Supplemental Information

Focal Adhesion Kinase: The Reversible Molecular Mechanosensor

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THE ROLE OF DOMAIN BONDING STRENGTH

We have commented in the main text that there is no accurate knowledge on the strength of interdomain bond for FERM-kinase linkage (which is known to contain a hydrophobic cluster composed of residues Y180, M183, N193, V196, and F596, an additional salt bridge D200-R598, and an electrostatic bond at E182, R184, K190 and N595, N628, N629, E636). The literature give the upper bound of this energy: $\Delta G_\text{o} \approx 28.5 k_B T$, and a lower bound: $\Delta G_\text{o} \approx 11 k_B T$, which has led us to take a median value of $17 k_B T$ in the main text. Here we shall present the alternative results for the two bound values of ΔG_o for comparison and discussion. Let us emphasize – we do not believe either bound is realistic, and consider the results presented in the main text valid.

The illustrations given in Figs. 1, 2, 3 show how sensitive the biologically meaningful response is to the value of interdomain bonding strength. In either case of strong bond, or weak bond, the rate of FAK opening (given by the universal model described in the main text) changes with the applied pulling force – or the changing substrate stiffness for a given force – in an entirely unreasonable way. For the strong bond, the overall magnitude of the K_+ rate is very low, making a single FAK molecule activating in several seconds – this, before even considering the re-folding rate K_- that would certainly prevent any activation dynamics. For the weak interdomain bond, we find (perhaps counterintuitively, until one considers the real physical reason for the [c]→[o] transition) that only very stiff substrates (bone, plastics and above) would give any sensitivity to the FAK complex – in the range where we know the cells respond most to the stiffness changes, this model predicts no response at all: just a uniform random opening of the weakly-bonded domains. As a result, we have concluded that the discussion presented in the main text represents the most biologically meaningful scenario.
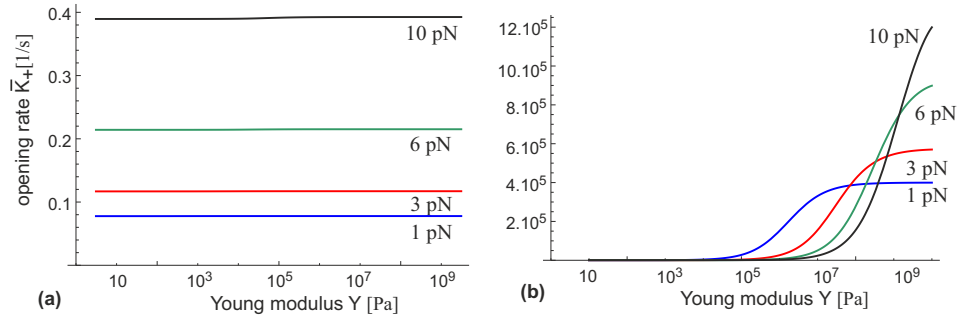

FIG. 1. The rate constant of the [c]→[o] transition $K_+(f, \kappa)$ plotted against the substrate stiffness (on logarithmic scale), for several values of the pulling force f corresponding to the plots in the main text. Plot (a) is for $\Delta G_0 = 28.5 k_B T$; plot (b) is for $\Delta G_0 = 11 k_B T$. Note the very low overall rates of opening, and a complete lack of mechanosensitivity for the strong interdomain bonding (a), and a regime of sensitivity shifted to unreasonably high stiffness for the low bonding energy in (b).
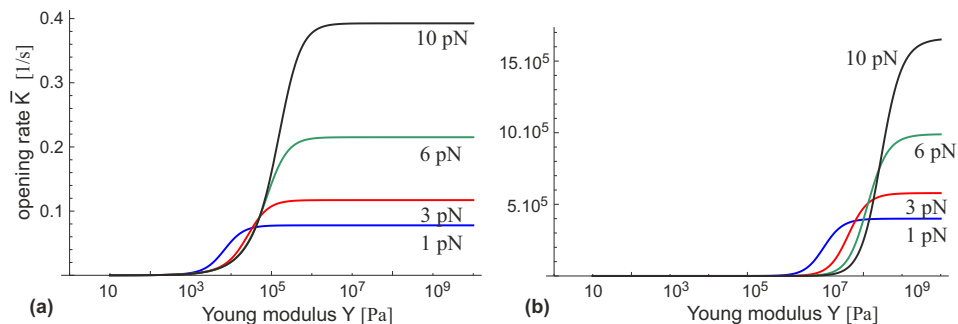
FIG. 2. The same 'sensor' plots as in Fig. 1, for $\Delta G_0 = 28.5 k_B T$ (a), and for $\Delta G_0 = 11 k_B T$ (b). This time we took a constant value of the substrate damping coefficient $\gamma_{\mathrm{sub}} = 0.01$ kg/s, so that the ratio $\zeta = \gamma_c/\gamma_{\mathrm{sub}} \approx 10^{-7}$ is fixed. Although the sensitivity is recovered, overall rates of opening are still extremely low for the strong interdomain bonding, while there is very little change of only high-stiffness sensitivity for the low bonding energy.
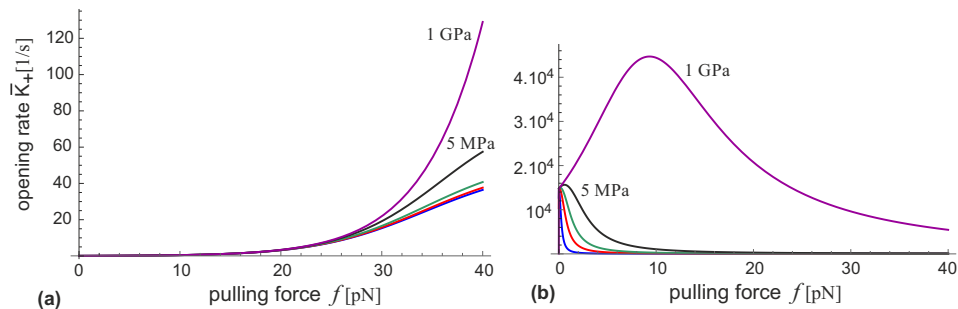

FIG. 3. The rate constant of FAK opening $K_+(f, \kappa)$ is plotted as a function of the pulling force f, for several values of substrate stiffness labelled on the plot. Again we compare the strong interdomain bond with $\Delta G_0 = 28.5 k_B T$ (a) with the weak bond for $\Delta G_0 = 11 k_B T$ (b). In the first case, we see that there is very little sensitivity, and very high forces required to achieve any FAK activation. In the weakly-bonded case, the 'sensor' responds with a negative feedback (effectively shutting itself down) for all substrates except the most stiff glass.

VISCOELASTICITY AND STRESS RELAXATION

Viscoelasticity is a broad and well-studied subject. However, because of the large literature available, sometimes different ideas get confused; in particular this is the case with the subject of stress relaxation. There are two very different effects: the long-time macroscopic relaxation of stress after e.g. a step-deformation was imposed at $t = 0$, and the internal relaxation of local fluctuations that is a much shorter-time process. The first effect is very easy to measure with simple equipment, and many rubbery and gel materials have a very long relaxation time: of hours if not days. This is a result of complex many-body correlation of individual relaxation processes, describing internal re-arrangement of chains and whole clusters. However, this is not what controls the damping of local fluctuations that determines the parameter γ_{sub} we need to estimate. This local damping is determined by an internal relaxation time (which we called $\tau_{\mathrm{sub}} = \gamma_{\mathrm{sub}}/\kappa$) and it manifests itself in a characteristic peak of the loss modulus (and the associated steepest-gradient point of the storage modulus). In simple viscoelastic models (such as Maxwell, Voigt, or Zener models) this internal relaxation is the only process, which directly enters into the model expressions for the frequency-dependent real and imaginary parts of the complex viscoelastic modulus Y^* (which is a function of non-dimensional product $\omega \tau_{\mathrm{sub}}$). Note that we are dealing with the Young modulus here, to make a better link with the arguments in the main text, while most of the literature on viscoelastic rheology presents the shear modulus $G^*(\omega)$ instead.

Our problem is that we wish to compare very different substrate materials, across a vast range of their equilibrium stiffness. Figure 4 shows the characteristic rheological traces for several typical materials (some data is taken from the literature, some measured by ourselves). In all cases, the regime $\omega \to 0$ gives the equilibrium Young modulus of the material, as labelled in the plots. The softest of all materials we could have access to was a colloidal gel, with $Y_0 = 40$ Pa (such a low modulus would not be able to support a droplet bigger than 1mm against gravity). We also used a very weakly crosslinked silicone elastomer with a low $Y_0 = 2$ kPa (which is a typical stiffness of a glial tissue; a typical muscle would have $Y_0 \geq 10$ kPa). Then we see a viscoelastic response of a typical rubber with the equilibrium
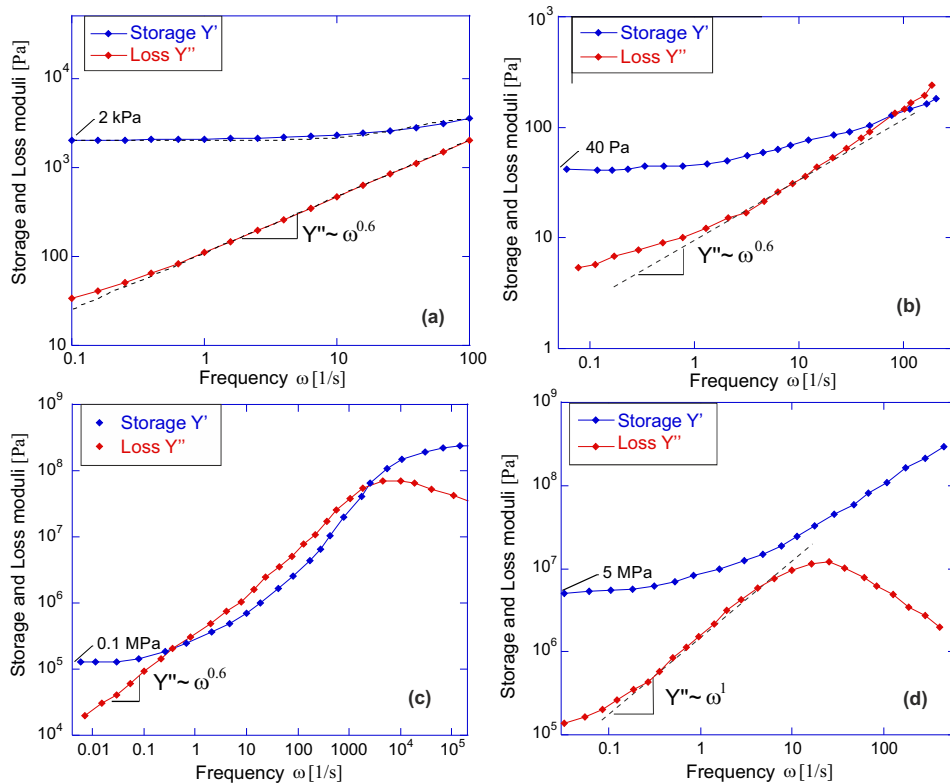
FIG. 4. Storage and loss moduli of viscoelastic materials: (a) a soft elastomer from weakly crosslinked Sylgard 527, (b) a very soft colloidal gel [ref: colloidal gel], (c) a typical rubber [A. M. Squires, A. R. Tajbakhsh, and E. M. Terentjev: *Dynamic Shear Modulus of Isotropic Elastomers*, Macromolecules **37**, 1652, 2004], and (d) PMMA plastic – all measured at room temperature. The plots illustrate the characteristic low-frequency regime: the equilibrium modulus Y_0, and the frequency-scaling of the loss modulus Y'' (labelled in each plot). The peak of Y'' marks the characteristic relaxation time we named τ_{sub}; in plots (a) and (b) the peak is not in the experiment range, so we had to fit the data to a viscoelastic model depending on $\omega\tau_{\mathrm{sub}}$.

stiffness $Y_0 = 100$ kPa (a bit higher than pre-calcified bone), and a PMMA plastic with $Y_0 = 5$ MPa. We do not give a measurement for the mineral glass, where $Y_0 > 1$ GPa is normally found.

The analysis in Fig. 4 gives a remarkably consistent value of the internal relaxation time, in spite of the vast difference in types of materials. In the two systems we did not have the peak of the loss factor explicitly within experimental range, the fitting of the basic model was used: the loss modulus $Y'' = Y' \cdot (\omega\tau_{\mathrm{sub}})^x$ at low frequency, using the independently determined scaling exponent x in each plot (which, in turn, has shown a remarkable consistency). In the two plots where the peak of loss modulus is evident, we simply take $\tau_{\mathrm{sub}} = 1/\omega_{\mathrm{peak}}$. The result is that in plots (a) and (b) we had $\tau_{sub} = 0.007$ s, and 0.011 s, respectively. In stiffer materials we had $\tau_{sub} \approx 0.001$ s in rubber, and 0.05 s in PMMA plastic. Combined with the literature data on the β-relaxation time in glass being also ~ 0.01 s (see main text), we follow a bold assumption, taking the substrate relaxation time $\tau_{\mathrm{sub}} \approx 0.01$ s always, and then calculating the damping constant $\gamma_{\mathrm{sub}} = \tau_{\mathrm{sub}}\kappa$ in all our analysis. It is certainly not perfect, but hopefully the arguments above explain that the values thus obtained are never far from accurate.