Supplementary Information

"How cells feel: stochastic model for a molecular mechanosensor"

Matteo Escudé, Michelle K. Rigozzi and Eugene M. Terentjev*

E-mail: emt1000@cam.ac.uk

Figure 1 reminds the reader about the construction of the model. The pictorial of the sequence of elements in the adhesion center illustrates the idea of how the TGF-β signal might be sent to the cell, but it also highlights the 'paradox'. On the short timescales of Brownian motion, at which the 'decision' about breaking or preserving the latent complex is made, there is no coherent motion in the overdamped macromolecular system – and therefore the force F is transmitted along the whole series of elements. This means that, whether the soft substrate deforms by this pulling force (as shown in the picture) or a stiffer substrate stays in its original position, the force acting on the latent complex is always F – and there could be no sensitivity to the degree of the above deformation (which is indeed measured by the stiffness κ).

The scheme in the right panel of Figure 1 illustrates the mechanical elements. Again, on a much longer time scales both the cell and the substrate may experience creep (irreversible deformation). However, on the time scales relevant to our problem, that is, when the Kramers-like 'escape' over the potential barrier $U(x)$ signifies the spontaneous breaking of the latent complex and the release of signalling TGF-β, both elements are elastic – in the sense that they each have a fixed equilibrium value of deformation induced by an external force. Of course, both elements must also have the energy dissipation (friction) mechanism to balance the energy input from the thermal motion (fluctuation-dissipation theorem), which is expressed by the friction constants γ_1 and γ_2.

*To whom correspondence should be addressed

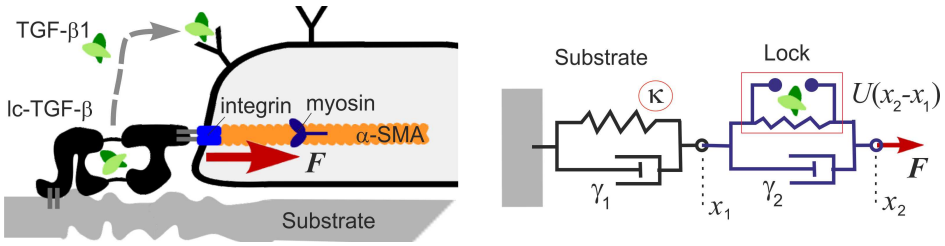

Figure 1: The reminder of the key points of the model: [left] the qualitative explanation for the release of active TGF-β signalling to the cell about the properties of the substrate; [right] the series of Voigt-like models describing the substrate (stiffness κ, friction constant γ_1 displacement of the binding point x_1) and the latent complex with the friction γ_2 and displacement x_2 controlled by the "lock" potential $U(x_2-x_1)$.

Equations (1) and (2) of the main paper express the Langevin dynamics of these two elements in series, as measured at the points measured by x_1 and x_2.

There are several ways of converting the microscopic stochastic problem described by Langevin dynamics into the kinetic equation(s) for the probability distributions. We follow the method summarized by Graham, which starts by expressing the Langevin equations for independent variables $q_\nu(t)$ in the 'standard' form:

$$\dot{q}_\nu = f_\nu(q) + g^i_\nu(q)\zeta_i(t), \tag{1}$$

where $f_\nu(q)$ are the corresponding dynamic forces and $\zeta_i(t)$ the stochastic force normalized to unity: $\langle \zeta_i(t)\zeta_j(t')\rangle = \delta_{ij}\delta(t-t')$ so that the actual intensity of the relevant stochastic force is expressed by a coefficient $g^i_\nu(q)$. In our case, for the two fluctuating variables $x_1(t)$ and $x_2(t)$ these parameters are quite simple:

$$f_1(x_1,x_2) = \frac{1}{\gamma_1}\left[-\kappa x_1 + \frac{\mathrm{d}U}{\mathrm{d}(x_2-x_1)}\right], \qquad f_2(x_1,x_2) = \frac{1}{\gamma_2}\left[-\frac{\mathrm{d}U}{\mathrm{d}(x_2-x_1)} + F\right]; \tag{2}$$

$$g_1 = \sqrt{2k_BT/\gamma_1}, \qquad g_2 = \sqrt{2k_BT/\gamma_2}. \tag{3}$$

Remaining in the overdamped limit (i.e. at timescales much greater than the characteristic relaxation time m/γ) allows us to dispense with the dependence on the corresponding velocity space in

the full Fokker-Planck formalism, and obtain directly:

$$\frac{\partial P(x_1,x_2,t)}{\partial t} = -\frac{\partial}{\partial x_1}\left[f_1(x_1,x_2)\cdot P\right] - \frac{\partial}{\partial x_2}\left[f_2(x_1,x_2)\cdot P\right] + k_B T\left[\frac{1}{\gamma_1}\frac{\partial^2}{\partial x_1^2} + \frac{1}{\gamma_2}\frac{\partial^2}{\partial x_2^2}\right] P.$$

The corresponding expressions for the diffusion currents take the explicit form:

$$J_1 = \frac{1}{\gamma_1}\left[-\kappa x_1 + \frac{\mathrm{d}U}{\mathrm{d}(x_2-x_1)} - k_B T\frac{\partial}{\partial x_1}\right] P, \qquad J_2 = \frac{1}{\gamma_2}\left[-\frac{\mathrm{d}U}{\mathrm{d}(x_2-x_1)} + F - k_B T\frac{\partial}{\partial x_2}\right] P. \quad (4)$$

In their current form it is not possible to write the components J_1 and J_2 as a single two-dimensional vector, because they are written with nominally different diffusion constants: $k_B T/\gamma_1$ and $k_B T/\gamma_2$. This issue can be overcome by scaling the variables: $x_1 = \sqrt{\gamma_2/\gamma_1}\tilde{x}_1$, $x_2 = \sqrt{\gamma_1/\gamma_2}\tilde{x}_2$. This allows us to write the current in general vector form:

$$\tilde{J}_i = -\frac{k_B T}{\sqrt{\gamma_1\gamma_2}} e^{-\tilde{V}_E/k_B T}\frac{\partial}{\partial \tilde{x}_i}\left(e^{\tilde{V}_E/k_B T} P\right) \qquad i = 1,2\,, \quad (5)$$

with a unique diffusion coefficient $\tilde{D} = k_B T/\sqrt{\gamma_1\gamma_2}$ and an effective potential acting in the plane (x_1,x_2): $V_E(x_1,x_2) = \frac{1}{2}\kappa x_1^2 - F x_2 + U(x_2-x_1)$. This effective potential surface represents the landscape over which the substrate and complex particles move, subject to collisions caused by thermal motion in the medium.

To apply the analysis to a real physical system, it is necessary to express $U(u)$, with the relative stretching of the 'lock' $u = x_2 - x_1$, in a particular functional form. In the 60 years past Kramers original work, many such forms were tried, with a great variety of barrier shapes. It is, however, clear that only two key features of such a potential are relevant: the distance u_0 of the barrier from the position of metastable minimum – and the barrier height Δ; in contrast, the shape of the potential around the minimum and around the barrier only contribute in a minor way to the pre-exponential factors. In recent years it becomes more and more common to use a cubic function that has a simple and explicit representation of the two mentioned key features, and also naturally excludes the possibility of rebinding by falling to negative infinity for large u. The form is adjusted

so that the minimum (point A) is at $u = 0$ and the maximum (point C) at $u = u_0$:

$$U(u) = \frac{3}{2}\Delta\left(\frac{u}{u_0} - \frac{1}{2}\right) - 2\Delta\left(\frac{u}{u_0} - \frac{1}{2}\right)^3 + \frac{\Delta}{2}. \tag{6}$$

When the external force F and the spring potential of the elastic substrate are added in the effective potential $V_E(x_1, u)$, we find that it has two extrema at:

$$x_1^* = \frac{F}{\kappa}, \qquad u_\pm^* = \frac{1}{2}u_0\left(1 \pm \sqrt{1 - \frac{2}{3}\frac{Fu_0}{\Delta}}\right). \tag{7}$$

The solution u_+^* corresponds to the saddle point (i.e. the barrier the systems needs to overcome) and the solution u_-^* marks the minimum of the two-dimensional well; the two extrema have the same x_1 coordinate; for $F_C = 3\Delta/2u_0$ the two extrema coincide so there is no longer any energy barrier to hold the latent complex together. The effective barrier height, Δ_E, is defined as the difference in potential between the minimum of the well and the saddle point: $\Delta_E = \Delta(1 - 2Fu_0/3\Delta)^{\frac{3}{2}}$. This expression, alongside the probability currents, are the starting point for the application of the Kramers theory.

Applying the Kramers theory to generalized multidimensional problems causes several computational problems; for this reason it is usually more convenient to reduce the system to a one-dimensional barrier escape. In our context this means we identify the path taken by the system in the effective potential landscape. We stay close to the original approach of Kramers, but differ in the technique used to evaluate the integrals for the path. Let us make the path dependent on a new single variable, u for convenience, which coincides with the previously defined $u = x_2 - x_1$ when $F = 0$. Starting with the assumption of steady current, we may write for the path A $\rightarrow$ C $\rightarrow$ B:

$$J\int_A^B \frac{\gamma}{k_B T} e^{V_E/k_B T}\,\mathrm{d}u = \left(P e^{V_E/k_B T}\right)\Big|_B^A. \tag{8}$$

Taking the potential at the point to which the particles 'escape' (B) to be such that $V(u_B) \sim -\infty$, which is consistent with the rapidly decreasing cubic describing $U(u)$, the expression for the con-

stant probability current can be simplified:

$$J = \frac{P(u_A)\mathrm{e}^{V_E(u_A)/k_BT}}{\int_A^B \frac{\gamma}{k_BT}\mathrm{e}^{V_E/k_BT}\mathrm{d}u}. \tag{9}$$

Moving $\mathrm{e}^{V_E(u_A)/k_BT}$ from the numerator to the denominator, the integrand of this expression becomes $I(u) = \mathrm{e}^{(V_E(u)-V_E(u_A))/k_BT}$. To approximate the value of this integral the original solution by Kramers uses the method of saddle-point integration, approximating the exponent of the integrand with a second order polynomial. This has been generally followed in the literature since. However, this classical method has a problem in the region of $F \to F_C$, that is, near the point where the barrier disappears and the system has no restriction escaping from its originally metastable state (A). This problem has been known since Kramers himself, and is usually avoided merely by assuming low forces: in the saddle-point method one has to extend the integration region to infinity (which is normally safe, since the Gaussian exponential cuts the integrand to zero far away from the barrier crest). So, although the 'curvature' $V_E''(u_C)$ goes to zero, actually as $\sqrt{F_C - F}$, the error of this saddle-point approximation increases. If one follows the classical recipe literally, it leads to the completely wrong result that $J \to 0$ as $F \to F_C$. Over the years, there were several much more accurate treatments of this problem, but we choose our own (as it seems all these methods are all worth each other: all converging to the classical Kramers result at $F \to 0$, while leading to the diverging flux at $F \to F_C$, in slightly different ways).

Our method is to fit a second order polynomial to the integrand $I(u)$ itself, integrating it from A to C, and then doubling this to approximate the value of the integral from A to B. The chosen polynomial shares its maximum with the maximum $I(u_C)$ and also passes through the minimum $I(u_A)$. The fitting parabola is given by $I(u) \approx -\left(I(u_C) - I(u_A)\right)(u-u_C)^2/(u_C-u_A)^2 + I(u_C)$, which may be substituted in the expression for the integrand:

$$\begin{aligned}\int_A^B \mathrm{e}^{(V(u)-V(u_A))/k_BT}\mathrm{d}u &\approx 2\int_A^C -\frac{\mathrm{e}^{(V(u_C)-V(u_A))/k_BT}-1}{(u_C-u_A)^2}(u-u_C)^2 + \mathrm{e}^{(V(u_C)-V(u_A))/k_BT}\mathrm{d}u \\ &= \frac{2}{3}(u_C-u_A)\left(1+2\mathrm{e}^{(V(u_C)-V(u_A))/k_BT}\right).\end{aligned} \tag{10}$$

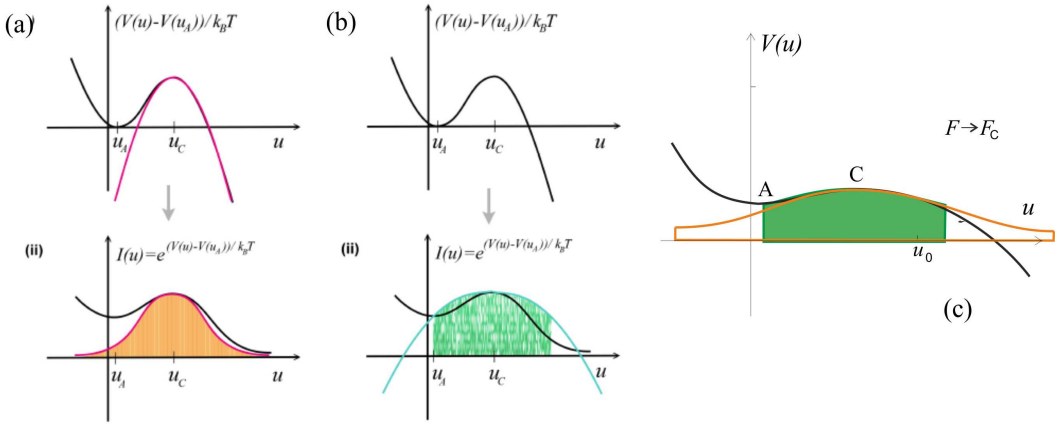

Figure 2: Comparison between the classical saddle-point integration (a) and the method of integration via model parabola used here (b): The exponent of the integrand (a)(i) is approximated by a second-order polynomial (shown in pink). In contrast, the integrand itself (b)(ii) is approximated in the method used here; the chosen second-order function shares the maxima of the integrand at u_C, passes though the functionŠs minima at u_A (shown in green), and the integral is taken over twice the area from u_A to u_C, indicated by the shaded region. (c) The limit of $F \to F_C$ illustrates the difference more explicitly, showing how the integral of the saddle-point approximation diverges, while the approximating by parabola with a fixed integration width gives a plausible result.

Here the distance between the extrema is $(u_C - u_A) = u_0\sqrt{1-2Fu_0/3\Delta}$ and the remaining energy barrier is expressed by $V(u_C) - V(u_A) = \Delta(1-2Fu_0/3\Delta)^{3/2}$ for our chosen potential energy. This expression for the integral in denominator may now be put back to obtain the probability flux:

$$J = \frac{k_B T}{\gamma} P(u_A) \frac{3}{2} \left[(u_C - u_A) \left(1 + 2\mathrm{e}^{(V(u_C)-V(u_A))/k_B T} \right) \right]. \tag{11}$$

The escape rate k is given by the flux J normalized by the numbers of particles in the well at A, ν_A. Quasi-stationary conditions are assumed, requiring the barrier to be large compared to thermal fluctuations ($\Delta \gg k_B T$). We are therefore justified to assume the Maxwell-Boltzmann distribution is valid in the neighborhood of (A), again following the classical Kramers analysis:

$$\mathrm{d}\nu_A = P(u_A)\mathrm{e}^{-V_E/k_B T}\,\mathrm{d}u, \tag{12}$$

which may be integrated to find the number of particles. Approximating the potential about the potential minimum at u_A by a Taylor series to second order, the number of particles may be ap-

proximated to

$$\nu_A = \frac{P(u_A)}{\omega_A}\sqrt{2\pi k_B T}\, e^{F^2/2\kappa k_B T}, \tag{13}$$

where ω_A is the curvature of the potential well at the minimum (A), while the exponential factor is a reminder that the depth of this minimum is no longer at $V_E = 0$ – which merely expresses the fact that the system states would accumulate more densely in a deeper energy minimum of the stretched substrate. We are now in the position to write down an expression for the ratio of the rate with a force a applied, $k(F)$, to the initial rate with no force, k_0, which frees the expression from constant factors:

$$\frac{k(F)}{k_0} = \frac{\omega_A(F)}{\omega_A(0)} e^{-F^2/2\kappa k_B T}\, \frac{u_{C0} - u_{A0}}{u_{CF} - u_{AF}}\, \frac{1 + 2e^{(V(u_{C0}) - V(u_{A0}))/k_B T}}{1 + 2e^{(V(u_{CF}) - V(u_{AF}))/k_B T}}. \tag{14}$$

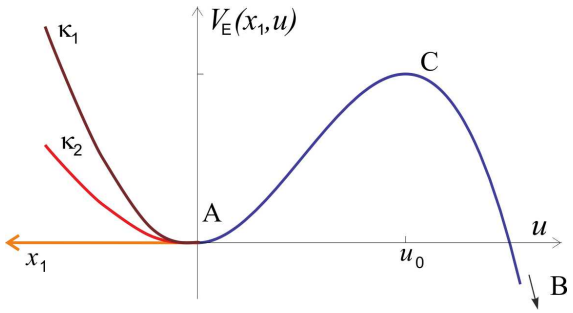

Figure 3: The illustration of a two-sided minimum of the effective potential $V_E(x_1,u)$, with the descent towards the minimum (nearly) along the x_1 direction has the curvature $\omega_{A1} = \sqrt{\kappa}$, while the climb towards the barrier along the u-direction has the curvature ω_{A2} given earlier in the text.

Whilst the positions and values of the potential at the different points are readily calculated from the previously obtained expressions, the curvature term ω_A requires further attention. The curvature we seek to find is composed by a first segment O $\rightarrow$ A and a second segment A $\rightarrow$ C, see Fig.3(b) in the main paper. To first order the curvature of the first segment will be simply $\omega_{A1}^2 = \kappa$, which may be visualized intuitively since, when working with forces safely below the critical, the path follows closely the x_1 axis. The curvature of the path A $\rightarrow$ C, when evaluated at the bottom of the well, may be taken to be equivalent to the modulus of the value of the second derivative of

the potential $U(u)$ at that point:

$$\omega_{A2}^2 = \frac{6\Delta}{u_0^2}\sqrt{1-\frac{2}{3}\frac{Fu_0}{\Delta}}. \tag{15}$$

It is therefore possible to approximate the total curvature ω_A^2 (which controls the result of the integration around u_A) as an average of these two values weighted by distance. That is, the weight of the first curvature will be the distance from O to A (F/κ to first order), while the weight of the second will be the distance from A to C, given above. As a result we obtain:

$$\omega_A^2 = \frac{6\kappa\Delta\left(1-\frac{1}{2}\frac{Fu_0}{\Delta}\right)}{u_0\left(F+\kappa u_0\sqrt{1-\frac{2}{3}\frac{Fu_0}{\Delta}}\right)}. \tag{16}$$

With this last piece in place we may proceed to substituting all the components into $k(F)=\frac{J}{v_A}$ to obtain:

$$k(F) = \frac{9\tilde{D}\Delta}{2\sqrt{2\pi k_B T}u_0^3}\frac{\mathrm{e}^{-F^2/2\kappa k_B T}\sqrt{1-2Fu_0/3\Delta}}{\left(1+2\exp\left[(1-2Fu_0/3\Delta)^{3/2}\Delta/k_BT\right]\right)\left(F/\kappa u_0+\sqrt{1-Fu_0/3\Delta}\right)} \tag{17}$$

As mentioned earlier, it is often considered useful to present the ratio of the breaking rate $k(F)$ and its 'bare' value at zero force. In doing so several constant factors cancel (in particular, the effective diffusion coefficient) and the expression allows the quick examination of the effects of the pulling force:

$$\frac{k(F)}{k_0} = \frac{\mathrm{e}^{-F^2/2\kappa k_B T}\sqrt{1-2Fu_0/3\Delta}\,(1+2\exp\left[\Delta/k_BT\right])}{\left(1+2\exp\left[(1-2Fu_0/3\Delta)^{3/2}\Delta/k_BT\right]\right)}. \tag{18}$$

Examining both expressions we see the natural dimensional parameters measuring the external pulling force relative to the characteristic returning force of the lock (the latent complex): $\tilde{f}=Fu_0/\Delta$, and similarly – the substrate stiffness: $\tilde{\kappa}=\kappa u_0^2/\Delta$. Similarly, the escape rate of the latent complex has its own natural units, making the non-dimensional measure $\tilde{k}=k\cdot 2\sqrt{2\pi k_BT}u_0^3/9\tilde{D}\Delta$. Of course, the non-dimensional ratio Δ/k_BT is the measure how strongly confined the latent complex is, and since there is no (or very little) spontaneous 'leakage' of TGF-β from it, we expect $\Delta/k_BT \gg 1$.

Two simplified expressions are presented and explored in the main text. One is simply the expansion of $k(F)$ in the limit of weak force ($\tilde{f} \ll 1$), however, retaining the key exponential factor $\exp[-F^2/2\kappa k_B T]$. The other expression is 'reconstructed' to interpolate a simple formula into the weak substrate limit ($\tilde{\kappa} \ll 1$) by lifting the linear expressions of the nature $(1-a)$ into the exponential form e^{-a}:

$$k \approx \frac{9\tilde{D}\Delta}{2\sqrt{2\pi k_B T u_0^3}}\, \mathrm{e}^{-(\Delta - F u_0)/k_B T}\, \mathrm{e}^{-F/\kappa u_0}\, \mathrm{e}^{-F^2/2\kappa k_B T}. \tag{19}$$