Supplementary Material for

Binding induced folding under unfolding conditions: Switching between induced fit and conformational selection mechanisms

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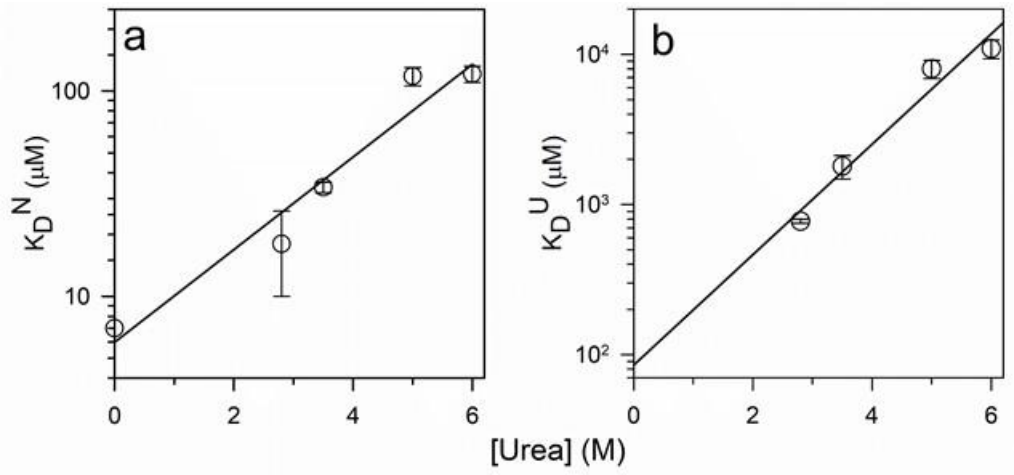

Figure S1. Dependence of K_D^N and K_D^U on urea concentration. (a) K_D^N was calculated from the t=0 points of the kinetic folding traces. (b) K_D^U was obtained from fitting the binding curves to Equation 2. The solid line through the data is a fit to a straight-line equation.

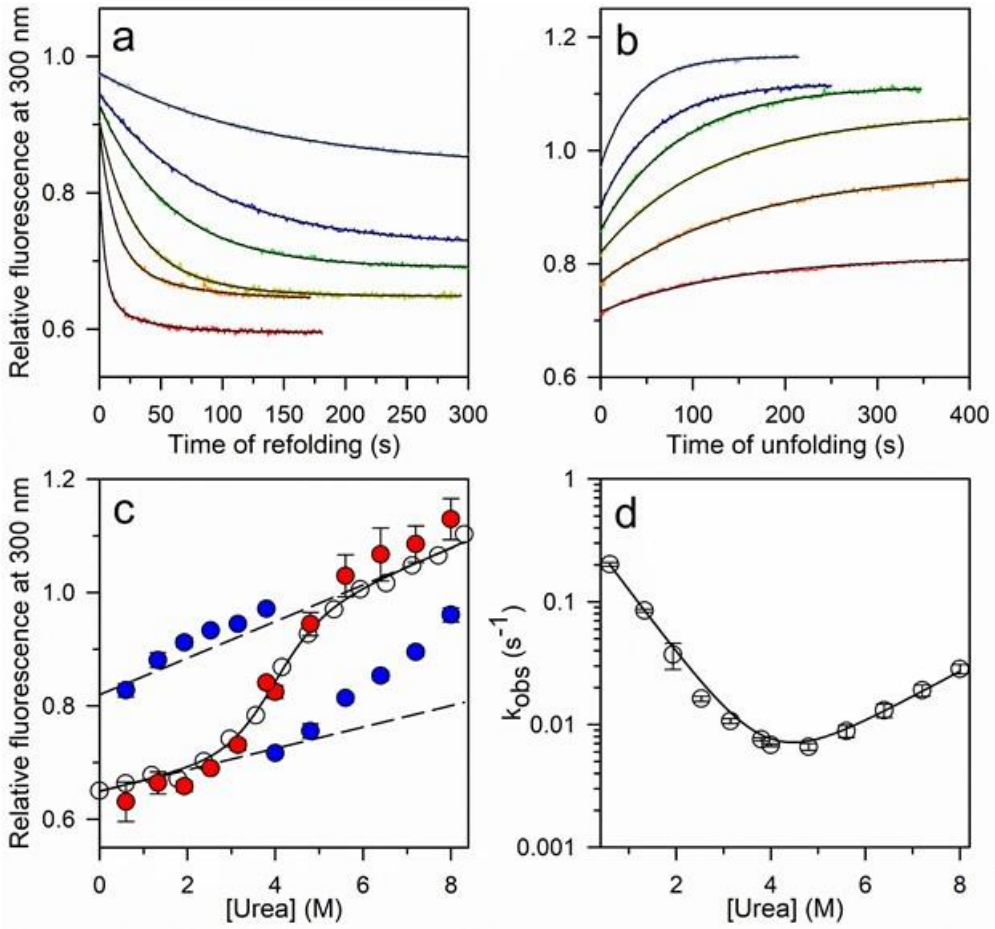

Figure S2. Urea-induced folding and unfolding of the PI3K SH3 domain. Urea-induced folding and unfolding kinetics were monitored at 300 nm upon excitation at 268 nm. (a) Kinetic traces of refolding at various urea concentrations: top to bottom, 3.8, 3.1, 2.5, 1.9, 1.3 and 0.6 M urea. (b) Kinetic traces of unfolding at various urea concentrations: top to bottom, 8, 7.2, 6.4, 5.6, 4.8 and 4 M urea. (c) Equilibrium unfolding transition of the PI3K SH3 domain (○). The t=0 (●) and t=∞ (●) points obtained from the kinetic traces are shown. The solid line through the data points is a fit to a two-state model of unfolding. All the data were normalized to the value of 1 for the fluorescence signal at 6 M urea. (d) Dependence of the observed rate constants of folding and unfolding on urea concentration. The data were fit to Equation 11. The error bars represent the standard errors obtained from two independent experiments.

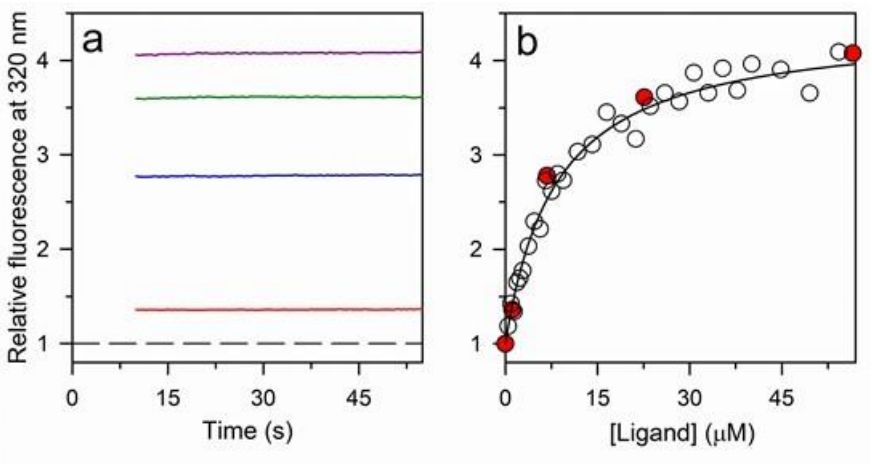

Figure S3. Kinetics of binding peptide ligand to PI3K SH3 domain under native conditions. (a) Binding was monitored by measurement of the change in the intrinsic Trp fluorescence signal at 320 nm, upon excitation at 268 nm. The protein was diluted to different concentrations of ligand, manually. The concentrations of the peptide ligand were, top to bottom, 57, 23, 8, 1.1 μM. Each trace was normalized to a value of 1 for the fluorescence signal of the protein in the absence of ligand. The reactions were carried out under pseudo-first order conditions. The dashed line represents the signal of the native protein in absence of the ligand. (b) Comparison of the burst phase amplitudes (●) to the equilibrium amplitudes of binding.

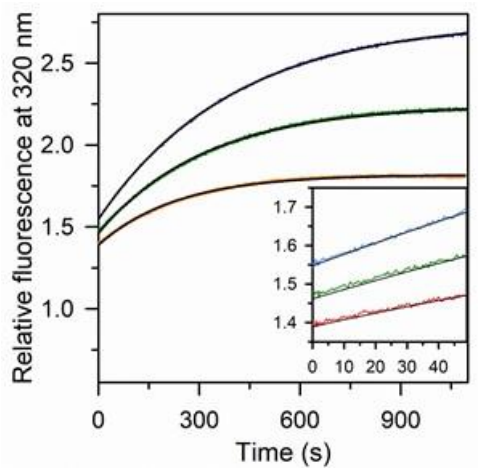

Figure S4. Stopped-flow kinetic traces of folding induced by the ligand, at 5 M urea concentrations. Ligand-induced folding was monitored by measurement of the change in the intrinsic Trp fluorescence signal at 320 nm, upon excitation at 268 nm. The protein at 5 M urea was diluted to different concentrations of ligand, manually, without changing the urea concentration. The concentrations of the peptide ligand were, top to bottom, 1500, 500 and 250 μM. Each trace was normalized to a value of 1 for the fluorescence signal of the protein in the absence of ligand. The reactions were carried out under pseudo-first order conditions, and the solid lines through the data are fits to a single exponential equation. The inset shows the zoomed in traces at lower time scales.

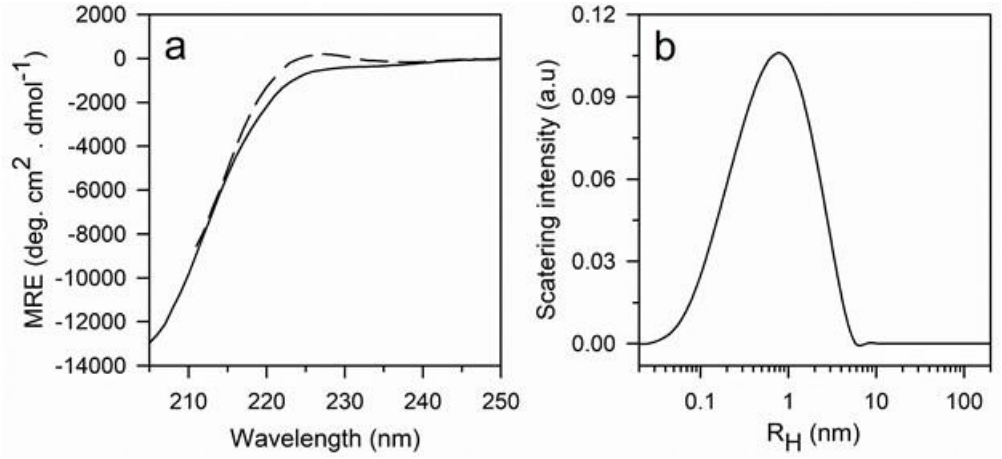

Figure S5. Characterization of the Pro-rich peptide ligand. (a) CD spectra of the peptide in 0 (solid black line) and 6 M urea (dashed black line) concentrations; (b) DLS measurements of 8 mM peptide ligand in 3.5 M urea. The R_h of the peptide ligand was ~1 nm which corresponds to the R_h of the monomer.

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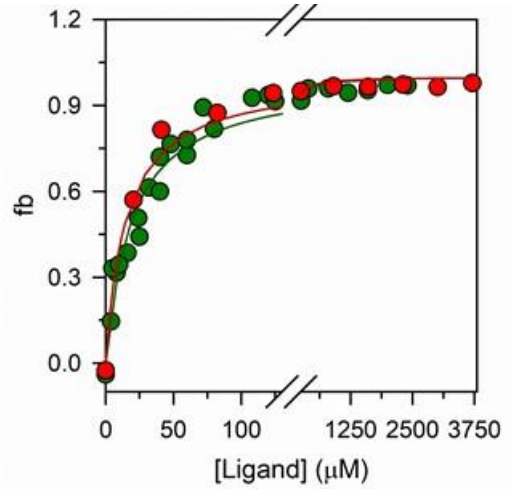

Figure S6. Binding curves of the PI3K SH3 domain obtained at 2.8 M urea, by measuring the change in the intrinsic Trp fluorescence signal at 320 nm, upon excitation at 268 nm (●) and by measuring the change in the fluorescence anisotropy (●). The data was obtained by equilibrating 2 μM of protein with varying ligand concentrations. In order to determine the fraction of the protein bound to ligand (fb), the data was normalized to values of 0 and 1 for the fluorescence signal of the completely unbound state and completely bound state, respectively. The solid lines through the points are fits the equation, $\mathrm{fb} = \frac{[\mathrm{L}]}{[\mathrm{L}] + \mathrm{K_D}}$.