Vt Variant	% Vt pelleted at 30 μM F-actin	$\Delta\Delta G_{bind}$ from co-sedimenation	$\Delta\Delta G_{bind}$ from calculation
Vt^{WT} (879-1066)	66 ± 1.3	N/A	N/A
Vt^{I997A}	35 ± 2.6	0.38	1.50
Vt^{V1001A}	48 ± 3.9	0.19	0.48
Vt^{Q1018K}	57 ± 0.6	0.09	1.25
Vt^{D882A}	67 ± 0.5	-0.01	0.02
Vt^{I948A}	63 ± 2.0	0.03	1.18
$Vt^{\Delta C5}$ (879-1061)	62 ± 0.3	N/A	N/A
Vt^{F885A}	76 ± 2.2	-0.09	0.08
Vt^{H906A}	94 ± 1.1	-0.21	0.35
Vt^{L928D}	75 ± 0.3	-0.08	-0.08
$Vt^{\Delta N}$ (893-1066)	78 ± 0.3	N/A	N/A

Table S1. F-actin binding properties of Vt variants and their agreement with the DMD model (related to Figure 4). Several Vt variants were generated and evaluated for their ability to bind F-actin. The percent of Vt that pellets with F-actin at 30 μM F-actin is reported as a measure of F-actin binding. All variants have been evaluated by NMR analyses (^{1}H-^{15}N 2D HSQC) and appear to fold properly (Figure S1, data not shown). The Vt variants are grouped into three categories based on their ability to bind F-actin. The mutation sites are mapped on the structure of Vt in Figure S4B.

Supplemental Experimental Procedures

IpaA peptide

The IpaA peptide (NNIYKAAKDVTTSLSKVLKNIN) with N-terminal acetylation and C-terminal amidation was synthesized by the UNC High-Throughput Peptide Synthesis and Array Facility and LifeTein. Peptide concentration was determined by absorbance at 280 nm using the extinction coefficient for tyrosine (1200 M^{-1} cm^{-1}).

Circular dichroism (CD) spectroscopy

Circular dichroism (CD) spectra were collected as reported (Palmer et al., 2009). Briefly, spectra were collected from 350-250 nm (near-UV) and 260-190 nm (far-UV) at protein concentrations of 450 and 5 μM, respectively. Data were collected on a Jasco J-815 CD Spectrometer at 25°C in 10 mM potassium phosphate, pH 5.5 or 7.5, 50 mM Na_2SO_4, and 1 mM dithiothreitol.

NMR spectroscopy

Vt samples for NMR were prepared from cells grown in M9 media with $^{15}NH_4Cl$ as the sole nitrogen source. The ^{15}N-Vt samples were exchanged into NMR buffer (10 mM potassium phosphate, pH 5.5, 50 mM NaCl, 0.1% NaN_3, 2 mM DTT, and 10% D_2O) and concentrated to 30 μM. Two-dimensional heteronuclear single quantum coherence (HSQC) spectra were collected on a Varian INOVA 700 MHz spectrometer at 37 °C (Palmer and Campbell, 2008). Processing was done with NMRPipe (Delaglio et al., 1995) and spectral analysis with NMRViewJ (Johnson and Blevins, 1994).

CLEANEX (Hwang et al., 1997) experiments were performed on a Varian INOVA 600 MHz spectrometer at 37 °C with mixing times of 0.2, 15.3, 30.4, 55.5, 85.6, 125.7, 180.8, and 300 msec. fast-HSQC (Hwang et al., 1998) experiments were performed on a Varian INOVA 700 MHz spectrometer at 37 °C . Samples for the fast-HSQC experiments were exchanged into 95% D_2O, 10 mM potassium phosphate, pD 5.9, 50 mM NaCl, 0.1% NaN_3, and 2 mM DTT using a pre-equilibrated PD-10 column (GE Healthcare Life Sciences). Ninety two data sets were collected at increasing intervals over 18 days. Protein samples were collected and added to NMR tubes at a concentration of 200 μM for CLEANEX and 700 μM for fast-HSQC. Experiments were processed with NMRPipe and rates were analyzed with NMRViewJ.

Heteronuclear NOE experiments (Farrow et al., 1994) were performed on a Varian INOVA 600 MHz spectrometer at 37°C. Proton saturation was achieved over 4.5 seconds.

Calculation of $\Delta\Delta G_{bind}$

Prior to estimation of change in binding free energy ($\Delta\Delta G_{bind}$), the structure of the Vt:F-actin complex was relaxed while maintaining a fixed backbone. $\Delta\Delta G_{bind}$ for all Vt variants was computed using Eris (Yin et al., 2007b). One thousand independent runs were performed for computing $\Delta\Delta G_{bind}$ for each of the Vt variants using the following relation:

$$\Delta\Delta G_{bind} = (\Delta G_{C-MUT} - \Delta G_{C-WT}) - (\Delta G_{V-MUT} - \Delta G_{V-WT})$$

where the subscripts MUT and WT refer to the mutant and wild-type forms of the complex (C) and Vt (V).

Measured ΔG values were calculated from the cosedimentation experiments using the following equation:

$$\Delta G_{bind} = -kT \ln(K)$$

where *k* is the Boltzmann constant, *T* is the temperature, and *K* is the mean percentage of Vt found in the pellet at 30 μM F-actin.

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