Supplemental Materials

Molecular Biology of the Cell

Pang et al.

Supplemental Materials for

Mechanical stability of αT-catenin and its activation by force for vinculin binding

Si Ming Pang [†, ¶], Shimin Le [†, ‡, ¶], Adam V. Kwiatkowski [§], and Jie Yan [†, ‡, ||, *]

† Mechanobiology Institute, National University of Singapore, Singapore 117411;

‡ Department of Physics, National University of Singapore, Singapore 117542;

§ Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261;

|| Centre for Bioimaging Sciences, National University of Singapore, Singapore 117546.

* Corresponding author

¶ These authors contributed equally to this work.

Supplemental Methods

Unfolding and folding rates of M1

The unfolding and folding rates of M1 were obtained from pseudo dwell time analysis from constant force experiments (Figure 3A). Using the unfolding transition as an example, the pseudo dwell times of the folded state were obtained by multiplying the time taken to the next unfolding transition event by a factor of 2 if the current state had both M1 domains folded, or multiplied by a factor of 1 if the current state had only one M1 domain folded (Cao *et al.*, 2008). The pseudo dwell times of the folded state were then fit to an exponential decay function to obtain the force-dependent unfolding rate. Bootstrap analysis was used to obtain the statistical error for the force-dependent unfolding rate (Supplemental Methods). A similar procedure was performed on the pseudo dwell time of the unfolded state to obtain the force-dependent folding rates (Figure 3B).

Folding energy of M1

The folding energy of M1, $-\mu$, was estimated based on the critical force, F_c, (of the equilibrium unfolding and folding transitions at which the unfolding and folding have the same rates) obtained in Figure 3B by using $\mu = -\int_0^{F_c} \left(x_{\text{folded}}(F') - x_{\text{unfolded}}(F')\right) dF'$, where $x_{\text{folded}}(F)$ is the force-extension relation of the folded state of M1 and $x_{\text{unfolded}}(F)$ is the force-extension relation of the unfolded state of M1. To obtain the range of the folding energy of M1, $-\mu$ was estimated using two methods. In the first method, the unfolded M1 was assumed to behave like a randomly coiled polypeptide, which $x_{\text{unfold}}(F)$ was modeled by the inverse worm-like chain, $\frac{FA}{k_B T} = \frac{1}{4\left(1 - x_{\text{unfold}}/L\right)^2} + \frac{x_{\text{unfold}}}{L} - \frac{1}{4}$, where A is the persistence length which is modeled here using a value of 0.8 nm (Rief *et al.*, 1999) and L = 50.5 nm is the contour length calculated with each amino acid having a contour length of 0.38 nm. The folded state of M1 was modeled as a rigid body with the force-extension relation of $x_{\text{folded}}(F) = b_{\text{folded}} \coth\left(\frac{F b_{\text{folded}}}{k_B T}\right) - \frac{k_B T}{F}$, with b_{folded} = 5.8 nm estimated based on I-TASSER predicted structure as well as that of αE-catenin M1 domain (PDBID: 4IGG). Using this method, the folding energy of M1 was estimated to be $-\mu = \sim -16\ k_B T$.

In the second method, the unfolded state of M1 was assumed to be a chain of four equal length α-helices with a short linker of 31 amino acids, as this model was found to best describe the behavior of M1 at low forces (Supplemental Figure S3). Therefore, the unfolded state of M1 was modeled as four rigid bodies, each of them with the force-extension relation of

$x_{\text{helix}}(F) = b_{\text{helix}} \coth\left(\frac{Fb_{\text{helix}}}{k_B T}\right) - \frac{k_B T}{F}$, with b_{helix} = 3.8 nm (each amino acid contributes 0.15 nm to the length of the α-helix), and a short flexible polypeptide region with the force-extension, $x_{\text{flexible}}(F)$, modeled using the inverse of the worm-like chain, $\frac{F\text{A}}{k_B T} = \frac{1}{4\left(1 - x_{\text{flexible}} / L_{\text{flexible}}\right)^2} + \frac{x_{\text{flexible}}}{L_{\text{flexible}}} - \frac{1}{4}$, with L_{flexible} = 11.8 nm (calculated with each amino acid having a contour length of 0.38 nm) and A = 0.8 nm (Rief *et al.*, 1999). The force-extension relation of the unfolded state can be written as: $x_{\text{unfolded}}(F) = 4x_{\text{helix}}(F) + x_{\text{flexible}}(F)$. The folded state of M1 was modeled as a rigid body as described in the first method. Using this method, the folding energy of M1 was estimated to be $-\mu$ = ~ -12 $k_B T$.

Number of amino acids in M1-3

The number of amino acids involved in the folding and unfolding transitions of the respective domains during the linearly increasing and constant force experiments was estimated from the stepsize, Δx_{step}, and corresponding transition force, F_t, using two methods. In the first method, it was assumed that the unfolded state is a flexible, fully uncoiled polypeptide with an extension-force relation, $x_{\text{unfold}}(F)$, which was well modeled by the inverse worm-like chain, $\frac{FA}{k_B T} = \frac{1}{4\left(1 - x_{\text{unfold}} / L\right)^2} + \frac{x_{\text{unfold}}}{L} - \frac{1}{4}$, where A is the persistence length which is modeled here using a value of 0.8 nm (Rief *et al.*, 1999) and L is the contour length to be solved. In addition, the force-dependent extension of the folded state, $x_{\text{fold}}(F)$, was assumed to behave similarly with a rigid body with an extension-force relation: $x_{\text{folded}}(F) = b_{\text{folded}} \coth\left(\frac{Fb_{\text{folded}}}{k_B T}\right) - \frac{k_B T}{F}$, where b_0 is the length of the folded state of each middle domain, estimated to be 5.8 nm based on I-TASSER predicted structure as well as that of αE-catenin M1 domain (PDBID: 4IGG). Since $\Delta x_{\text{step}} = x_{\text{unfold}}(F_t) - x_{\text{fold}}(F_t)$, the contour length L can be obtained by solving the following equation:

$$\frac{F_t A}{k_B T} = \frac{1}{4\left(1 - \left(\Delta x_{\text{step}} + x_{\text{fold}}(F_t)\right) / L\right)^2} + \frac{\Delta x_{\text{step}} + x_{\text{fold}}(F_t)}{L} - \frac{1}{4}$$

The contour length, *L,* obtained can then be converted into the number of amino acids by dividing it by 0.38 nm, which is the contour length per amino acid (Carrion-Vazquez *et al.*, 1999).

In the second method, it was assumed that the M1 domain unfolded into four equal length rigid α-helices and a short region of flexible polypeptide. Each α-

helix was modeled as a rigid rod, $x = b\left(\coth\left(\frac{Fb}{k_B T} \right) - \frac{k_B T}{Fb} \right)$, with length b = 3.8 nm and extension x at force F. The extension of the short flexible polypeptide at force F, $x_{\mathrm{WLC}}(F)$, was modeled using the inversed of the worm-like chain (similar as above) with contour length L = 31 amino acids × 0.38 nm = 11.8 nm. The folded state of M1 was modeled as a rigid rod, $x = b_0 \coth\left(\frac{Fb_0}{k_B T} \right) - \frac{k_B T}{Fb_0}$, with length b_0 = 5.8 nm and extension x at force F as described above. Based on these, the length of each of the rigid alpha α-helix, b, was estimated by solving:

$$\Delta x_{step} = 4b\left(\coth\left(\frac{F_t b}{k_B T} \right) - \frac{k_B T}{F_t b} \right) + x_{\mathrm{WLC}}(F_t) - b_0 \coth\left(\frac{F_t b_0}{k_B T} \right) - \frac{k_B T}{F_t b_0}$$

In an α-helix, each amino acid is known to contribute 0.15 nm to b, hence from the b obtained, the number of amino acids in M1 can then be estimated by dividing by 0.15 nm.

Force-dependent vinculin dissociation step size

The vinculin binding site in M1 was estimated to have ~ 36 amino acids in the α-helix structures that were stabilized by VD1 binding (Yonemura *et al.*, 2010; Choi *et al.*, 2012). Upon VD1 dissociation at high forces of more than 20 pN, the α-helix instantly unfolded into flexible uncoiled polypeptide chain. The force-dependent step size observed for this unfolding was calculated by finding the force-dependent extension difference between the flexible polypeptide chain and the α-helix of 36 amino acids. The extension of the flexible polypeptide chain, x, at force F was modeled using the inverse of the worm-like chain, $\frac{FA}{k_B T} = \frac{1}{4(1 - x/L)^2} + \frac{x}{L} - \frac{1}{4}$, where A is the bending persistent length, and L = 13.7 nm is the contour length which is calculated with each amino acid having 0.38 nm in length. The extension of the α-helix, x_{VBS}, at force F can be modeled as a rigid body, $x_{\mathrm{VBS}}(F) = b_{\mathrm{VBS}} \coth\left(\frac{Fb_{\mathrm{VBS}}}{k_B T} \right) - \frac{k_B T}{F}$, where b_{VBS} = 5.4 nm is the rigid body length calculated with each amino acid contributing 0.15 nm. At a force range of 20-40 pN, the extension difference due to the α-helix unfolding in VBS was calculated to be ~ 3-4 nm (Supplemental Figure S5), in agreement with experimental observation.

Bootstrap analysis

Bootstrap analysis was used to obtain statistical error for M1 unfolding/folding rates and VD1 binding probabilities. The procedure was, for a given number (N) of data points, 1000 data sets of the same size were generated by N times of random data picking from the original set with replacement. The data in

each data set were then analyzed, and the average value was reported, while the standard deviation was reported as error.

For the pseudo dwell times of the M1 folded/unfolded state, the pseudo dwell times in each data set were fit with an exponential decay function to obtain the unfolding/folding rate. Since there were 1000 data sets, a total of 1000 unfolding/folding rates were obtained at each force. The mean of the 1000 unfolding/folding rates was then reported, with error reported as the standard deviation of the 1000 rates.

For the VD1 binding experiments, the number of binding events in each data set was divided by the total number of cycles for the time interval (at which M123 was subjected to ~ 8 pN) to obtain the binding probability. Since there were 1000 data sets, a total of 1000 binding probabilities were obtained for each time interval. The mean of the 1000 binding probabilities was then reported, with error reported as the standard deviation of the 1000 binding probabilities.

Supplemental Figures:

Fig. S1. **Sequence comparison of the VBS in αE-, αT- and αN-catenin.** Residues 300-360 in αE-catenin were identified as the VBS (Yonemura *et al.*, 2010; Choi *et al.*, 2012). This amino acid sequence was aligned with the αT-catenin (residues 295-355) with 82 % identity. In addition, these residues were aligned with αN-catenin (residues 298-358) with 95% identity.

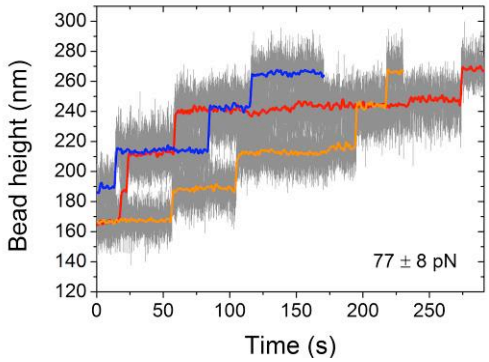

Fig. S2. **I27 domain unfolding as a positive control.** At a high force of 77 ± 8 pN, four stepwise unfoldings that correspond to the four I27 repeats (which act as positive control) were observed, indicating that the tether is attached to the correct M123 construct.

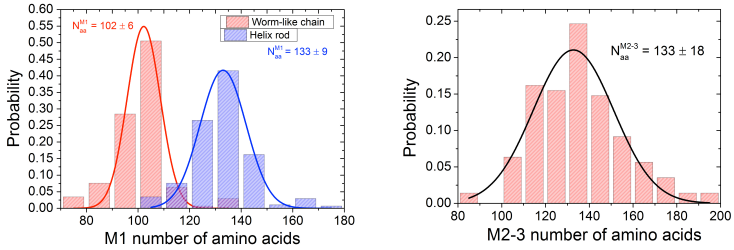

Fig. S3. **Number of amino acids in αT-catenin M123 domains.** (A-B) The number of amino acids involved in the unfolding/folding transition of M1 domain (A) and M2-3 domains (B). The number of amino acids in M1 and M2-3 domains were first estimated with the assumption that the domains unfolded into fully uncoiled polypeptides (red histograms) and fit with a Gaussian. (A) For M1, a separate estimation was done with the assumption that it unfolded into a series of four α-helices and a short uncoiled polypeptide (blue histogram) and fit with a Gaussian.

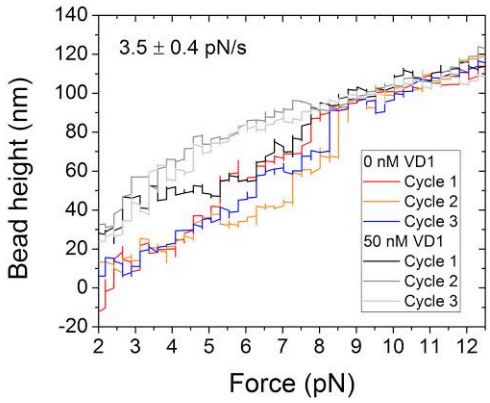

Fig. S4. **Vinculin head domain 1 blocks αT-catenin M1 domain from refolding.** Representative changes in the height of the bead tethered to a 2M1 construct when subjected to a linearly increasing (3.5 ± 0.4 pN/s) force loading rate before (colored traces) and after addition of 50 nM VD1 (grey traces), smoothed over 0.1 s. Before addition of 50 nM vinculin head domain 1, two stepwise unfolding events were observed, which correspond to the two M1 domains. Upon addition of VD1, only one unfolding transition occurred in the first cycle with no unfolding transition in the subsequent cycle (gray traces), indicating that the VD1 had bound to the VBS in M1 and blocked M1 from refolding.

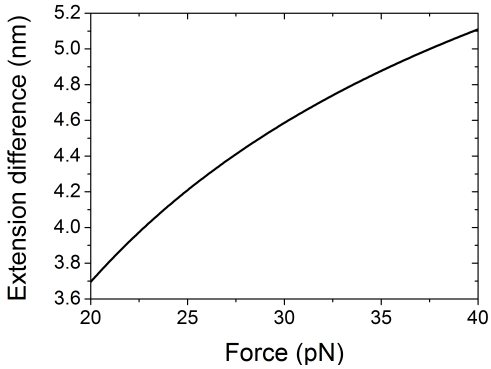

Fig. S5. **Extension change of the α-helical VBS at high force**. Upon vinculin dissociation at high force, the α-helix in VBS (~ 36 amino acids) unfolded into a flexible polypeptide. The flexible polypeptide was modeled using the worm-like chain, while the α-helical VBS was modeled using a rigid body (Supplemental Methods). The extension change of this unfolding at force of 20-40 pN was ~ 3-4 nm, in agreement with experimental observation (Figure 4, A and C).

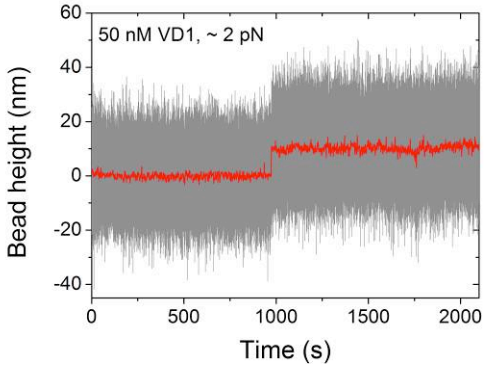

Fig. S6. **VD1 binding onto VBS in M1 at low force.** A representative time traces of the height change of a bead tethered to an originally folded M123 construct in 50 nM VD1 held at a constant force of ~ 2 pN. A ~ 10 nm stepwise extension increase occurred at ~ 960 s. This indicated VD1 binding to the VBS in M1 domains in the absence of applying a transient force of ~ 8 pN for two minutes to unfold M1. Subsequently, the bead height remained unchanged for 1000 s, indicating that VD1 did not dissociate.

Supplemental References

Cao, Y., Kuske, R., and Li, H. (2008). Direct observation of Markovian behavior of the mechanical unfolding of individual proteins. Biophys. J. *95*, 782-788.

Carrion-Vazquez, M., Marszalek, P.E., Oberhauser, A.F., and Fernandez, J.M. (1999). Atomic force microscopy captures length phenotypes in single proteins. Proc. Natl. Acad. Sci. *96*, 11288-11292.

Choi, H.-J., Pokutta, S., Cadwell, G.W., Bobkov, A.A., Bankston, L.A., Liddington, R.C., and Weis, W.I. (2012). αE-catenin is an autoinhibited molecule that coactivates vinculin. Proceedings of the National Academy of Sciences *109*, 8576-8581.

Rief, M., Pascual, J., Saraste, M., and Gaub, H.E. (1999). Single molecule force spectroscopy of spectrin repeats: low unfolding forces in helix bundles. J. Mol. Biol. *286*, 553-561.

Yan, J., Yao, M., Goult, B.T., and Sheetz, M.P. (2015). Talin dependent mechanosensitivity of cell focal adhesions. Cell. Mol. Bioeng. *8*, 151-159.

Yonemura, S., Wada, Y., Watanabe, T., Nagafuchi, A., and Shibata, M. (2010). α-Catenin as a tension transducer that induces adherens junction development. Nature cell biology *12*, 533.