Supporting Information

Jagannathan et al. 10.1073/pnas.1201800109

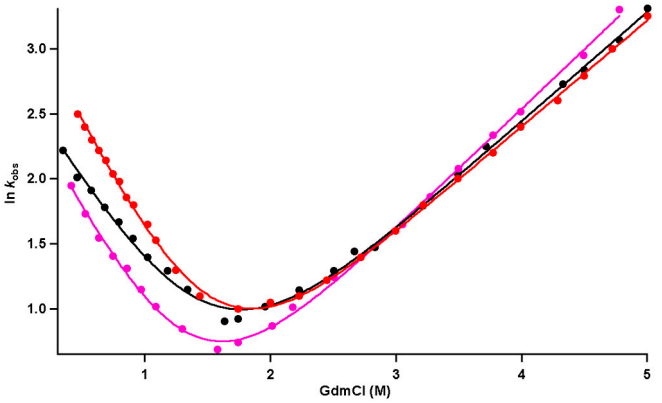

Fig. S1. Ensemble kinetic chevron plots for cysteine-free, wild-type src SH3 (pink), A7C/N59C src SH3 (black), and R19C/N59C src SH3 (red). The stopped-flow experiments were performed at 25 °C under the same buffer conditions as those used in the optical tweezers (10 mM Tris pH 7.0, 250 mM NaCl).

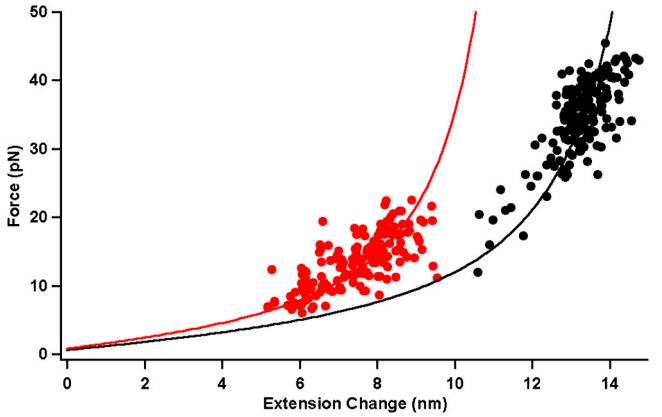

Fig. S2. The measured extension changes in force ramp experiments for A7C/N59C (black) and R19C/N59C (red) src SH3. The extension changes were fit to a worm-like chain model (solid lines) using a persistence length of 0.65 nm and a contour length of 0.36 nm/residue. The distances between the cysteine residues in the folded state was estimated from the structure of src SH3 (Protein Data Bank ID code 1SRL).

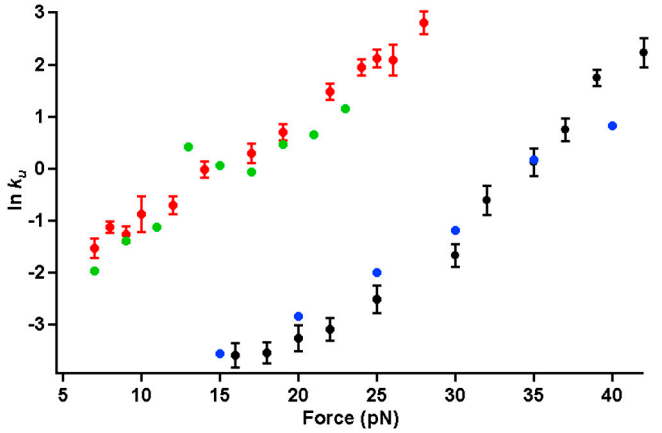

Fig. S3. Comparison of the force-dependent unfolding rates measured in our force-jump experiments (A7C/N59C, black; R19C/N59C, red) to the rates extracted from the unfolding force histograms (A7C/N59C, blue; R19C/N59C, green) using the method described by Dudko et al. (1).

1 Dudko OK, Hummer G, Szabo A (2008) Theory, analysis, and interpretation of single-molecule force spectroscopy experiments. *Proc Natl Acad Sci USA* 105:15755–15760.

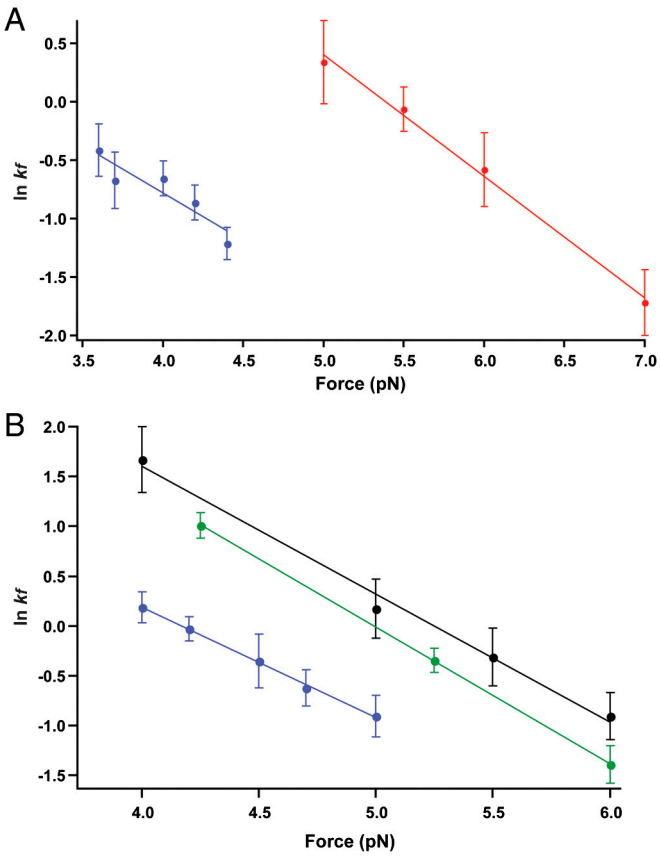

Fig. S4. Force dependence of refolding rates for the I34A (blue) and S47A (green) variants of src SH3 along the (*A*) unzipping and (*B*) shearing geometries. The plots for the parent proteins, R19C/N59C and A7C/N59C, are shown in red and black, respectively. The refolding rates of R19C/N59C/S47A could not be determined.

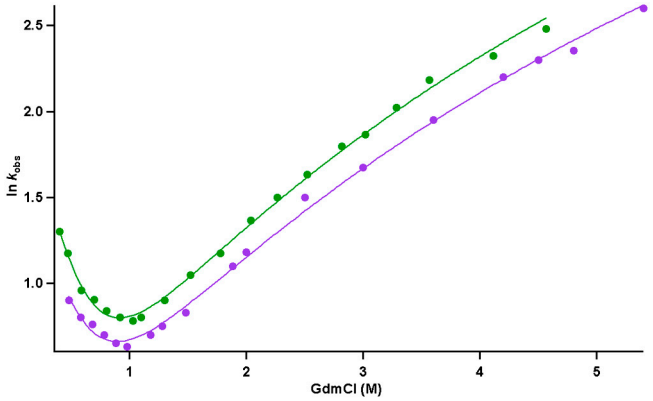

Fig. S5. Ensemble kinetic chevron plots of the S47A src SH3 variant in the wild-type (purple) and A7C/N59C (green) background. The solid lines represent polynomial fits to the data (2).

2 Maxwell, et al. (2005) Protein folding: Defining a "standard" set of experimental conditions and a preliminary kinetic data set of two-state proteins. *Protein Sci* 14:602–616.

Table S1. Results from a two-state fit of the chevron plots from stopped-flow kinetic experiments on the src SH3 constructs

src SH3 construct	k_{f,H_2O}, s^{-1}	k_{u,H_2O}, s^{-1}
Wild-type	13.0 ± 0.8	0.30 ± 0.02
A7C/N59C	15.0 ± 0.6	0.40 ± 0.02
R19C/N59C	29.0 ± 0.7	0.42 ± 0.01
A7C/N59C/I34A	2.05 ± 0.25	0.25 ± 0.01
A7C/N59C/S47A	7.00 ± 0.55	1.20 ± 0.30
R19C/N59C/I34A	5.50 ± 0.25	0.15 ± 0.01
R19C/N59C/S47A	6.50 ± 0.65	0.40 ± 0.01

Table S2. Average unfolding forces (F_u) and distances to the transition states ($X_u^{\ddagger}$) of src SH3 variants under shearing and unzipping forces

src SH3 construct	F_u, pN	$X_u^{\ddagger}$, nm
A7C/N59C	35.0 ± 0.5	0.45 ± 0.05 (low force) 1.40 ± 0.15 (high force)
A7C/N59C/I34A	35.0 ± 0.3	0.45 ± 0.06 (low force) 1.30 ± 0.20 (high force)
A7C/N59C/S47A	34.0 ± 0.8	0.25 ± 0.05 (low force) 1.25 ± 0.20 (high force)
R19C/N59C	14.0 ± 0.3	0.70 ± 0.05
R19C/N59C/I34A	17.5 ± 0.5	0.72 ± 0.05
R19C/N59C/S47A	11.0 ± 0.3	0.65 ± 0.10