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Supplemental Information

Kinetic Ductility and Force-Spike Resistance of Proteins from Single-Molecule Force Spectroscopy

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Supporting Information: Kinetic ductility and force-spike resistance of proteins from single-molecule force spectroscopy

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Supporting Text

High Speed AFM

The theory of force-ramp experiments based on Main Text Eq. 3 is valid only when the pulling speed is so slow that the system can relax to equilibrium at each instant of time. In addition, the linkers must be sufficiently soft that the effect of the pulling apparatus is negligible. One can examine the influence of the linkers and of the AFM tip using the two-dimensional free energy surface [1]

$$G(x,q) = G_o(x) + \frac{1}{2}\kappa_L(q-x)^2 + \frac{1}{2}\kappa_S(vt-q)^2, \tag{S1}$$

where x and q are the molecular and measured extensions, respectively; $G_o(x)$ is the molecular free energy profile, v is the pulling velocity, κ_L and κ_S are the spring constant of the linker and cantilever, respectively. The dynamics is assumed to be diffusive and the diffusion coefficients D_x along x and D_q along q are in general different.

In a pulling experiment, the rupture force is commonly found from the extrapolated average force at rupture. When the pulling speed is slow then this rupture force can be approximated as $F = \kappa_s(vt - q_\cup(t))$ where t is the time at which rupture occurs and $q_\cup(t)$ is the force-dependent minimum (i.e.,

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the value of q for which $\partial G/\partial x = \partial G/\partial q = 0$). For larger pulling speeds a better estimate of the rupture force is to replace $q_\cup(t)$ by the average value of q, $\langle q(t)\rangle$ before rupture, so the observed force is $F_{\rm obs} = \kappa_S(vt - \langle q(t)\rangle)$. To find $\langle q(t)\rangle$, one can show, for example by averaging the underlying stochastic differential equations and using the fact the average of the random force is zero, that

$$\begin{array}{rcl} \frac{d\langle q(t)\rangle}{dt} & = & -\beta D_q \langle \frac{\partial G}{\partial q}\rangle, \\ \frac{d\langle x(t)\rangle}{dt} & = & -\beta D_x \langle \frac{\partial G}{\partial x}\rangle. \end{array} \tag{S2}$$

If we approximate $G_o(x)$ by a harmonic potential at its minimum (which we take as 0 without loss of generality) $G_o(x) = \frac{1}{2}G_o''(0)x^2 = \frac{1}{2}\kappa_M x^2$ then the differential equations for $\langle q(t)\rangle$ and $\langle x(t)\rangle$ are linear and can be solved analytically. For the initial conditions $\langle q(0)\rangle = \langle x(0)\rangle = 0$, we find that at long times $\langle q(t)\rangle = q_\cup(t) - \delta$ where the lag δ is

$$\delta = \frac{v(\kappa_e^\cup)^2}{\beta\kappa_S}\left(\frac{1}{D_x\kappa_M^2} + \frac{1}{D_q\kappa_{ML}^2}\right), \tag{S3}$$

with $(\kappa_e^\cup)^{-1} = \kappa_S^{-1} + \kappa_M^{-1} + \kappa_L^{-1}$ and $\kappa_{ML}^{-1} = \kappa_M^{-1} + \kappa_L^{-1}$, so that the average value of q lags behind its minimum value. The measured rupture force can then be written as

$$F_{\rm obs}(t) \approx \kappa_S(vt - q_\cup(t)) + \kappa_S\delta, \tag{S4}$$

where δ is given by Eq. S3. The effective potential surface experienced by the molecular coordinate x at time t has its minimum, on average, at $x_\cup^e(t) = \kappa_L\langle q(t)\rangle/(\kappa_M + \kappa_L) = \kappa_L[q_\cup(t) - \delta]/(\kappa_M + \kappa_L)$. This shift in the minimum from $x = 0$ can be interpreted as the result of an effective force $F_{\rm eff}(t) = \kappa_M x_\cup^e(t)$ acting on the molecular coordinate x. The difference between the observed force $F_{\rm obs}(t)$ and this effective molecular force $F_{\rm eff}(t)$ is an approximate correction for drag effects,

$$\Delta F_{\rm drag} \equiv F_{\rm obs}(t) - F_{\rm eff}(t) = (\kappa_S + \kappa_{ML})\,\delta, \tag{S5}$$

where the time-dependent terms exactly canceled. If this drag correction $\Delta F_{\rm drag}$ is subtracted from the $F_{\rm obs}$ then our previous analysis [1] of the relations between the rate at constant force and the rate that determines the rupture force histogram via Main Text Eq. 3 is still valid within the framework of the quasi-adiabatic approximation. For stiff molecules ($\kappa_M \gg \kappa_L$)

that relax rapidly ($D_x \gg D_q$) the force correction term is

$$\Delta F_{\rm drag} \approx \frac{v}{\beta D_q}\left(1+\frac{\kappa_L}{\kappa_S}\right)^{-1}. \tag{S6}$$

However, for finite D_x/D_q and stiff apparatus and linkers, the drag correction in Eq. S5 can also exceed $v/\beta D_q$.

For very high speeds not only the quasi-adiabatic approximation breaks down but it becomes difficult to define the rupture force when the fluctuations in q are slow compared to the rupture time. The breakdown of the quasi-adiabatic approximation has been recently analyzed for the one dimensional harmonic-cusp potential [2]. It will be interesting to see if this analysis can be extended to two dimensional free energy surfaces as given in Eq. S1.

Supporting Figures

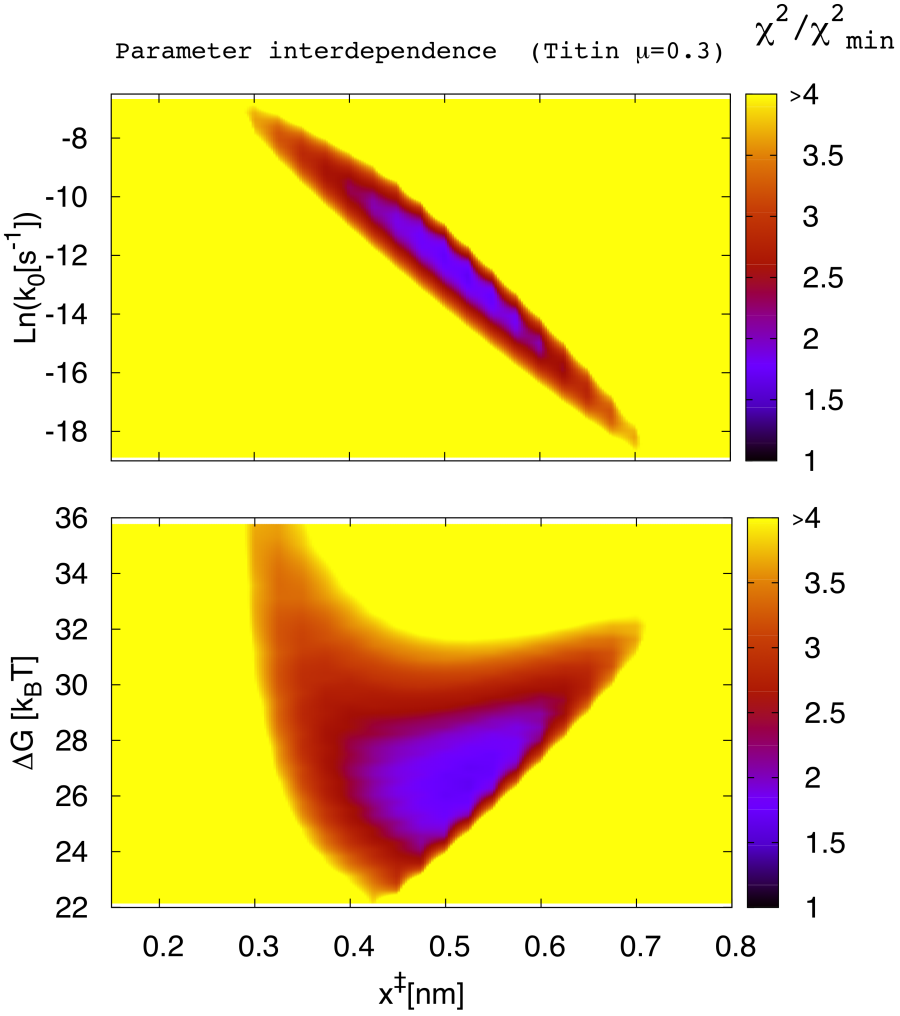

Figure S 1: **Parameter interdependence in unrestricted fits to hsAFM [3] data for titin I91 domain.** For μ=0.3, the relative χ^2 is shown as a function of $\ln(k_0[\mathrm{s}^{-1}])$ and $x^{\ddagger}$[nm] (top), and of $\Delta G^{\ddagger}[k_BT]$ and $x^{\ddagger}$[nm] (bottom). Best fits exhibit a clear linear interdependence with $\ln(k_0[\mathrm{s}^{-1}])/x^{\ddagger}[\mathrm{nm}] \sim -26$, for $10^{-10} \leq k_0 \leq 10^{-3}$ s^{-1}, and $0.3 \leq x^{\ddagger} \leq 0.7$ nm. The parameter interdependence can be reduced by using additional information, such as restricting k_0 to the bulk unfolding rate 4.9×10^{-4} s^{-1}.

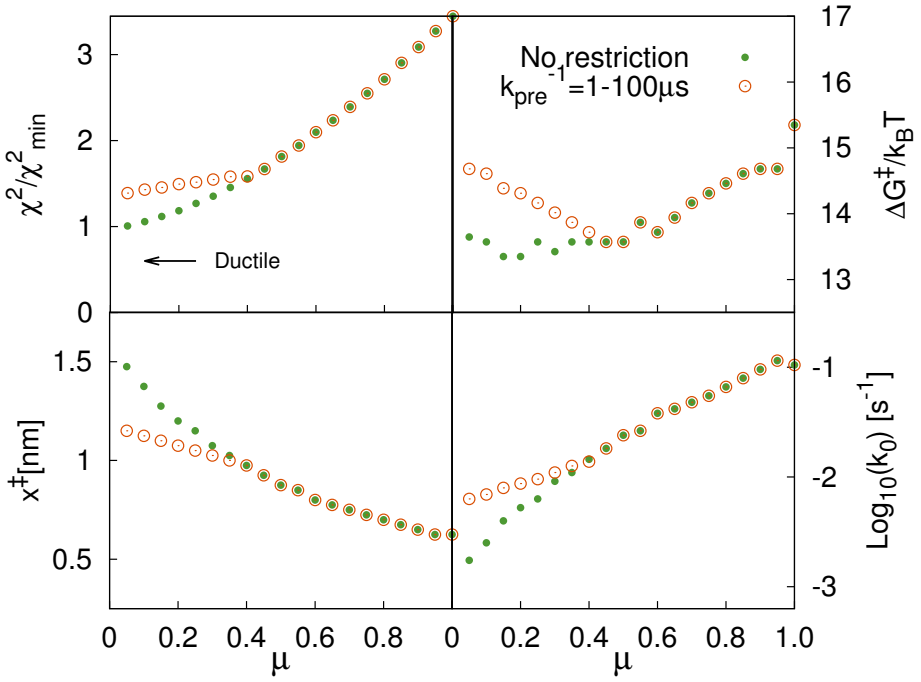

Figure S 2: **Ductility of the ddFLN4 domain.** We performed restricted fits to the experimental ddFLN4 unfolding rates, enforcing that the kinetic prefactor, $k_{pre} = k_0 e^{\beta \Delta G^{\ddagger}}$, is within the range expected from the transition path time measurements [4] for proteins of comparable size ($1/\mu\text{s} - 1/100\mu\text{s}$). Relative error χ^2 (top left) of the fits to the ddFLN4 unfolding rates, activation barrier $\beta \Delta G^{\ddagger}$ (top right), transition state $x^{\ddagger}$ (bottom left), and base ten logarithm of the intrinsic rate, $\log_{10}(k_0)$ (bottom right), as a function of μ. Solid green circles are for the unrestricted fits (Main Text Fig. 5), and open magenta circles show the results with the restriction.

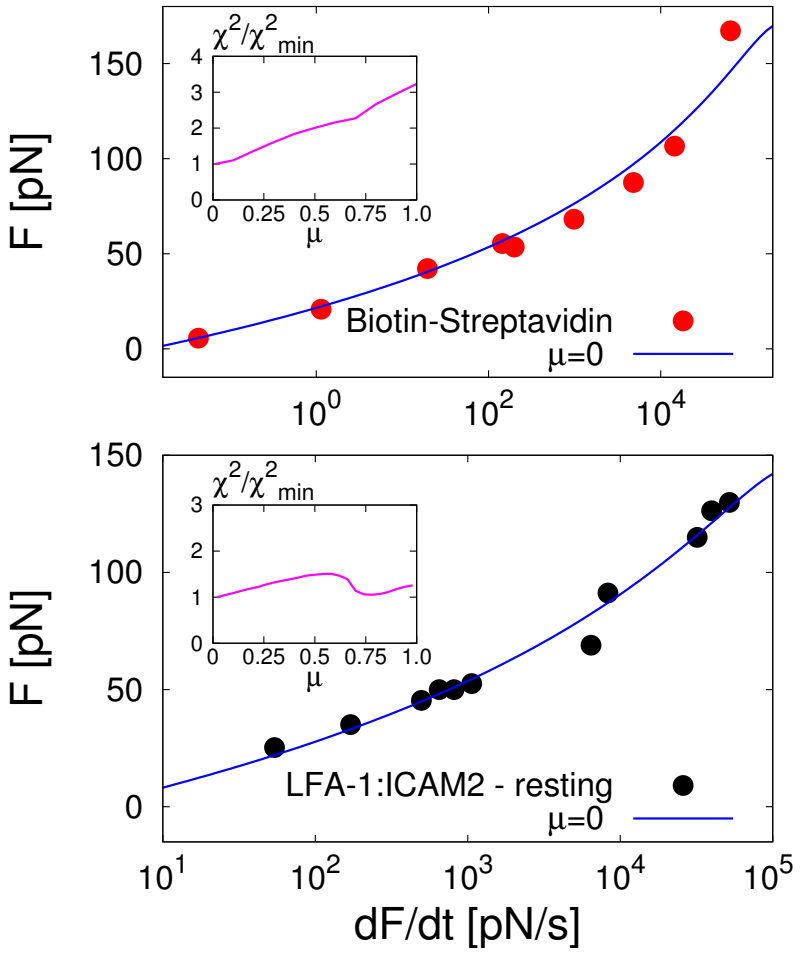

Figure S 3: **Ductility of the biotin-streptavidin and LFA-1:ICAM1 complexes.** Mean rupture force of the biotin-streptavidin (top) and LFA-1:ICAM1 (bottom) complexes [5] as a function of the logarithm of the force-loading rate. Fits of $\langle F \rangle$ are shown as lines for the best model $\mu = 0$ (blue). The inset shows the error χ^2 relative to the best fit.

Supporting References

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[2] Bullerjahn, J. T., S. Sturm, and K. Kroy, 2014. Theory of rapid force spectroscopy. *Nat. Commun.* 5.

[3] Rico, F., L. Gonzalez, I. Casuso, M. Puig-Vidal, and S. Scheuring, 2013. High-speed force spectroscopy unfolds titin at the velocity of molecular dynamics simulations. *Science* 342:741–743.

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