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Comment on "Experimental Free Energy Reconstruction From Single-Molecule Force Spectroscopy Using Jarzynski's Equality"

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Harris, Song and Kiang [1] (HSK) describe their results on reconstructing the free energy profiles for both the stretch of the titin polymer, and the unfolding of an individual I27 domain. The new finding reported in [1] is the measurement of the free energy barrier (or activation energy) to unfolding the I27 domain. Due to a misinterpretation of the mechanics involved, the free energy surface (and thus the energy barrier) to unfolding the I27 domain was not measured.

As sketched in figure 1, the experimental control parameter, λ, is given by the combination of the extension of each component in the series,

$$\begin{aligned} \lambda &= \Delta z + z_P + z_D \qquad\qquad (1)\\ &= F_s(c_s + c_P(F_s) + c_D(F_s)), \end{aligned}$$

where F_s is the tension, Δz, z_P, and z_D are respectively the extensions of the cantilever, unfolded polymer, and folded domain, and c_s, $c_P(F_s)$, and $c_D(F_s)$ are the respective compliances. The cantilever compliance is simply the inverse of the spring constant $c_s = 1/k_s$. HSK analyze the work performed on the system over the coordinate $z = \lambda - \Delta z = z_P + z_D$. The incremental change in work done on this system is given by,

$$dW = Fdz = Fdz_P + Fdz_D. \qquad\qquad (2)$$

Clearly this work is a combination of the work done on the polymer and the domain. Because the compliance of the polymer is far greater than the folded domain, the polymer overwhelmingly absorbs the stretching energy. Thus in order to isolate the work done on the domain, one would need to have independent knowledge of the compliance of the polymer $c_P(F_s)$ [2].

It is shown in Fig. 3(b) in [1] that HSK envision a very different mechanism of accumulating work than that described by Eqs. (1) and (2). The schematic suggests the following. First the work is only exerted to stretch the polymer by some amount. Then, the polymer extension is held fixed, as to not permit further stretching. Finally, work begins accumulating on extending the domain until unfolding occurs. This process would require that the domain is not attached to the system until the instance that the polymer is held fixed. It is not clear how this complicated mechanism could occur.

HSK also claim that the reconstructed profile, $G(z)$, extends up to the transition state to unfolding. However, the highest possible yielding force is not located
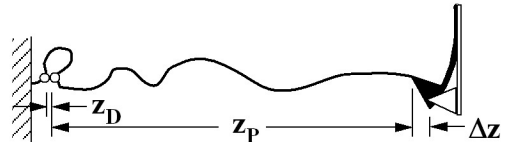

FIG. 1: Illustration of the extensible components involved when loading a folded domain with an intervening polymer linkage. The length added by stretching and eventually unfolding the domain is given by z_D.

at the transition state, but rather it corresponds to the maximum gradient of the energy surface. Even to reach such a small ($< x_u^{\ddagger}$), critical distance requires that the effective barrier to unfolding has vanished, which is highly improbable given that thermally activated unfolding will stochastically occur *before* this point [3]. The data in Fig. 2(a) of [1] attest to this point — the unfolding location occurs at a distribution of extensions, not a single location.

Under the model of stretching viewed by HSK, extracting the barrier to unfolding, $\Delta G_u^{\ddagger}$, is rather trivial. Since the slope near the unfolding event of $G(z)$ is nearly constant (Fig. 3(b) in [1]), HSK *effectively* multiply this slope by a literature value of the transition state, $x_u^{\ddagger} = 0.6$ nm, hence, $\Delta G_u^{\ddagger} = x_u^{\ddagger} \cdot \partial_z G(z)$. This value for $\Delta G_u^{\ddagger}$ is meaningless because, *i*) $G(z)$ is dominated by the energy of polymer stretching, and *ii*) the work on the domain accumulates over the entire process (not over some time close to unfolding). The roughly constant slope of $G(z)$ near the unfolding event also explains why HSK find that $\Delta G_u^{\ddagger}$ is reproducible over the 4 nm range (15 - 19 nm) of stretch lengths considered.

In summary, the mechanism of stretching the titin system envisioned by HSK is non-physical. The free energy reconstruction, $G(z)$, is dominated by the energy of stretching the unfolded polymer and thus reveals little information on the energy of unfolding a domain. By determining the compliance of the polymer component of titin independently [2], the free energy reconstruction of an individual domain can in principle be carried out.

[1] N.C. Harris, Y. Song, and C.H. Kiang, Phys. Rev. Lett. **98** (2007).

[2] G. Hummer, and A. Szabo, Acc. Chem. Res. **38**, 504 (2005).

[3] G. Hummer, and A. Szabo, Proc. Natl. Acad. Sci. USA **98**, 3658 (2001).