change in N_{tot} with the stiffness k would lead to change in $\{a, b, c\}$. Then these parameters could no longer be considered constants and equation (1) would no longer be satisfied.

Let us now consider a schematic representation of the contractile machinery of the cell (Fig. S4). The N_{tot} contractile units are distributed over N fibers linking the rigid and flexible plates of our setup (*i.e.*, pulling in parallel). Each fiber contains n contractile units arranged in series. This very simple representation takes into account the essential features needed to express $\{a, b, c\}$ as functions of $\{a_u, b_u, c_u\}$, namely the fact that part of the contractile units work in series, and others in parallel. At a given deflection δ_0, a cell pulling on a plate of stiffness k is submitted to a force $F = k\delta_0$. F is then distributed over the N parallel fibers, each fiber being submitted to a force:

$$F_f = \frac{F}{N} \tag{3}$$

The speed of shortening V_f of the fibers is equal to that (V) of the whole cell:

$$V_f = V \tag{4}$$

One can then express the force f and the speed of shortening v of each contractile unit as functions of F and V. Along a given fiber, the tension is equal to F_f, thus:

$$f = F_f = \frac{F}{N} \tag{5}$$

The contractile units being submitted to the same force f, and conforming to the Hill equation (2), they all shorten at the same speed v. Moreover, in a given fiber, n contractile units are arranged in series. Thus, the strains of the individual units add to each other and the speed of shortening of the fiber is simply n times v:

$$V = V_f = nv \tag{6}$$

Combining equations (6) and (2) one can express the cell speed of shortening V as a function of the features of an individual contractile unit:

$$V = n[\frac{c_u}{f + a_u} - b_u] \tag{7}$$

Taking into account (5), the latter equation can be expressed as a function of the force F experienced by the cell, instead of f:

$$V = n[\frac{c_u}{\frac{F}{N} + a_u} - b_u] \tag{8}$$

The latter equation is equivalent to:

$$V = \frac{Nnc_u}{F + Na_u} - nb_u \tag{9}$$

This equation can then be identified to the Hill equation observed at the scale of the whole cell (eq. (1)) and the cell Hill constants $\{a, b, c\}$ can be expressed as functions of the characteristics $\{a_u, b_u, c_u\}$ of one contractile unit:

$$a = Na_u \tag{10}$$

$$b = nb_u \tag{11}$$

$$c \;=\; Nnc_u = N_{tot}c_u \tag{12}$$

What these last three equations illustrate is that the observation of a Hill behavior at the molecular level ($\{a_u, b_u, c_u\}$ constant) as well as at the cell scale ($\{a, b, c\}$ constant, independent of k) is incompatible with myosin recruitment (N and n must be constants independent of k also). We argue as follows, the data shown in Fig. 3 demonstrate that when the stiffness k of the plate changes, the traction force $F = k\delta_0$ and the speed of shorting $V(\delta_0)$ vary in such a way that they are related by Hill equation (1). Thus $\{a, b, c\}$ are constants defining the cell's response to stiffness variations. Furthermore, $\{a_u, b_u, c_u\}$ are constants characterizing the acto-myosin response to load at the molecular level (Hill behavior of individual contractile units [2]). Thus, equations (10), (11), and (12) imply that N and n are also constants. In other words, cells observed at the same stage of their spreading (*i.e.* at the same flexible plate deflection δ_0) have the same mean number of contractile units for all stiffness values (no myosin recruitment, Fig. S4). In this scenario, the cell behaves like a skeletal muscle: its response to load reflects that of the individual acto-myosin units. A Hill-type F-V relationship at the cell scale (our results) would just be the "integrated" expression of a load-dependent binding kinetics at the molecular level.

The previous argument is based on the assumption that individual contractile acto-myosin units are characterized by a Hill f-v relationship. This is true in the skeletal muscle and has also been demonstrated in motility assays on single actin filaments [2]. However, strictly speaking, we did not show that this was true in our isolated cells. Nevertheless, one has to keep in mind that Hill behavior is based on physical or passive processes (namely load-dependent binding kinetics), as opposed to active ones that involve chemical regulation. Thus, even in isolated cells, individual contractile units should conform to Hill behavior *unless specific biochemical loops are implemented to modify acto-myosin response to load.* One could then imagine a scenario involving myosin recruitment controlled by a complex and specific combination of chemical regulatory loops allowing the cell to coordinate the number and the activity of its contractile units to ensure an overall Hill behavior[6]. Such a recruitment-based scenario is questionable, however. At first, it implies a complex combination of biochemical loops to ensure the same overall cell response (Hill F-V relationship) that can be readily obtained by a muscle-like behavior with no regulation at all. Moreover, it is conceptually surprising that regulatory loops should be implemented, on the one hand to modify the regular (physical, "automatic") Hill acto-myosin response to load at the local contractile fibers scale, while imposing, on the other hand, an overall Hill-like F-V relationship at the cell scale.

To sum up, we showed that the single cell response to stiffness (dF/dt as a function of k, Fig. 2b) is equivalent to

a Hill F-V relationship (Fig. 3 and 4). This Hill behavior can be explained by two different mechanisms, depending on whether or not myosin recruitment is involved:

- in the simplest scenario, the cell acts like a skeletal muscle. Its Hill F-V relationship at the cellular level corresponds to the integrated Hill responses of its contractile acto-myosin units. Hill-like behavior is then dictated by the load-dependent binding kinetics of myosin to actin, and no myosin recruitment is involved.
- a more complex and speculative scenario would involve sophisticated biochemical loops to combine myosin recruitment and modification of the f-v relationship of acto-myosin contractile units (at the local molecular level) to ensure a global Hill F-V relationship at the cell scale.

In both cases, our findings ($dF/dt(k)$ and Hill F-V relationship) lead to important conclusions.

The muscle-like scenario implies that acto-myosin units act themselves as mechanosensors. That is the interpretation we favor because of its simplicity. Indeed, acto-myosin interaction has been identified as a universal mechanism to generate contractility, force and movement in a wide variety of organisms, for different biological functions, and at length scales ranging from the microscopic (stress fibers) to the macroscopic level (muscles of vertebrates). Thus, it is reasonable to speculate that the ubiquitous characteristics of acto-myosin interaction are used for rigidity sensing by the cell.

The recruitment-based scenario would make the Hill behavior a biological rule that must be obeyed regardless of the physical responses of the cell contractile machinery. If true, this would be a new and important information. However, it is somewhat implausible to assume that the living cell should have developed a chemical control process tuned to ensure a Hill F-V relationship at the cell scale, while neutralizing the characteristic Hill f-v relationship of acto-myosin units.

B. Recruitment of adhesion complexes

There is no conflict between our findings and recruitment of adhesion complexes. One can even imagine that acto-myosin based rigidity sensing and sensitivity of adhesion complexes to load might be synergistic processes. In fact, it is well documented that focal complexes and adhesions are sensitive to applied forces. In particular, it was shown that the area of focal contacts was proportional to the magnitude of an externally applied force, suggesting that focal contacts bear a constant stress ([3]). Indeed, when the cell traction force increases, the adhesion complexes must be reinforced to avoid breakage of the mechanical link between the cell and its environment. Thus, as cells pull

harder on stiffer substrates (myosin-based sensitivity, Fig. 1), adhesion complexes may grow in size as the stiffness k is increased.

The important point is that adhesive complexes are not, on their own, able to produce force. Thus the fact that these contacts may increase in size when plate stiffness is increased should have no consequence on the F-V relationship. This would be the case only if adhesions recruitment would induce an increase of the number of contractile units, which is incompatible with Hill F-V relationship, as mentioned in the previous section.

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[4] R. Cooke and W. Bialek. Contraction of glycerinated muscle fibers as a function of the ATP concentration. *Biophysical Journal*, 28(2):241–258, 1979.

[5] N. Desprat, A. Guiroy, and A. Asnacios. Microplates-based rheometer for a single living cell. *Review of Scientific Instruments*, 77:055111, 2006.

[6] Back to equations (10), (11), and (12), it appears that if N and/or n are to increase with increasing plate stiffness k (myosin recruitment), $\{a_u, b_u, c_u\}$ must be tuned to ensure constancy of $\{a, b, c\}$. For instance, if N increases with k, a could be maintained constant if the cell was able to induce a decrease of a_u proportional to $1/N$. In fact, tuning $\{a_u, b_u, c_u\}$ is possible. It is well known, for instance, that Hill F-V relationship is highly dependent on ATP concentration [4]. More recently, Debold *et al.* [2] showed that increasing the concentration of ATP increased the maximum speed $v_{max} = 4b_u$ and decreased the stall force $f_{max} = 4a_u$ of actin filaments sliding on a mini-ensemble of myosin heads. It is noteworthy that change in ATP concentration induces opposite evolutions of a_u and b_u in that case. Thus tuning the molecular activity of acto-myosin to compensate for changes in the number of contractile units is possible. However, as illustrated in the latter example, a scenario based on recruitment implies numerous and sophisticated regulatory processes. In our schematic model, at least three regulation loops are needed: one to increase the number N_{tot} of contractile units with increasing external rigidity, the second one to coordinate the evolutions of N and n, and the third one to adapt ATP delivery to compensate for the increase of N_{tot} and maintain an overall Hill behavior of the cell).

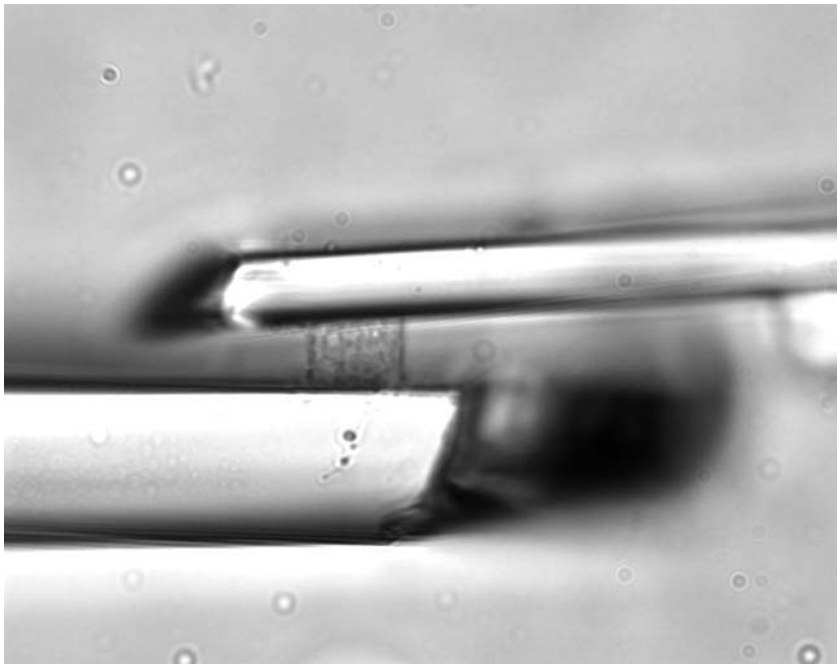
Figure S1: Preliminary results obtained for 3T3 fibroblasts. The behaviors **(a)** of the force F(t), **(b)** of the rate of force increase $\frac{dF}{dt}(k)$, and **(c)** of the speed of cell shortening $V(F)$ and mechanical power $P(F)$ were similar to those observed for C2.7 myoblasts (Fig. 2 and 4). In particular, for low stiffness values, the force generated by the fibroblasts increased faster when pulling on stiffer plates. V and P data were fitted to the Hill equation of muscle (acto-myosin) contraction.

Figure S2: The process of cell migration on an anisotropic substrate. The stiffness of the substrate varies continuously from k_{min} to k_{max} depending on the direction considered. Initially, the cell is migrating along the direction of the speed vector V_1. At the cell front, new cell-substrate adhesions are formed and submitted to traction forces perpendicular to the cell leading edge. Our results (Fig. 2) indicate that force will increase faster on adhesions formed along directions of high rigidity. After a time Δt (step duration), forces exerted by the substrate to the cell (which are opposit to those exerted by the cell to the substrate) are higher along the stiffer directions. For instance, $F_3 > F_2 > F_1$ (For purpose of simplicity and clarity, we only represented few characteristic force vectors). When the rear of the cell detaches, the center of mass of the cell is submitted to the sum of the force vectors exerted to the cell front, here F_{Tot}. This vector indicates the new direction of cell displacement. As illustrated here, the direction of migration rotates towards the stiffer direction of the substrate (rotation from speed vector $V_1(t)$ to speed vector $V_2(t + \Delta t)$ in this example).

Figure S3: Cell migration on an anisotropic substrate. Sequence of migration steps following the scheme of Fig. S3 (A1 to A5), and the corresponding successive positions and displacements of the cell (B).

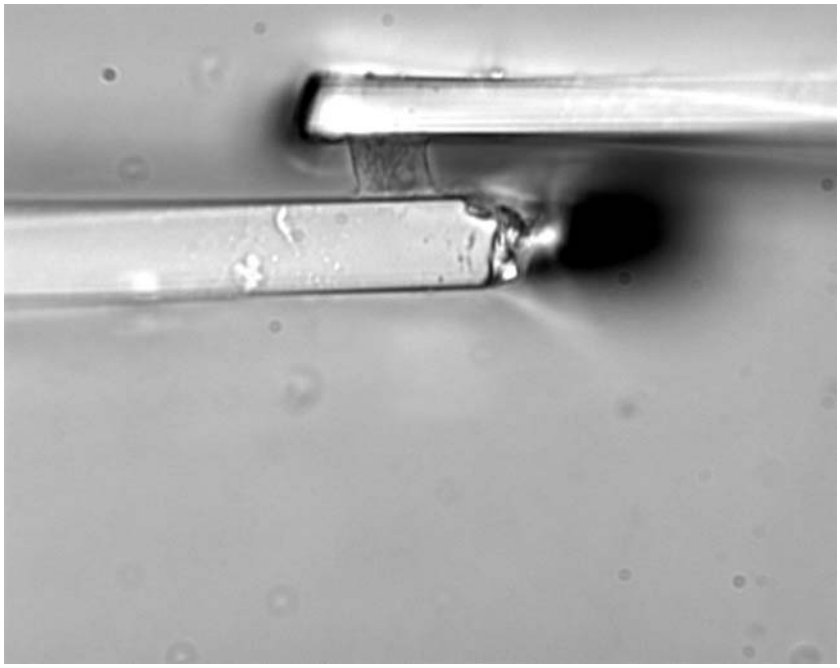
Figure S4: Diagram illustrating the effect of myosin recruitment. The cell contractile machinery is represented by contractile fibers (red lines) linking the microplates. With ongoing time and plate deflection (horizontal sequence, control parameter δ), the cell spreads between the plates. This is a slow process (typical time scale of tens of minutes as shown on $F(t)$ curves) where change in cell shape is necessarily associated with cytoskelatal remodeling. Myosin recruitment can occur as illustrated by the increased number of contractile fibers. Experiments with varying plate stiffnesses (vertical sequence), were performed at the same plate deflection and hence cell shape. The observation of a Hill-type F-V relationship is then in conflict with a stiffness-dependent myosin recruitment process, as illustrated here by the same number of contractile fibers for all stifness values.

Figure S5: F-V relationships measured at **(a)** $\delta = 0.1\ \mu m$ and **(b)** $\delta = 0.5\ \mu m$. Already at these early stages of cell spreading and contraction, $V(F)$ could be fitted following the Hill equation.



Movie S1. A single myoblast pulling on a plate of low stiffness (k = 21 nN=μm), bringing the plates into contact after ~12 min.

Movie S1 (AVI)



Movie S2. Feedback-controlled experiment to mimic the effect of an infinite plate stiffness on force generation. The position of a ~10 nN=μm flexible plate was controlled such that the plate-to-plate distance was constant regardless of cell force and plate deflection (5).

Movie S2 (AVI)

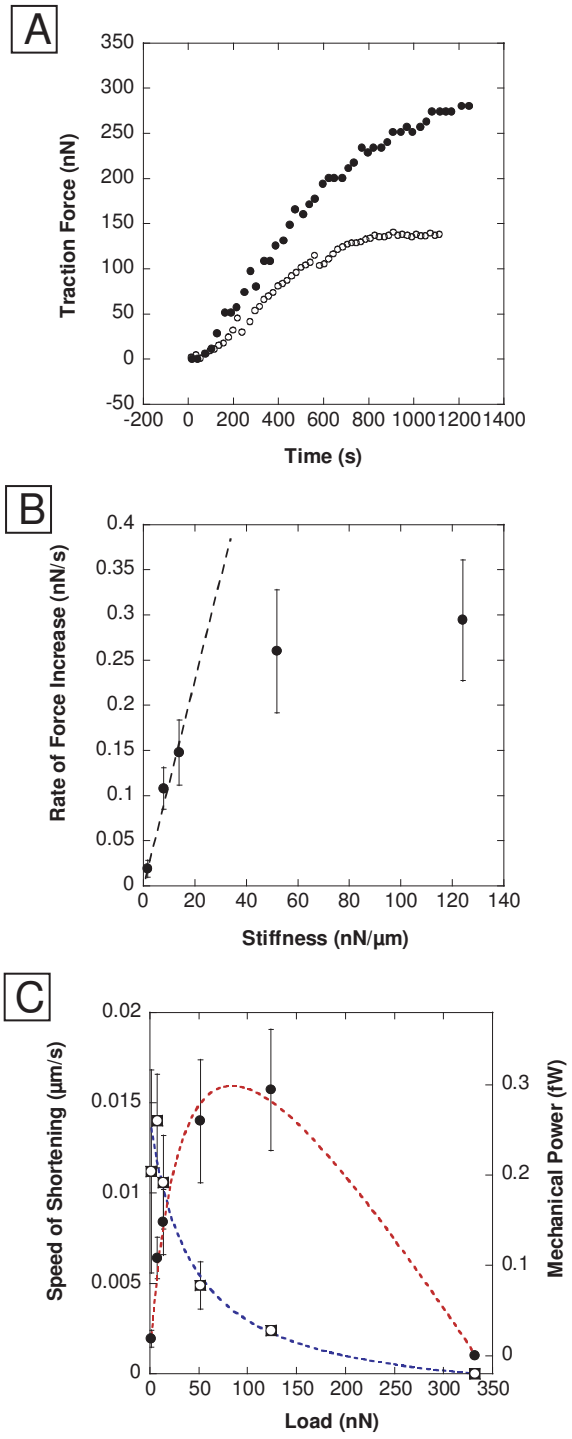

A
Traction Force (nN)
Time (s)
B
Rate of Force Increase (nN/s)
Stiffness (nN/µm)
C
Speed of Shortening (µm/s)
Mechanical Power (fW)
Load (nN)

Figure S1

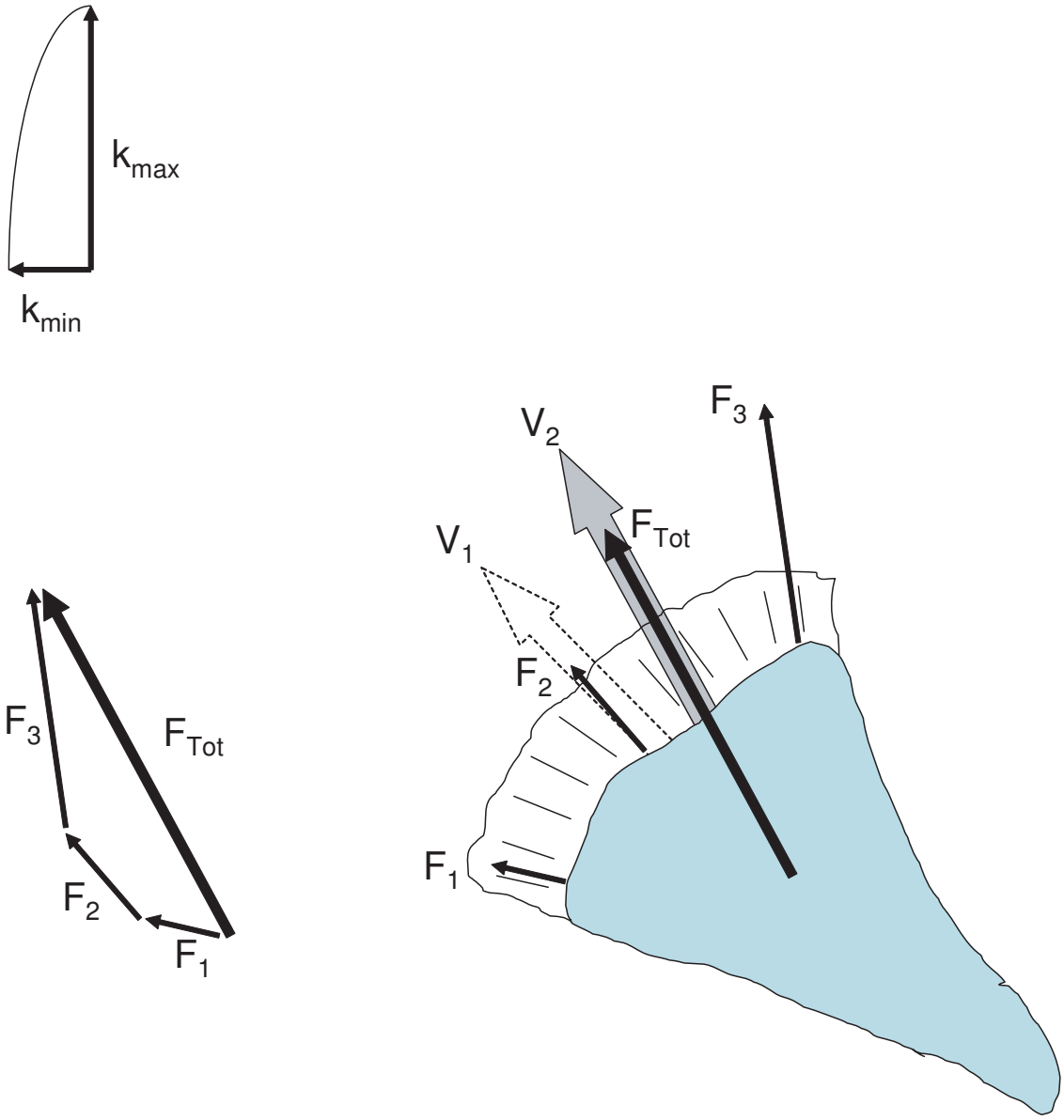


Figure S2

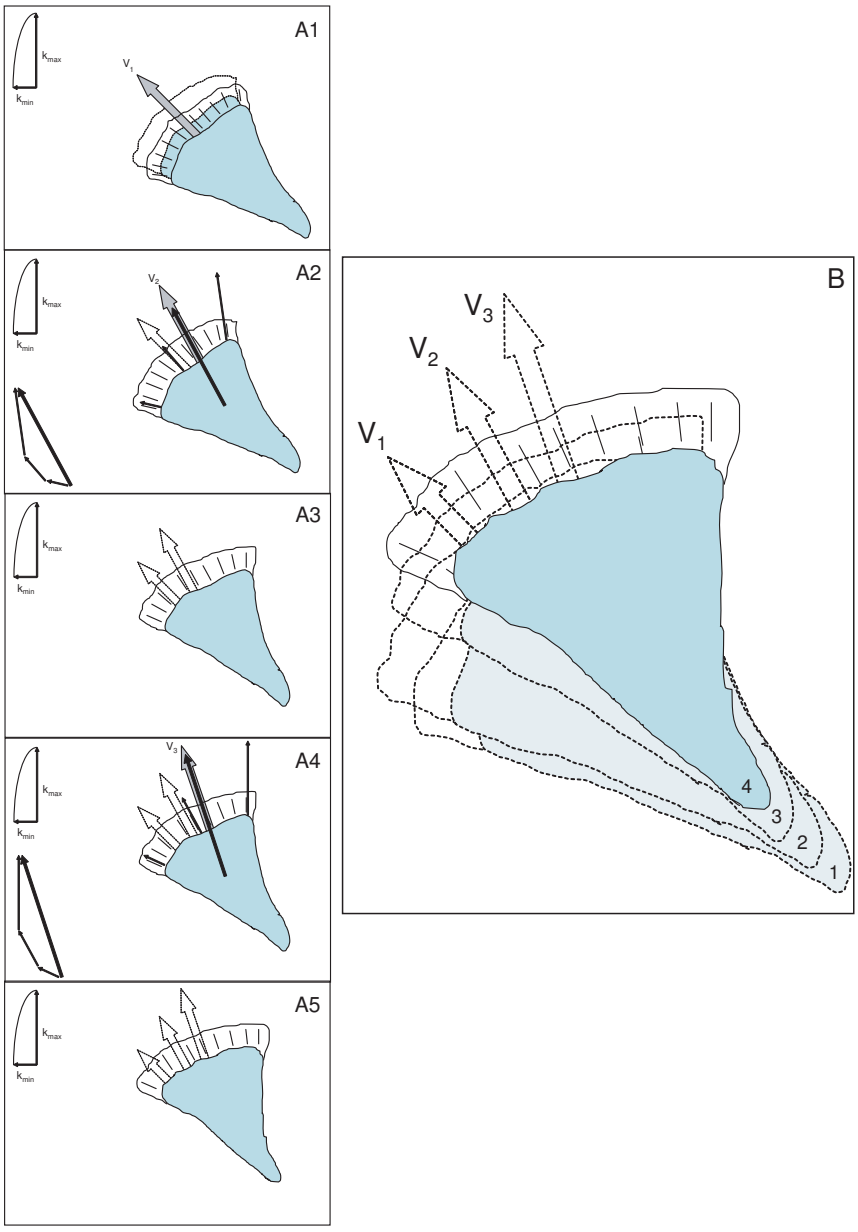


Figure S3

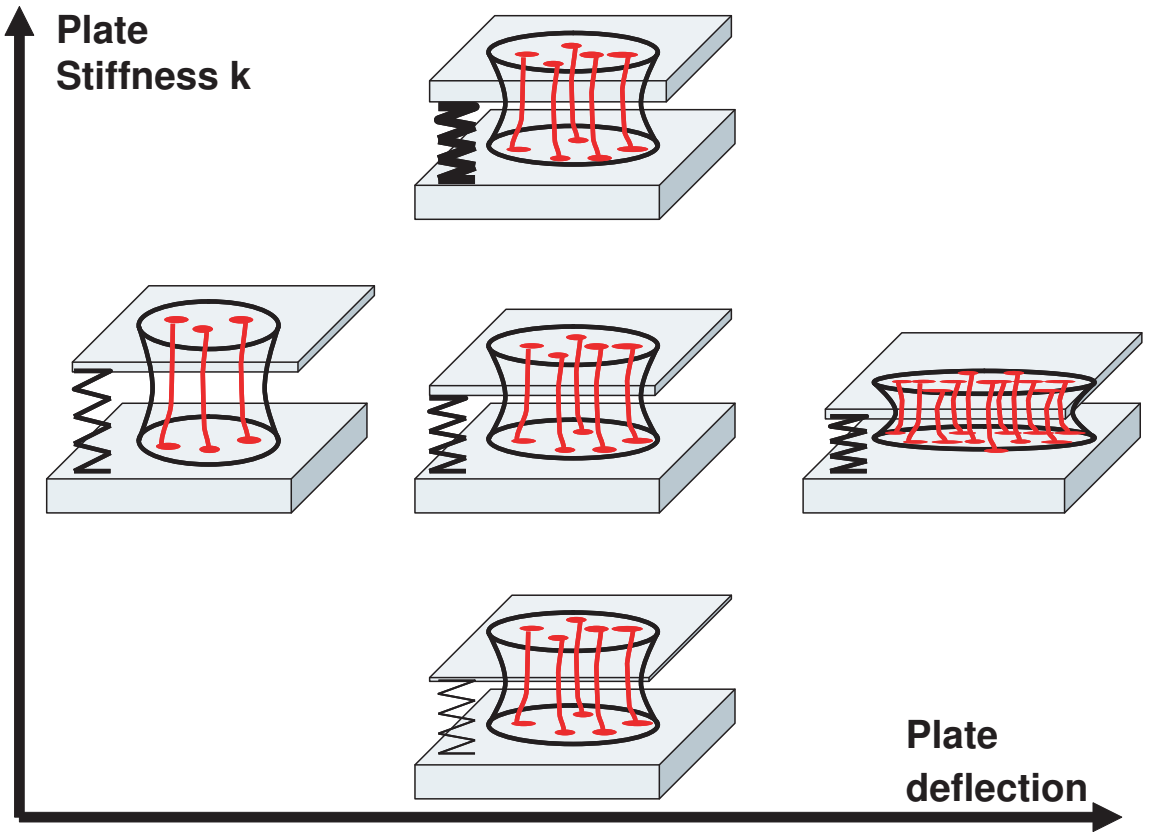


Figure S4

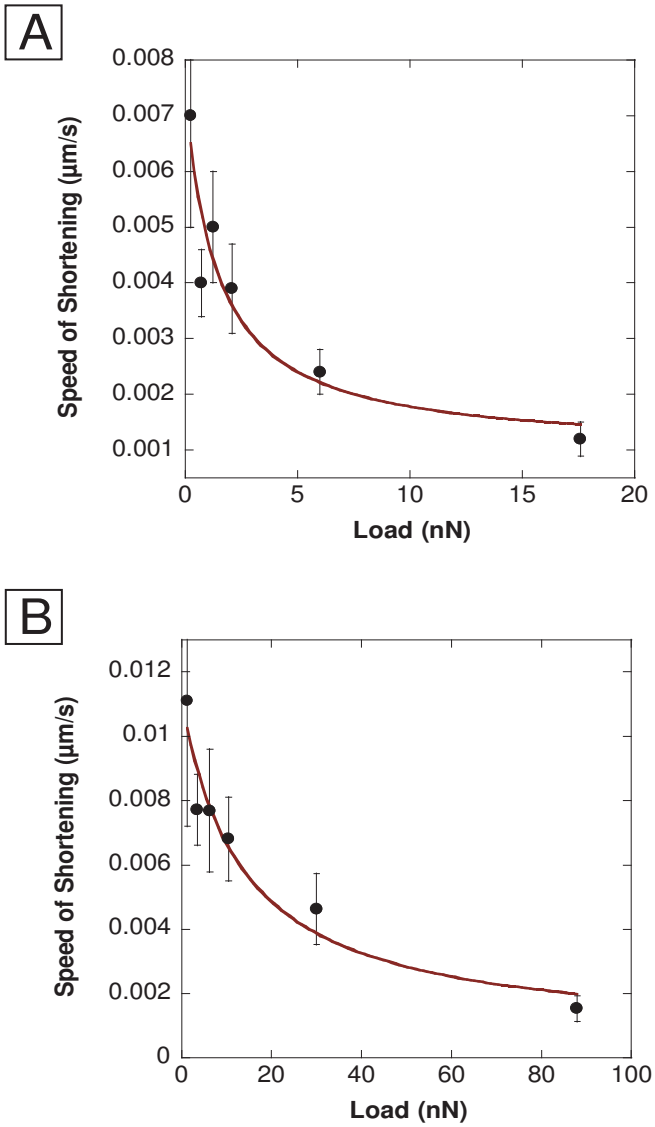

A
Speed of Shortening (µm/s)
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
5
10
15
20
Load (nN)
B
Speed of Shortening (µm/s)
0.012
0.01
0.008
0.006
0.004
0.002
0
0
20
40
60
80
100
Load (nN)

Figure S5