**Supplementary Material for:**

# Different members of a simple 3-helix bundle protein family have very different folding rates and fold by different mechanisms.

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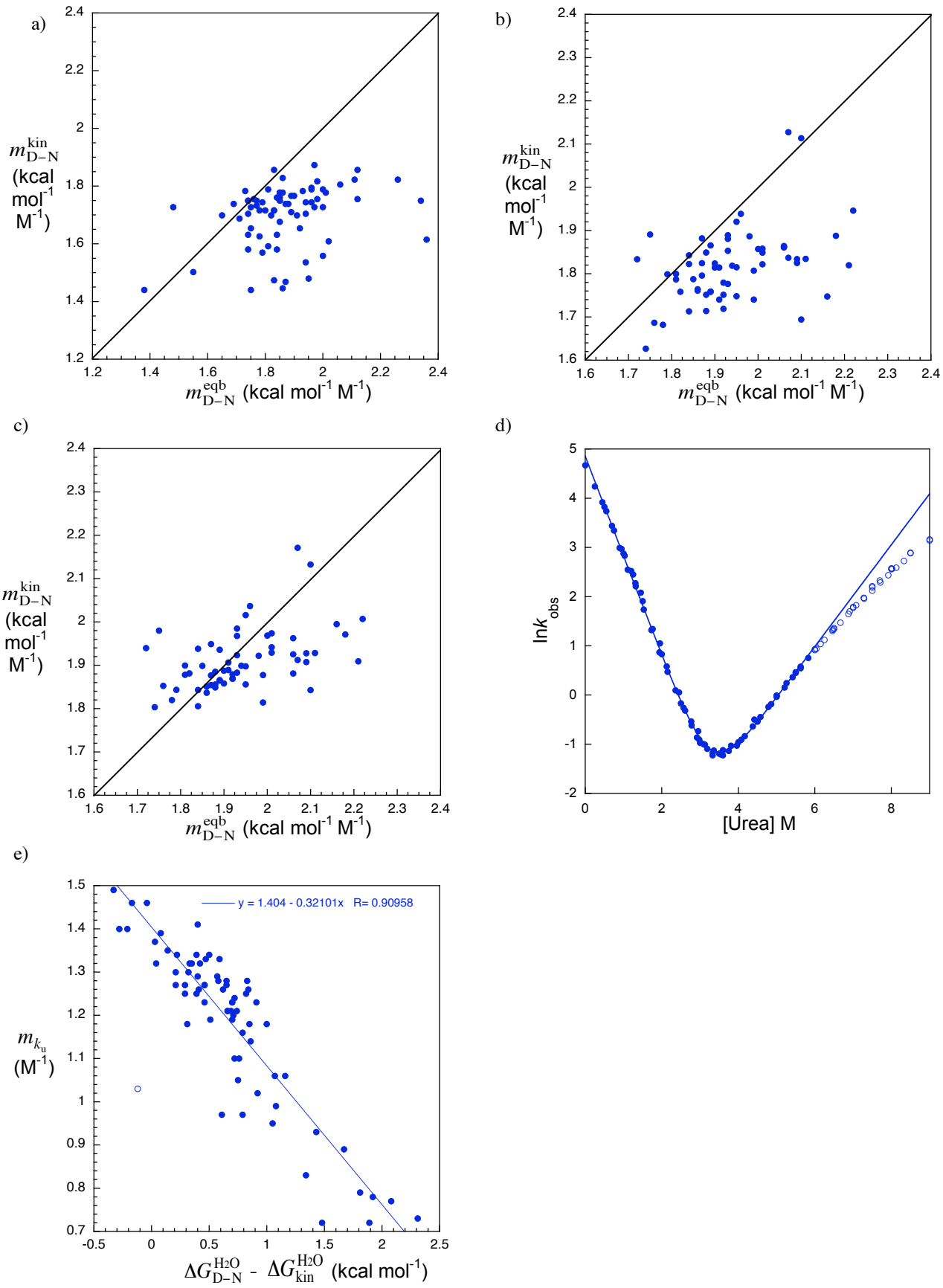
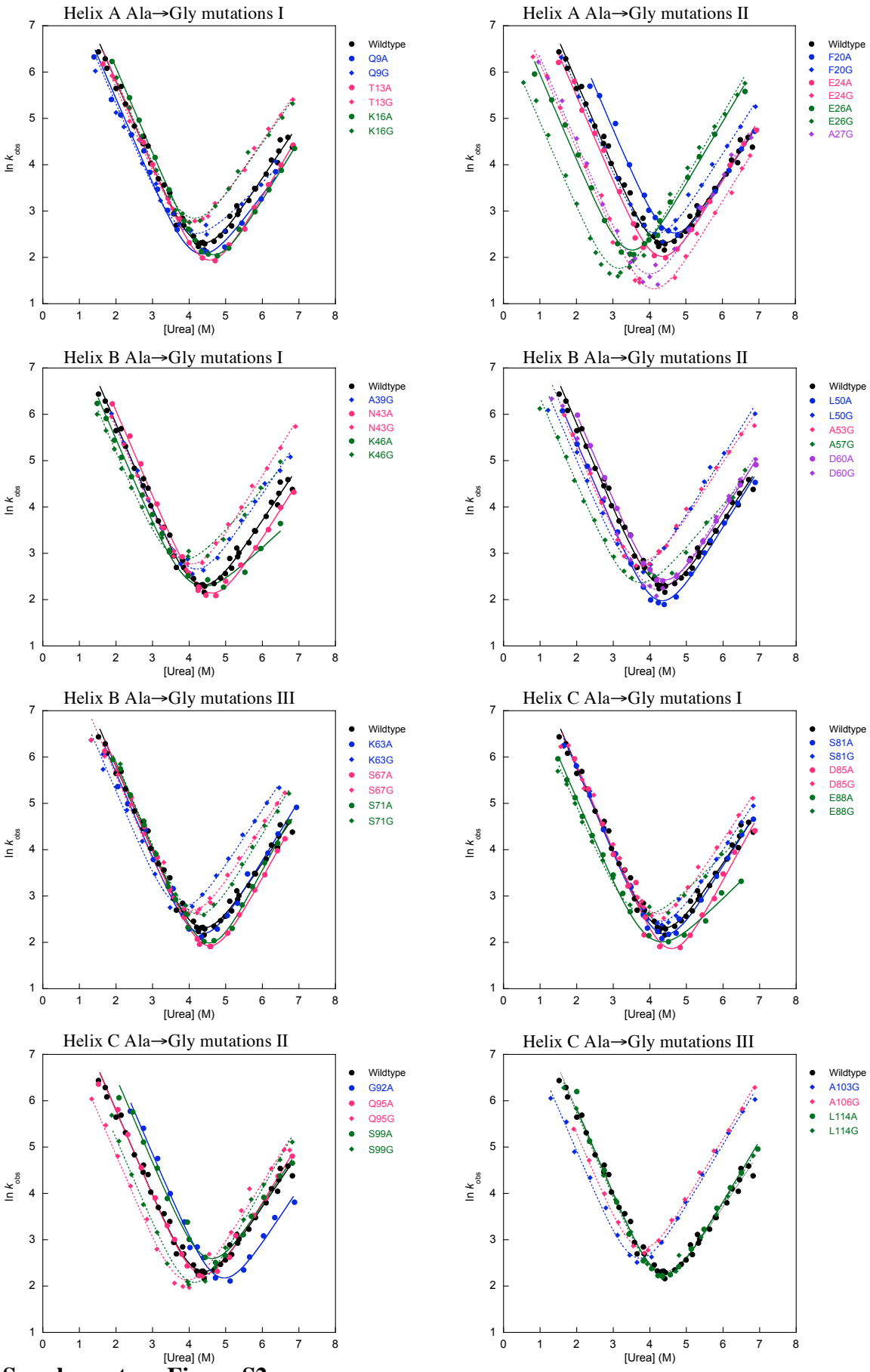


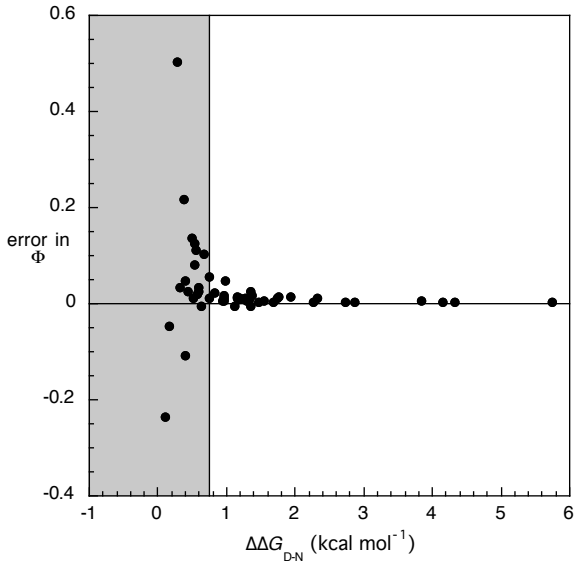

**Supplementary Figure S1.** The variation seen in $m_{k_u}$ and its effects. (a) – (c) Comparisons of equilibrium ($m^{eqb}_{D-N}$) and kinetic ($m^{kin}_{D-N}$) $m_{D-N}$ for (a) wild-type R15 and all mutants, (b) wild-type R16 and all mutants using only data collected between 0 and 6 M urea and fit with a

standard two-state chevron fit and (c) R16 and all mutants using all data collected and fit with a sequential transition state fit[1,2]. Lines have the equation $y = x$. (d) The chevron plot for wild-type R16 at 25 ˚C. Data collected > 6 M urea have open circles and were not included in the fit. The continuous line represents the fit of the filled circles to a standard two-state chevron fit with parameters: $k_f^{H_2O} = 128\ s^{-1}$, $k_u^{H_2O} = 5 \times 10^{-3}\ s^{-1}$, $m_{k_f} = 2.0\ M^{-1}$, $m_{k_u} = 1.0\ M^{-1}$. (e) Effect of $m_{k_u}$ on $\Delta G_{D-N}^{H_2O} - \Delta G_{kin}^{H_2O}$ for wild-type R15 and all mutants. The equation shows a linear fit to the data. The open circle is I55A, which has a very short unfolding arm due to a low stability and low Φ-value, and has been excluded from the fit.




**Supplementary Figure S2**

**Supplementary Figure S2.** Chevron plots and fits for R15 Ala-Gly scanning mutations. Continuous lines represent the fit for each mutant to a globally fitted two-state fit with a shared $m_{k_f}$.




**Supplementary Figure S3.** The errors in Φ are low where $\Delta\Delta G_{\text{D-N}} \leq 0.75$. This was the cut-off used in our study.

## References


1. Scott, K. A. & Clarke, J. (2005). Spectrin R16: broad energy barrier or sequential transition states? *Protein Sci*. **14**, 1617-1629.
2. Scott, K. A., Randles, L. G. & Clarke, J. (2004). The folding of spectrin domains II: phi-value analysis of R16. *J. Mol. Biol*. **344**, 207-221.