Supplementary Materials

"Native state interconversion of a metamorphic protein requires global unfolding"

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Material and Methods

Production Expression, Purification and Refolding. All human Lymphotactin used in this study were produced using established laboratory protocols described in reference 7 of this report.

Stopped-flow experiments for determining interconversion and unfolding rates. Stopped-flow kinetic experiments were preformed on an Applied Photophysics SX18.MV stopped-flow spectrophotometer equipped with photomultiplier for detection of fluorescence signals. Detector was positioned perpendicular to sample cell with incident signal passing through a 320 nm long-pass filter prior to data collection. All experiments were initiated by excitation of protein samples at 282 nm. Sample chamber was equipped with a water-jacketed mixing manifold that allowing temperature control between 283 K and 323 K using an ISOTEMP 1013P water-bath. A final protein concentration of 30uM protein monomer was used in all experiments. Resulting fluorescence curves were fit to single exponential decays using pro Fit 6.1.11 software from QuantumSoft. Kinetic rates were determined by averaging 4-8 traces per sample point. Interconversion into Ltn10 was accomplished by mixing 30μM of protein with 200mM NaCl in 20mM phosphate buffer pH 6 (final concentrations) monitored at temperatures described in text. Interconversion into Ltn40 was accomplished by mixing 30μM of Ltn protein with 60μM of low molecular weight heparin (Sigma Chemicals) in 20mM phosphate buffer pH 6 (final concentrations). An estimation of Ltn10 and Ltn40 populations at temperatures described in text are shown in Table S1 and are based on 1D NMR measurements described in reference 8 of this report.

All unfolding experiments were conducted by rapid mixing with urea (Sigma chemicals) at concentrations displayed in Figure S1. Selection of each Ltn conformer present at the start of denaturant mixing experiments was accomplished by adjustment of temperature and solution conditions. Ltn10 unfolding was measured by buffering solutions in 20mM NaHPO4, with 200mM at temperatures < 298K , while the Ltn40 unfolding was measured by buffering in 20mM NaH2PO4, with no added salt at temperatures > 313K. The resulting data appeared consistent with the equation:

$\ln(k_{obs}) = \ln(k_{0M\ urea}) - m_f[urea]$

that describes the relationship between observed unfolding rates and chemical denaturant for two-state unfolding. Intrinsic unfolding rates used in Eyring analysis were determined by linear extrapolation to 0 M urea (y-intercept of $\ln(k_{0M\ urea})$) as depicted in Figure S1. Error bars associated with extrapolations and Eyring plots were determined by error propagation of standard deviations of rate averages.

Table S1

Temperature (K)	Ltn10 Population	Ltn40 Population
291	0.7	0.3
294	0.6	0.4
298	0.5	0.5
303	0.4	0.6
308	0.3	0.7
311	0.2	0.8
315	0.1	0.9
318	0	1.0
323	0	1.0

Estimates of fractional populations of Ltn10 and Ltn40 at temperatures employed in interconversion fluorescence measurements.

Figure Legends

Figure S1. Semilogarithmic plots of kinetics of Ltn unfolding as a function of urea concentration. Top: Extrapolation plots of Ltn10 unfolding rates to 0M urea determined for the temperature range 283 K to 291 K. Bottom: Extrapolation plots of Ltn40 unfolding rates to 0M urea determined for the temperature range 313 K to 323 K.

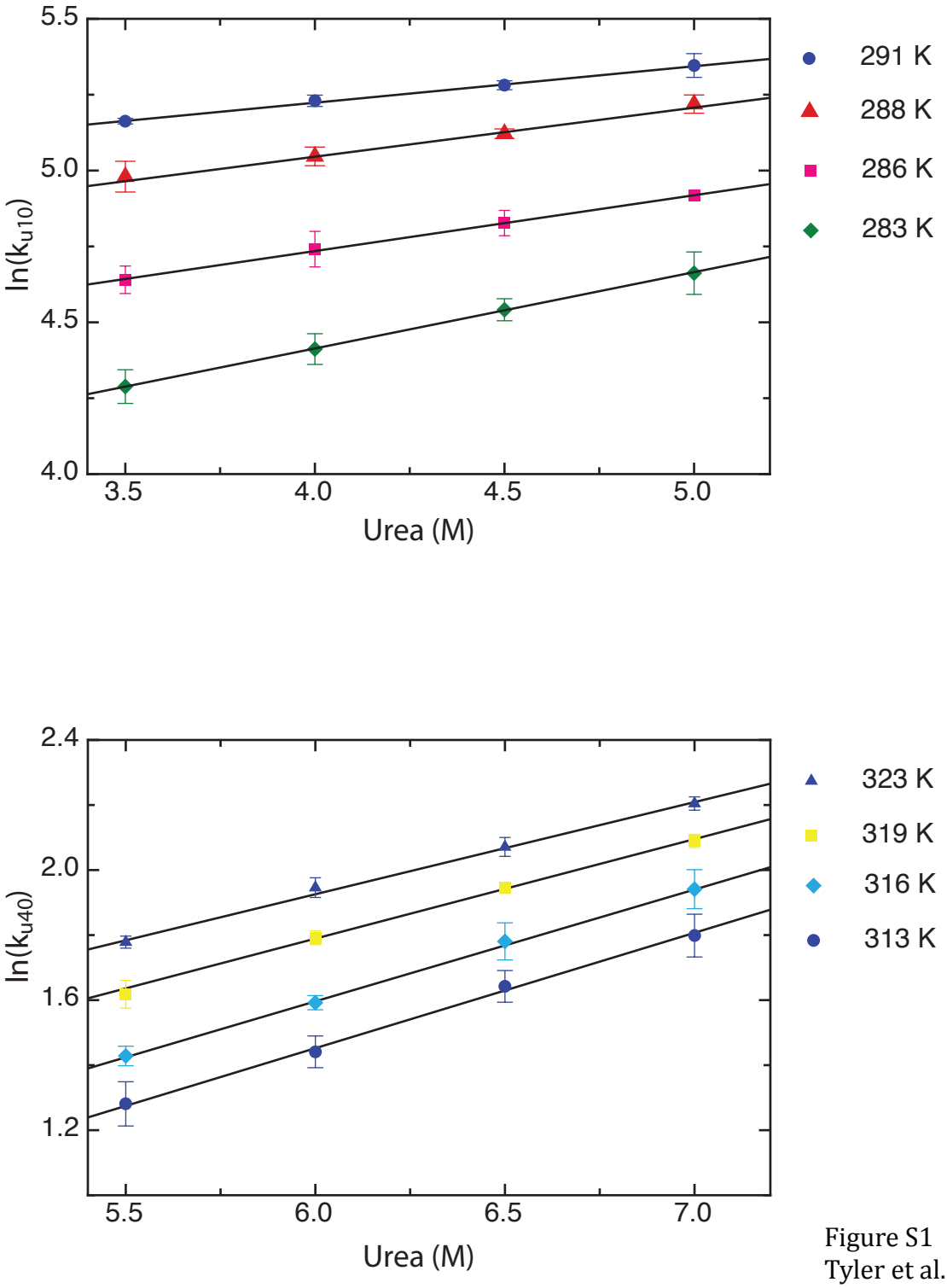

Figure S1
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