Supplementary Materials

"Slow transition between two β-strand registers is dictated by protein unfolding"

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Materials and Methods

Measuring the +3/WT conformation interconversion rate in ARNT PAS-B Y456T

Point mutants of ARNT PAS-B were created, expressed and purified using methods described previously[1]. 400 μL of 1.2 mM ARNT PAS-B Y456T protein suspended in 50 mM Tris (pH 6.6), 17 mM NaCl and 5 mM DTT buffer was injected over a 1 mL MonoQ column located in a 4°C refrigerator at a rate of 0.5 ml per minute. The chromatogram resulted in three peaks, in the absence of any salt gradient. The first eluted peak fraction yielded 350 μL of a 40 μM sample, which was enriched ~88% for the +3 conformation, as established by ^{15}N-^{1}H HSQC spectra. The two peaks that eluted after this first fraction remained in an equal mixture. Due to the low protein concentration of the first fraction, multiple injections were required to obtain a reliable measurement of the relative intensities. Upon elution from the column, the protein was placed on an ice slurry to slow any interconversion while an additional two FPLC runs were conducted. The total time to run all three FPLC experiments was approximately 15 min. The first fraction from all three runs were pooled together and concentrated to a final volume of 500 μL, which typically equated to a 140 μM concentration. To directly detect the rates of interconversion, NMR experiments were carried out on a Varian Inova 600 MHz NMR spectrometer with samples of uniformly ^{15}N, ^{13}C-labeled ARNT PAS-B Y456T mutant in 50 mM Tris (pH 6.6), 17 mM NaCl and 5 mM DTT solutions. A series of 1D ^{13}C edited ^{1}H NMR experiments were recorded until equilibrium was established, with the total time set to

four times the expected time constant for each temperature. Peak intensities over time were plotted using the DosyView utility in NMRDraw[2] and the respective rates were measured. Chemical shift assignments for ARNT PAS-B Y456T were transferred from prior solution NMR studies of the wt[3] and +3[1] conformations.

The procedure by which the +3 conformation of ARNT PAS-B P449A/Y456T was separated using ion exchange column is the same as that detailed for Y456T under the same solution conditions. Since this enriched fraction was kinetically trapped for at least 100 hours, the experiment was repeated and this time, after 24 hr at 298 K, GdmHCl was added. As such, 500 μL of a 190 μM concentration of P449A/Y456T was denatured in 3.5 M Gdn·HCl, for a final volume of 1 mL for four hours. The sample was then diluted to a final concentration of 1.0 M GdmHCl and re-concentrated down to 500 μL and analyzed by $^{15}N/^{1}H$ HSQC spectra on the refolded fraction.

Stopped-flow Experiments for Measurements of Protein Unfolding/Folding Rates

Rapid mixing experiments were conducted using a Bio-Logic SFM-3 stopped flow apparatus equipped with a 1 cm path length quartz cell. All experiments were carried out at 25°C in 50 mM sodium phosphate buffer (pH 7.5) and 17 mM NaCl. A monochromator was used for excitation at 280 nm and the intensity of fluorescence emission was recorded. Experiments were controlled by BioKine 16 V 3.03 software and had an estimated dead time of 6 ms. Kinetic rates were measured by fitting the average of 10-18 traces per sample point to a single exponential curve, using BioKine 16 software. Twenty-four hours prior to recording the refolding rates, the proteins were denatured to 4 M Gdn·HCl and placed at 25°C to ensure equilibrium was achieved.

Figure Legends

Figure S1. Two conformations of the Iβ−strand within ARNT PAS-B. Sidechains from residues in the Iβ strands of wildtype (blue; structure of wildtype ARNT PAS-B, PDB code: 1X0O[3]) and +3 (green; structure of F444Q/F446A/Y456T ARNT PAS-B, PDB code: 2K7S[1]) conformations adopt similar positions despite different chemical groups.

Figure S2. Eyring plot for the temperature dependence of the interconversion rate of ARNT PAS-B Y456T starting from the +3 conformation and returning to a wt:+3 equimolar distribution.

Figure S3. Chevron plot for Gdn•HCl-dependent folding and unfolding studies of ARNT PAS B wildtype and mutants. Rates of folding and unfolding are graphed for wildtype (black), Y456T (blue) and F444Q/F446A/Y456T (red) at 298 K. Error bars represent the standard deviation for each data point consisting of at least 10 measurements.

Figure S4. Measuring the temperature dependence on the unfolding rates for ARNT PAS-B Y456T. a. Unfolding rates measured over a range of temperatures (black, 278 K; red, 284 K; green, 291 K; and blue, 298 K). Error bars represent the standard error for each data point consisting of at least 10 measurements. **b.** Analysis of the Eyring plot for the unfolding process yields an enthalpic barrier of 11.8 kcal/mol and an entropic barrier of -9.8 kcal/mol at 298 K.

Figure S5. MonoQ separation of P449A/Y456T. Following injection over a MonoQ column, one fraction is enriched (>95%) for the +3 conformation. After 80 hours of incubation at 298 K,

this fraction remains >95 percent for the +3 conformation, suggesting it is kinetically trapped. Refolding this fraction from a denatured state, generated by the addition of Gdn•HCl, re-establishes the equilibrium (28:72; wt:+3).

Figure S6. Chevron plot for various ARNT PAS-B mutants. The rates of folding and unfolding are graphed for both wildtype (black), Y456T (blue), P449A/Y456T (magenta) and F444Q/F446A/Y456T (red).

References

(1) Evans, M. R.; Card, P. B.; Gardner, K. H. *Proc Natl Acad Sci U S A* **2009**, *106*, 2617-22.
(2) Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J Biomol NMR* **1995**, *6*, 277-93.
(3) Card, P. B.; Erbel, P. J.; Gardner, K. H. *J Mol Biol* **2005**, *353*, 664-77.

Additional Reference Information – Full Author List for Ref. 9

Maxwell, K. L.; Wildes, D.; Zarrine-Afsar, A.; De Los Rios, M. A.; Brown, A. G.; Friel, C. T.; Hedberg, L.; Horng, J. C.; Bona, D.; Miller, E. J.; Vallee-Belisle, A.; Main, E. R.; Bemporad, F.; Qiu, L.; Teilum, K.; Vu, N. D.; Edwards, A. M.; Ruczinski, I.; Poulsen, F. M.; Kragelund, B. B.; Michnick, S. W.; Chiti, F.; Bai, Y.; Hagen, S. J.; Serrano, L.; Oliveberg, M.; Raleigh, D. P.; Wittung-Stafshede, P.; Radford, S. E.; Jackson, S. E.; Sosnick, T. R.; Marqusee, S.; Davidson, A. R.; Plaxco, K. W. Protein Sci 2005, 14, 602-16.

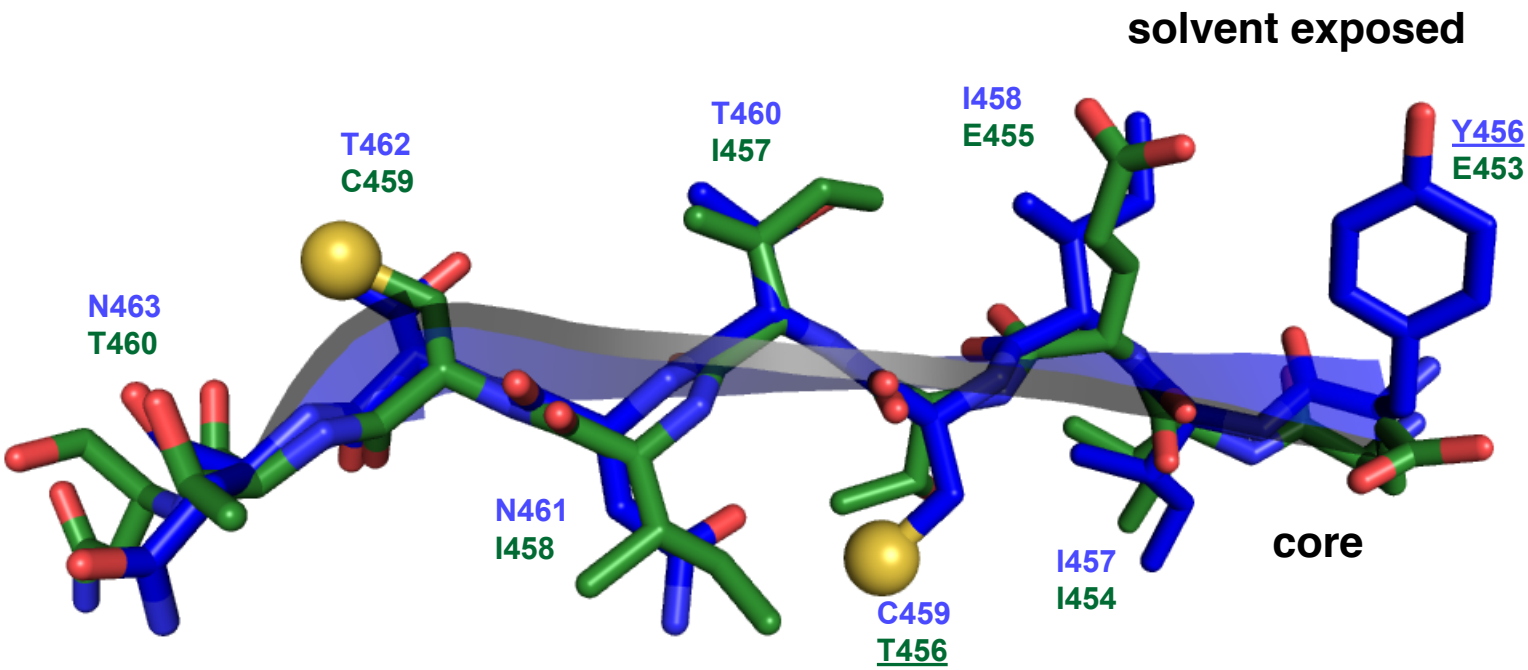

Figure S1
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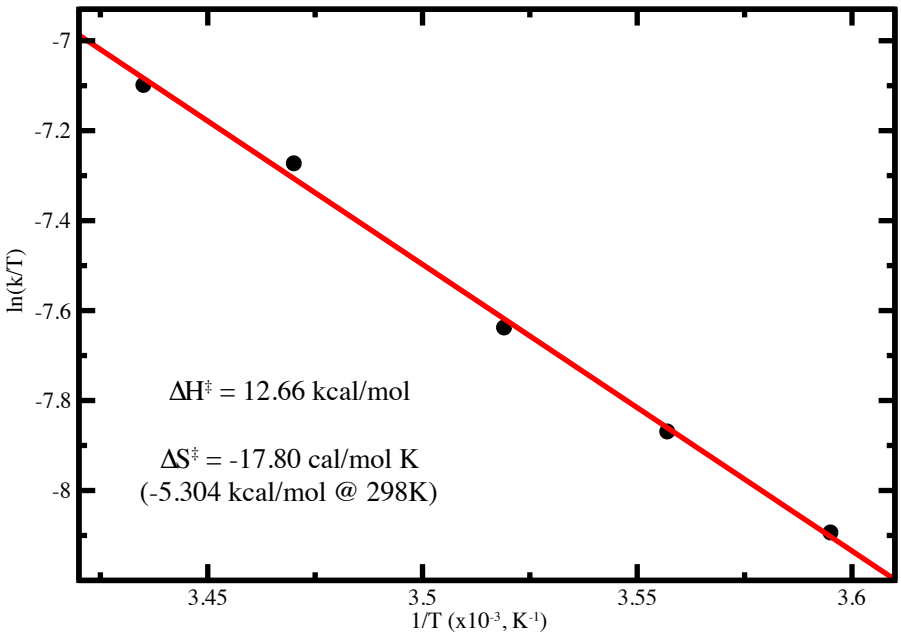

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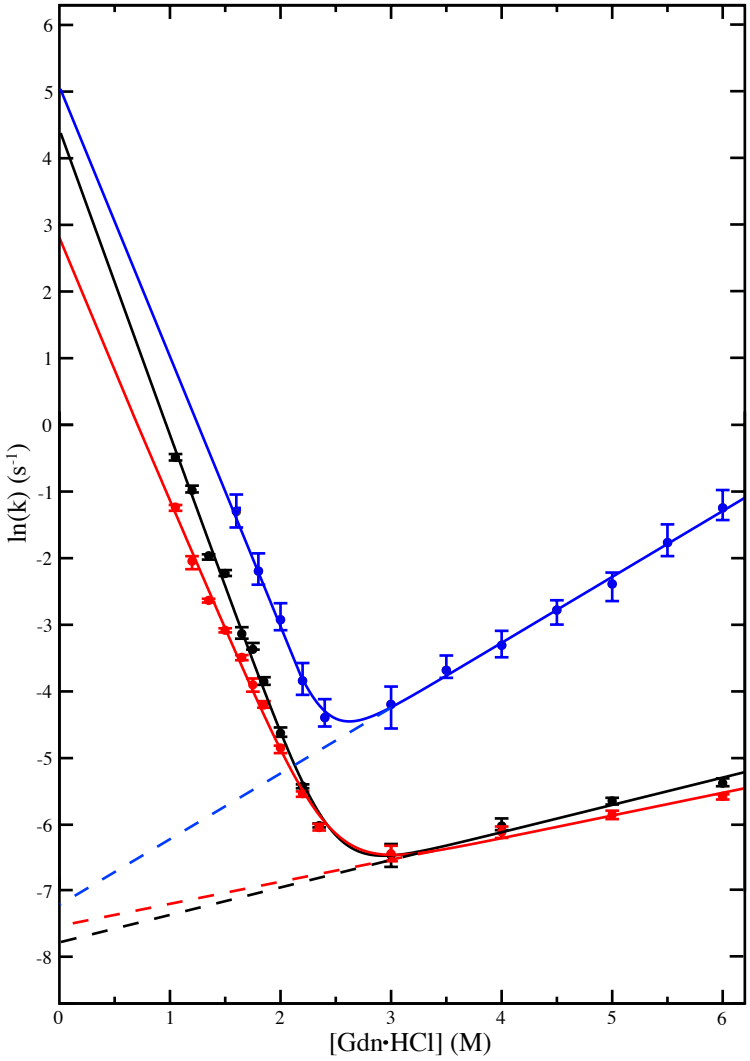

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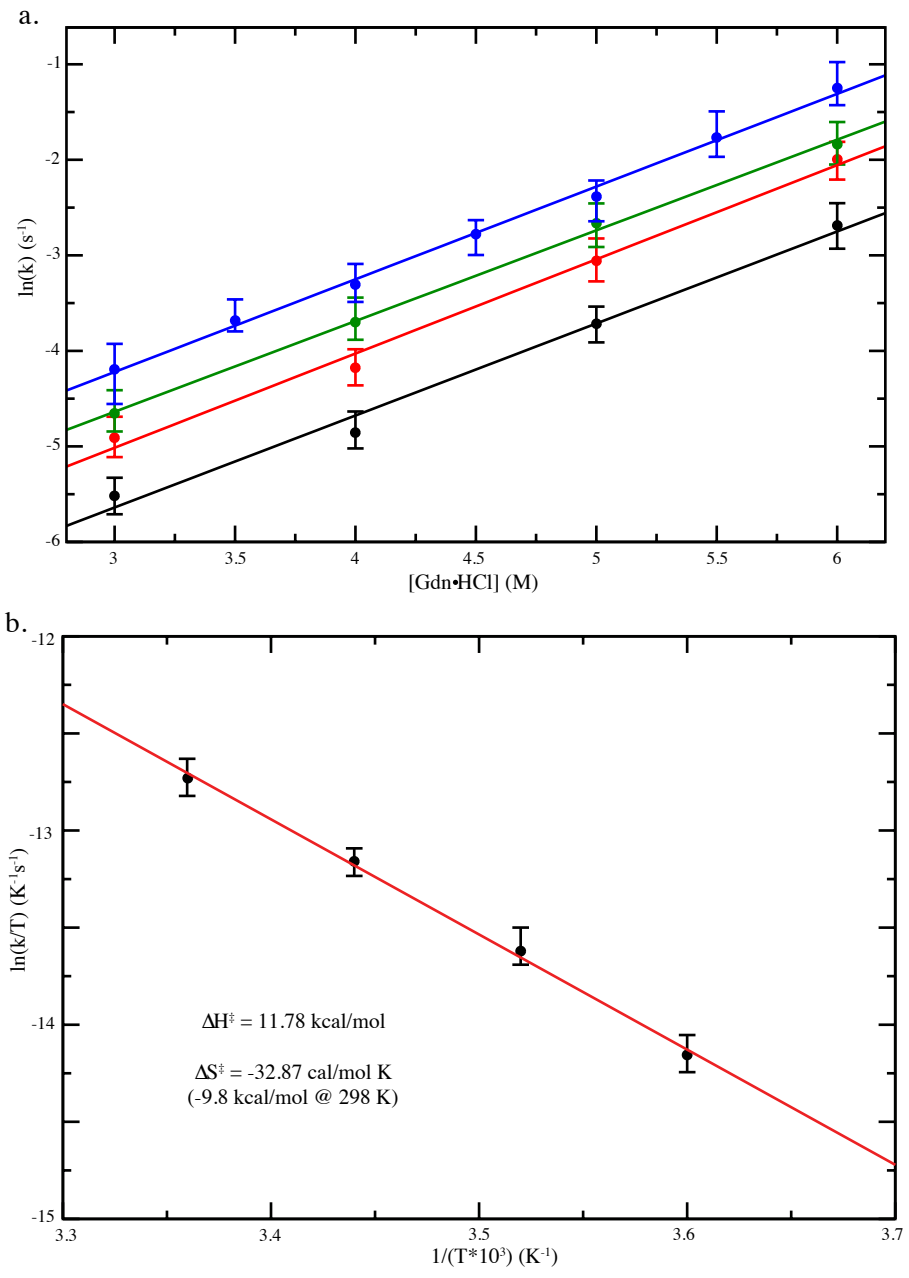

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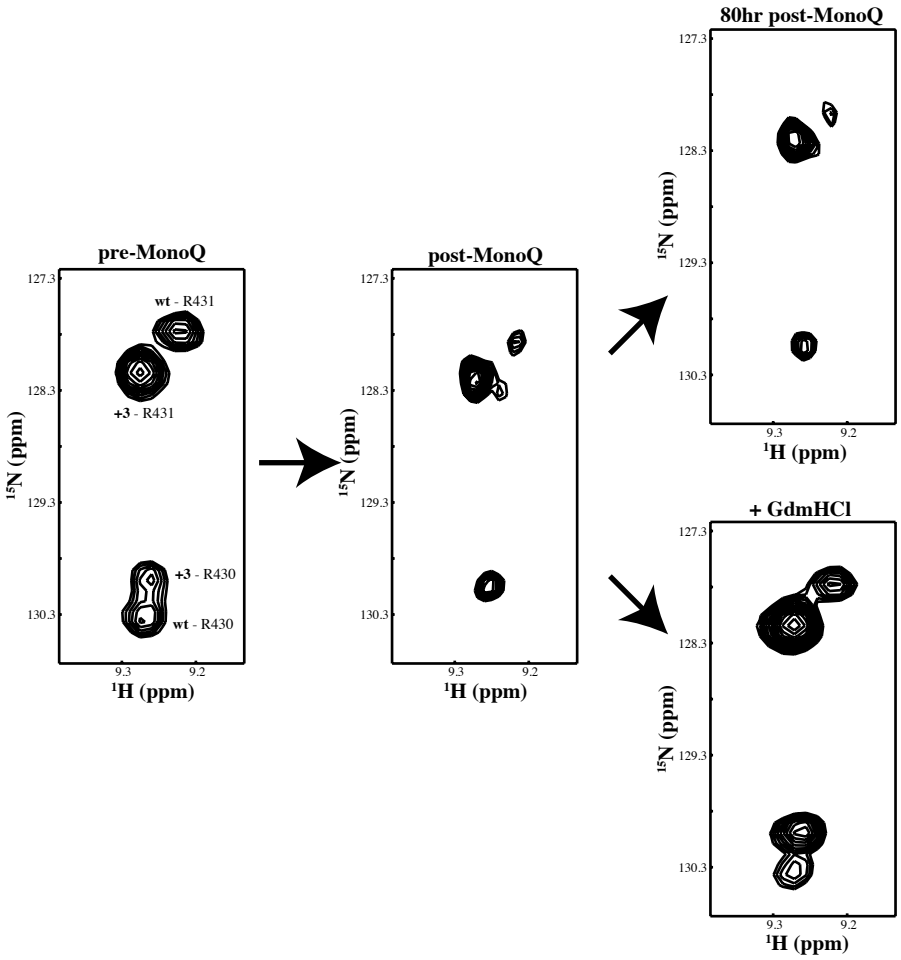

Figure S5
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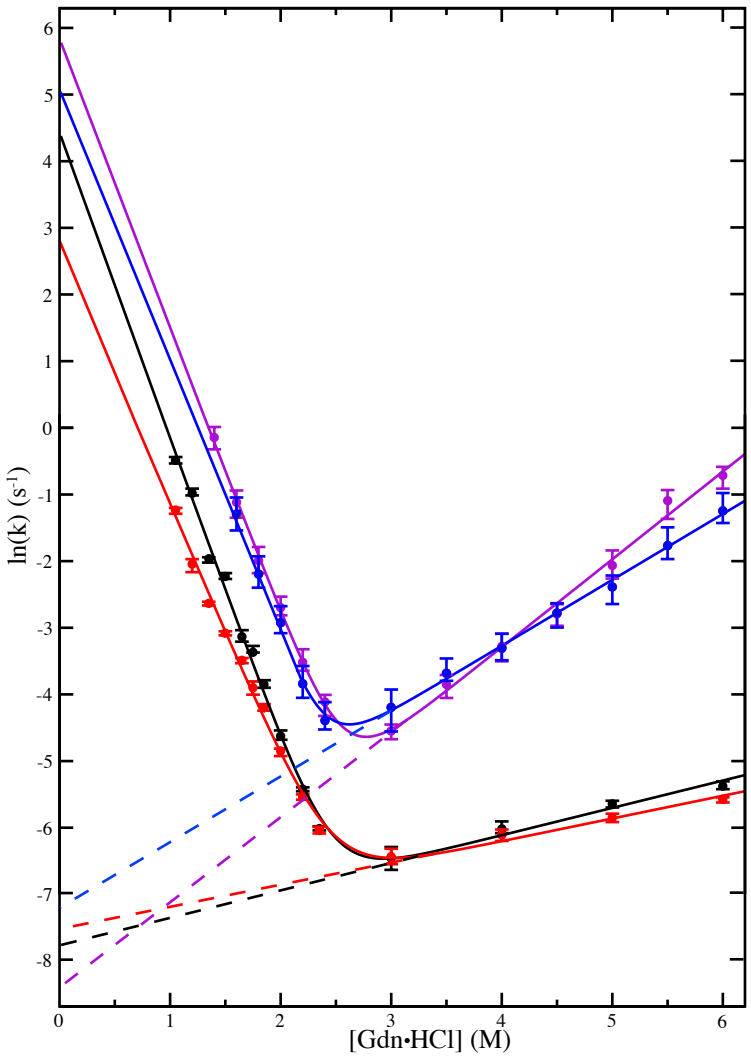

Figure S6
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