The effect of consensus mutation on the folding properties of IκBα

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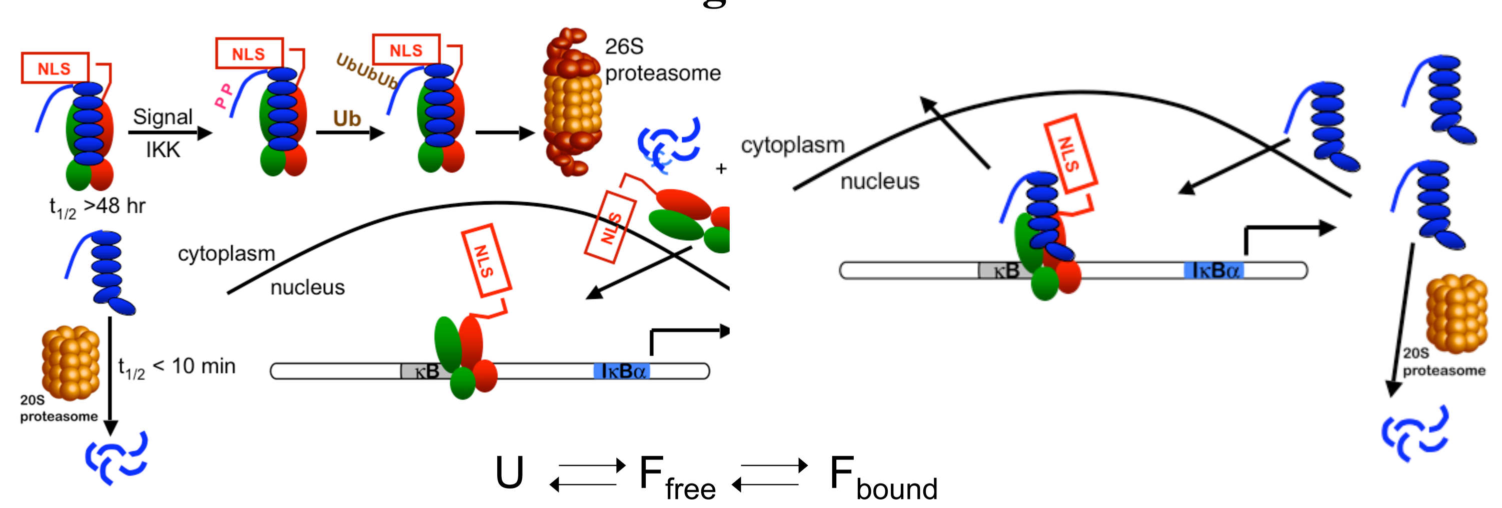
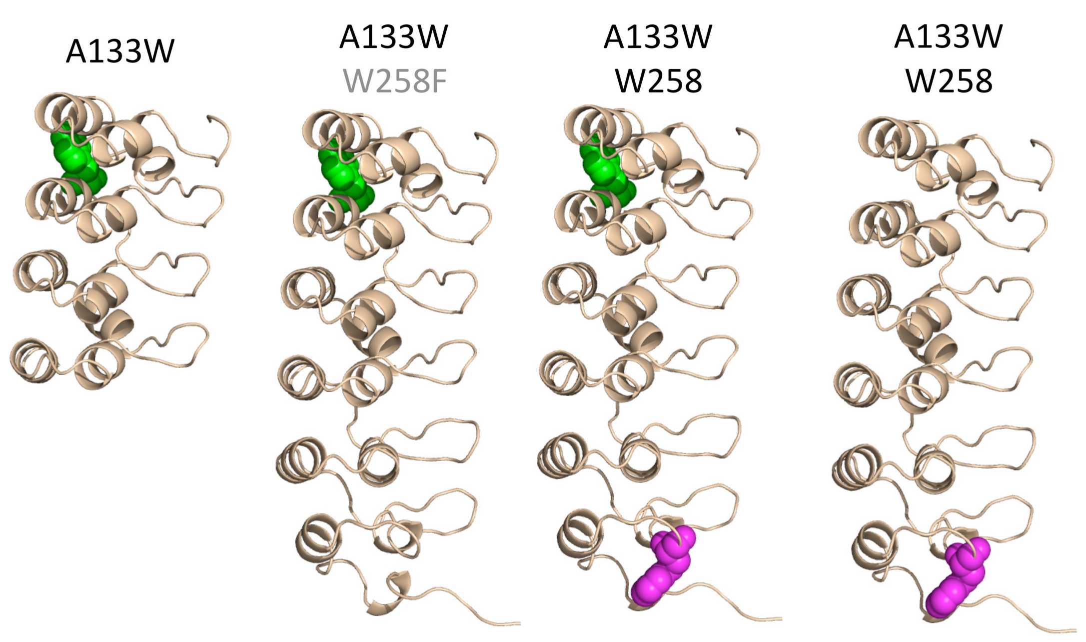
Abstract: The Inhibitor of Kappa B (IκBα) regulates the activity of the Nuclear Factor kappa B (NF-κB) by keeping NF-κB inactive and sequestered in the cytoplasm. It has been shown that parts of IκBα fold upon binding to NF-κB, suggesting that the folding process plays a role in IκBα function. We are investigating the folding of IκBα to learn more about how folding is related to function in this protein. We have begun by investigating the folding of the first four ankyrin repeats of IκBα with a tryptophan reporter engineered into repeat 2 ($I\kappa B\alpha_{67-206}W$). Mutations based on the ankyrin repeat consensus and showed that restoration of the consensus stabilized the protein while destruction of the consensus had the reverse effect. Meanwhile, kinetic effects were delineated structurally. Only mutants in helix 2 of ARs 3 and 4 affected the folding rate, suggesting folding is initiated in this region. Further studies on the folding of the 6AR construct ($I\kappa B\alpha_{67-287}$) show somewhat more complex kinetics than the 4AR construct, though mutants have similar effects.

Repeats 5&6 weakly folded in free IκBα, fold on binding to NF-κB

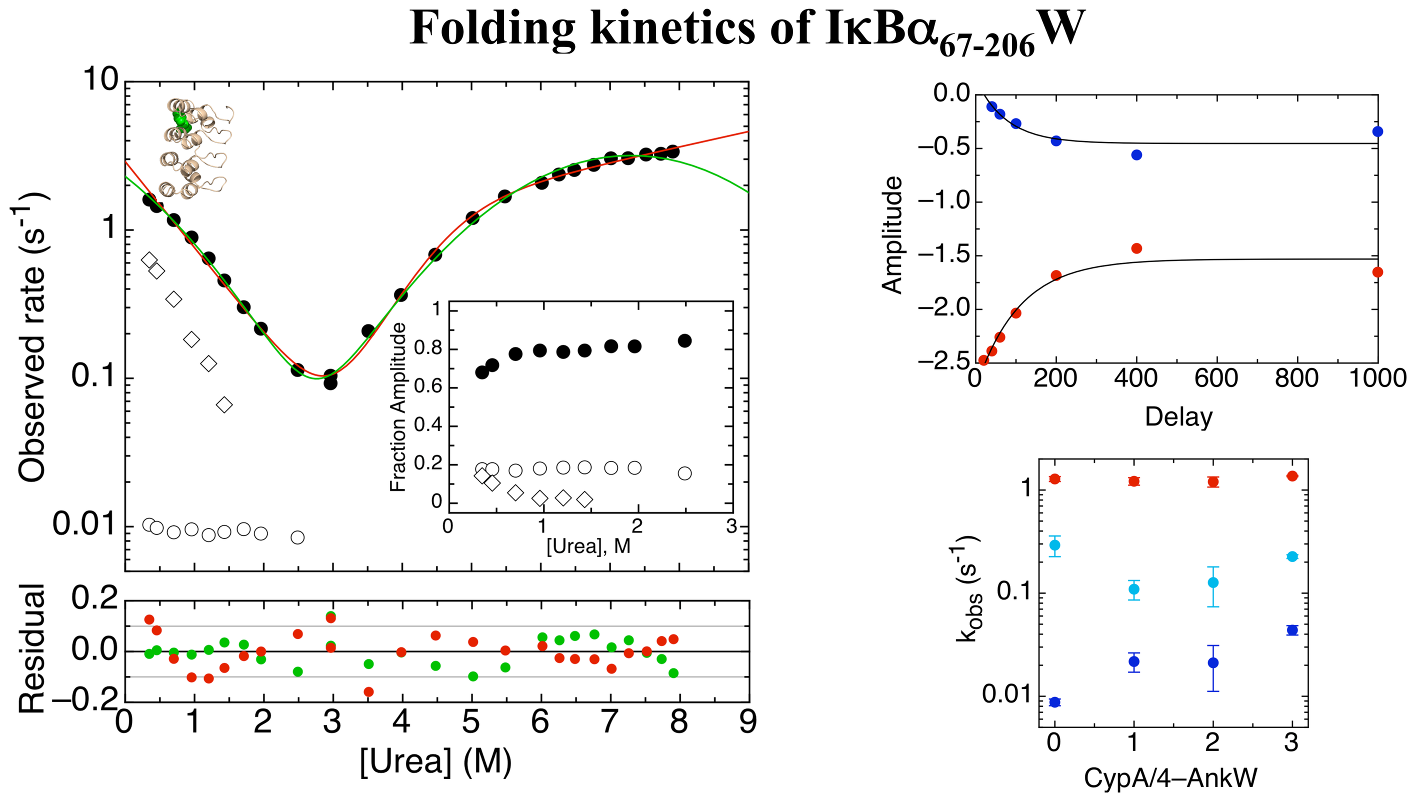


Tryptophan reporters in AR2 and AR6 can track both transitions



A133W: Engineered Trp in AR2; follows main (cooperative) transition
W258: Natural Trp in AR6; follows transition of weakly folded region (non-cooperative transition)

Folding kinetics of $I\kappa B\alpha_{67-206}W$



Folding kinetics show three refolding phases and one unfolding phase:

- Minor phases likely due to proline-isomerization
- Main refolding phase and unfolding phase fit to 2 folding models:

Two-state model

$$k_{obs} = k_{f,H_2O}\exp(-m_{kf}[Urea] - m^*[Urea]^2) + k_{u,H_2O}\exp(-m_{ku}[Urea] - m^*[Urea]^2)$$

Three-state model

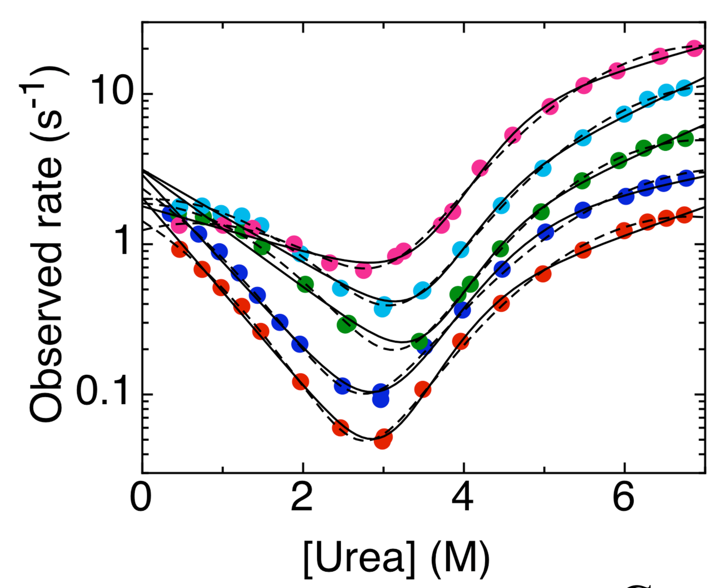
$$\lambda_{1,2} = \frac{-B \pm \sqrt{B^2 - 4C}}{2}$$

$$B = -(k'_{12} + k'_{21} + k'_{23} + k'_{32})$$

$$C = k'_{12}(k'_{23} + k'_{32}) + k'_{21}k'_{32}$$

$$k'_i = k_i \exp(m_i[Urea])$$

Temperature dependance of main phase



The curvature of the refolding arm of the chevron plot increases with temperature, suggesting that the transition state may change with temperature to some extent. Equilibrium stability is within error over this range of temperatures; at higher temperatures (15°C and above), ΔG obtained from kinetics and equilibrium do not agree.

Consenus mutants in $I\kappa B\alpha_{67-206}W$

Mutants designed based on the AR consensus. Residues deviating from the consensus were mutated to the consensus. Residues agreeing with the consensus were mutated conservatively.

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AR1  KQQLTEDGDSFLHLAIIHEEKALTMEVIRQVKGDLAF
AR2  LNFQNNLQQTPLHLAVITNQPEIAEALLGWGCD
AR3  PELRDFRGNTPLHLACEQGCLASVGVLTQSCTTPHLHSI
AR4  LKATNYNGHTCLHLASIHGYLGIVELLVSLG
Consensus---G-TPLHLA---G----V--LL--GA-----
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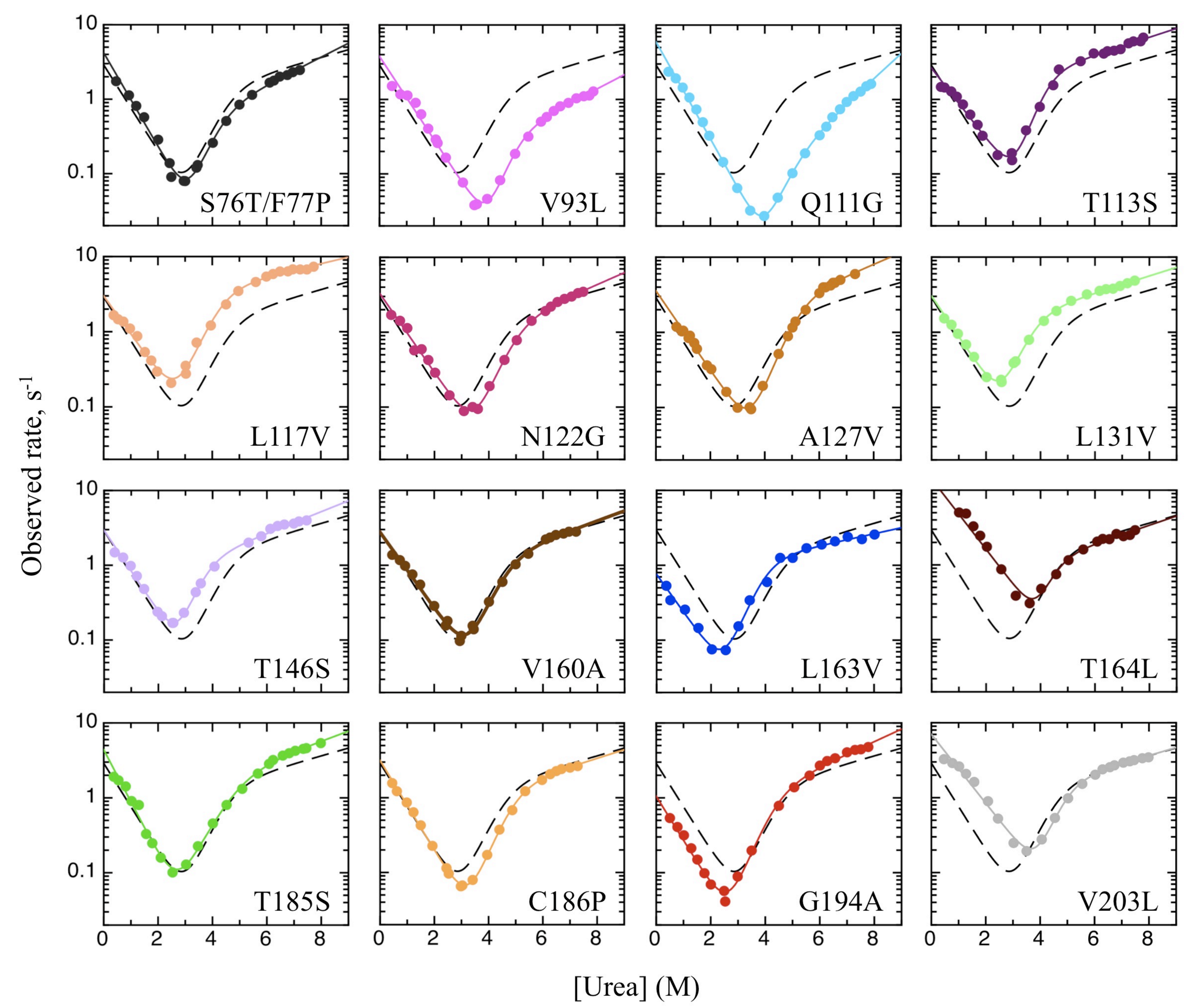


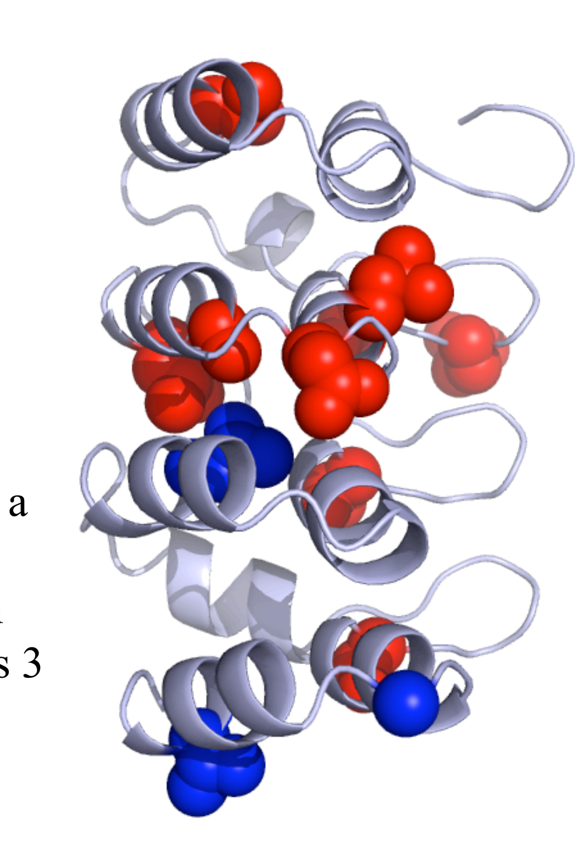
Folding kinetics of mutants were compared to $I\kappa B\alpha_{67-206}W$ and can be categorized into several distinct groups:

Minimal/no effect:	Unfolding effect:
S76T/F77P	V93L
V160A	Q111G
C186P	T113S
Refolding effect:	L117V
L163V	N122G
T164L	A127V
G194A	L131V
V203L	T146S
	T185S

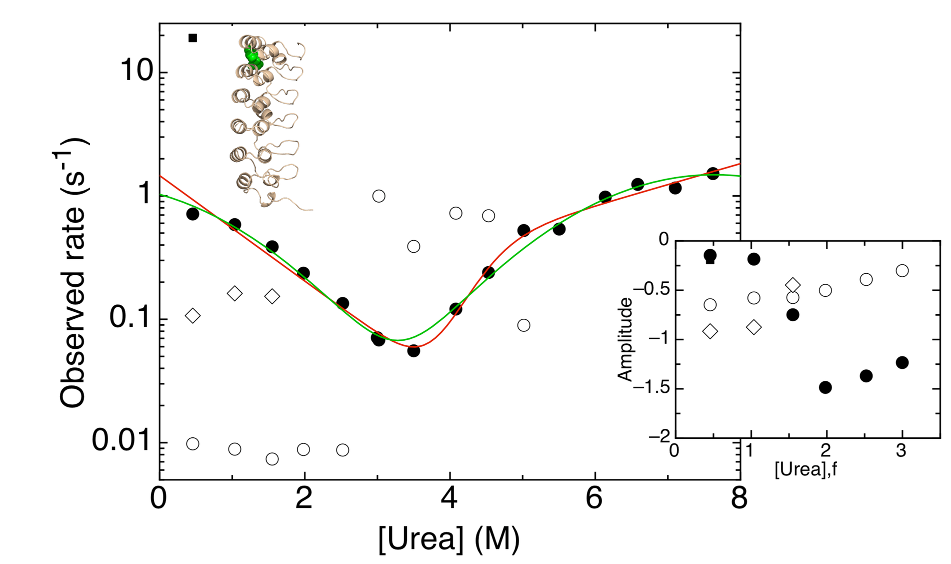
Φ-value analysis

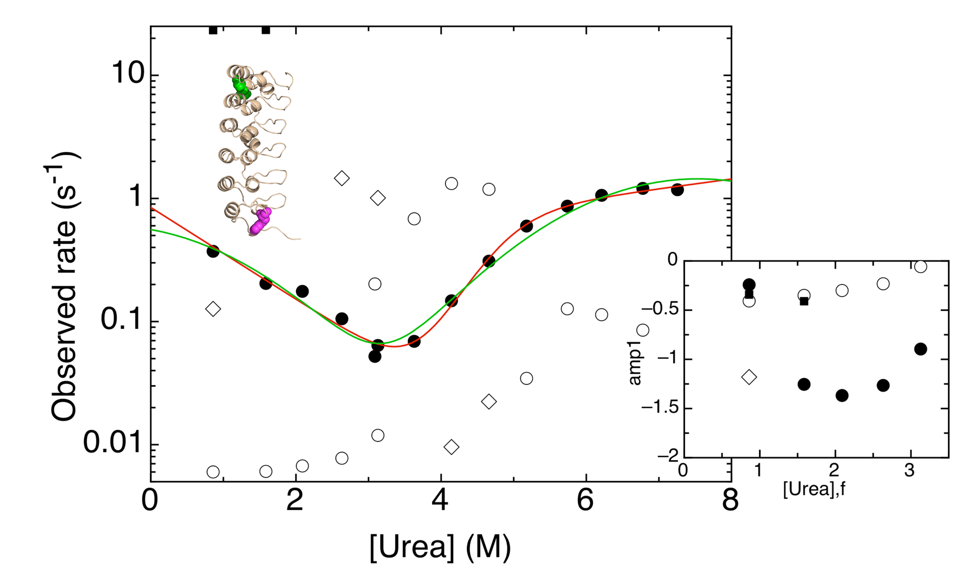
$$\Phi = \frac{RT\ln\left(\frac{k_f^{WT}}{k_f^{mut}}\right)}{\Delta\Delta G_{eq}}$$

Phi-value analysis shows a polarized transition state suggesting that folding in initiated in helix 2 of ARs 3 and 4.



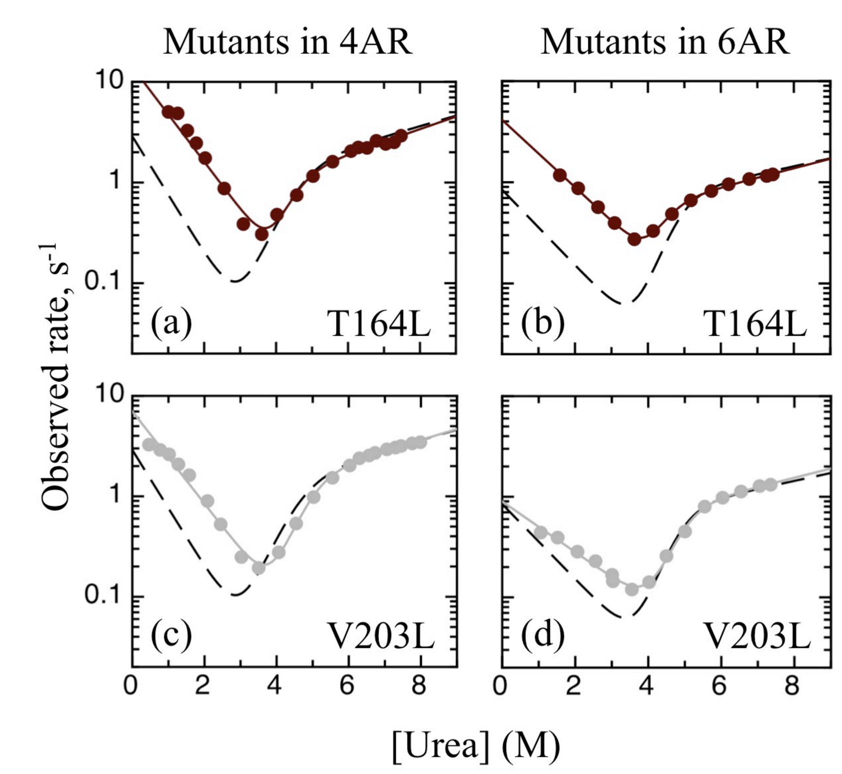
Folding kinetics of $I\kappa B\alpha_{67-287}$ Trp variants





Folding kinetics of $I\kappa B\alpha_{67-287}$ Trp variants (5°C) is similar to $I\kappa B\alpha_{67-206}W$. However, the main phase is not the phase with the largest amplitude at low [Urea]. Additional phases are also present at higher [Urea] than in $I\kappa B\alpha_{67-206}W$.

Mutants show similar effects on kinetics in 4AR and 6AR



Some of the mutants with the largest effects in $I\kappa B\alpha_{67-206}W$ were examined in the $I\kappa B\alpha_{67-287}$ A133W W258 background. T164L and V203L showed very similar effects (compared to $I\kappa B\alpha_{67-287}$ A133W W258).

Future Directions

Having examined in depth the folding of free IκBα, we will look at the effect of NFκB on the folding kinetics using the different IκBα Trp variants as well as the pre-folded YLTA mutant.

Acknowledgements

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