Determining the Foldedness of IκBα P261F and IκBα E282W

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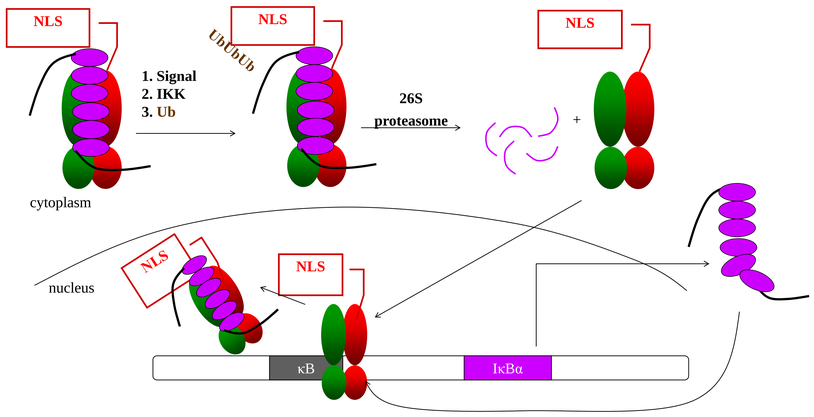
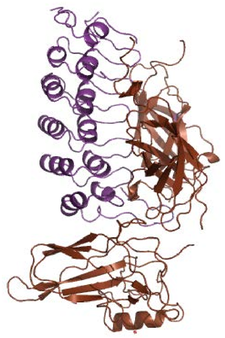
Background

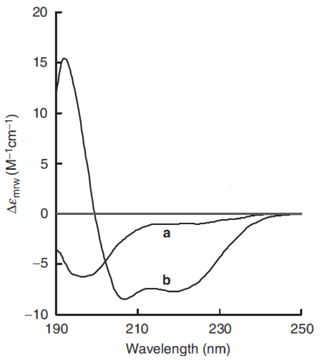
IκBα Interaction with NFκB



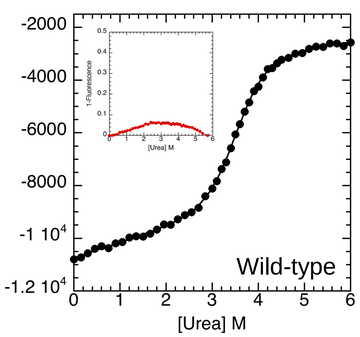
IκBα



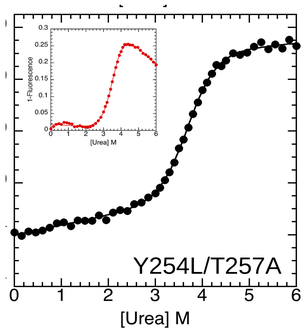
Above: Nuclear factor kB (NFkB) and its inhibitor, IκBα. PDB 1IKN.



Above: Circular dichroism (CD) spectrum to determine the foldedness of a protein. (a) non-structured protein and (b) a-helical protein.[1]



Above: Fraction unfolded in equilibrium denaturation experiments with $IκBα_{67-287}$ monitored by CD signal at 225 nm (filled circles) and fluorescence (red circles).



Above: Urea denaturation curves followed by the CD signal at 225 nm of IκBα Y254L/T257A (filled circles) and fluorescence (red circles).[2,3]

Protein Expression and Purification

Transformation

Incubate Cells with DNA
1 ng DNA was added to 50 µL BL21 (DE3) cells and incubated on ice 20 min

Heat Shock
Cells were incubated at 42 °C 45 s and then incubated at 4 °C 2 min

Recover Cells
400 µL Luria Broth were added to the transformation

Shake
Cells were incubated at 37 °C 1 h shaking

Plate Transformation
100 µL of transformation were plated on LB_{amp} plates

Growth

Starter Culture
Inoculate 10 mL M9-Zn_{amp} with 1 colony

Transfer Starter Culture
Transfer M9-Zn_{amp} culture to 1 L M9$_{amp}$ media and shake at 37 °C

Shake and Grow
Grow cells until OD_{600} ~ 0.6

Pre-Induction
Spin down and re-suspend pellet in 50 µL of sup and then mix1:1 in 2X reducing buffer

Induction
Put flask in shaker at 18 °C, 150 rpm and induce with 0.1 mM IPTG for 16 h

Isolation

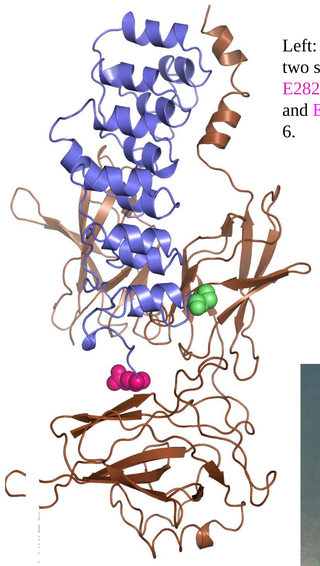
Lyse Cells
Resuspend cell pellet with 70 mL of lysate buffer (50 mM Tris pH 7.5, 150 mM NaCl…)

Sonicate cells
Pulse cells at output of 8.0 for 3-45 s intervals with 2 min interim

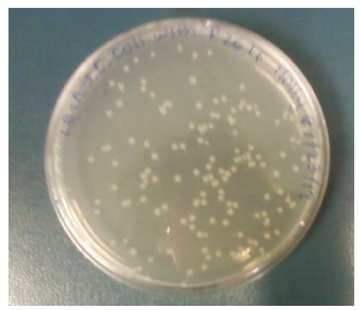
Centrifuge
Spin down lysate 45 min 12000 RPM

High Load Q-Sepharose Column
Load supernatant onto column. Elute with 225 mM – 700 mM NaCl gradient.

Size Exclusion
Load column with 2 mL purified HLQ fraction and elute in 10 mM $P0_4$, 50 mM NaCl, 1 mM DTT, 0.5 mM EDTA

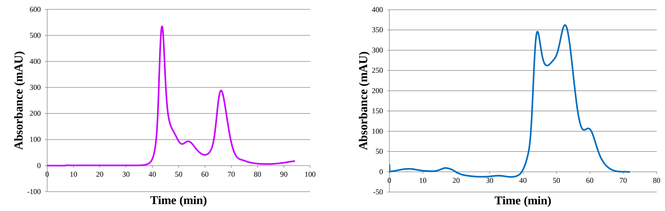


Left: The structure of IκBα shows that two site mutants which are at P261 and E282. Mutant P261 is in Ankyin Repeat 6 and E282 is at the end of Ankyin Repeat 6.



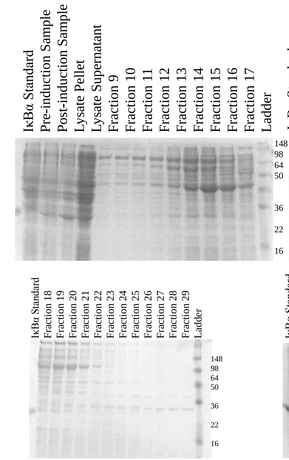
Above: Transformation of BL21 (DE3) *E. coli* with pET11a/IkBa P261 plasmid on an LB_{amp} plate that was incubated at 37 °C overnight.

Data and Results

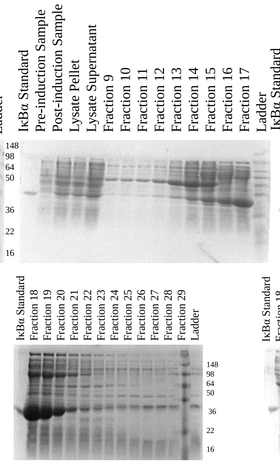


Above: S75 size exclusion chromatograms for IκBα WT (fraction 17) and IκBα E282W (fraction 14).

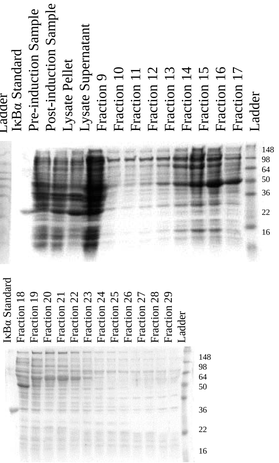
IκBα E282W HLQ



IκBα WT HLQ



IκBα P261F HLQ



Conclusions and Future Directions:

- IκBα WT was successfully expressed and purified.
- IκBα P261F and E282W mutant plasmids were transformed into BL21 (DE3) *E. coli* cells.
- The expression of the IκBα P261F and E282W mutants was not optimal under traditional IκBα expression conditions.
- I plan to optimize the expression of IκBα P261F and E282W mutants.
- I will analyze the foldedness of the IκBα P261F and E282W mutants by urea denaturation circular dichroism experiments.
- Once the mutants are purified, I will test the binding affinities of the mutants to NFκB, and I will test the abilities of the mutants to "strip" NFκB from DNA.

References

1. Martin, SR and Schilstra, MJ (2008) Circular Dichroism and Its Application to the Study of Biomolecules, 84 Method In Cells Biology, Chapter 10.

2.Devries, I., Ferreiro, D. U., Sánchez, I. E. and Komives, E. A. (2011) Folding Kinetics of the Cooperatively Folded Subdomain of the IκBα Ankyrin Repeat Domain. J. Mol. Biol. 408, 163-76.

3. Ferreiro, D. U., Cervantes, C. F., Truhlar, S. M. E., Cho, S. S., Wolynes, P. G., and Komives, E. A. (2007) Stabilizing IkBalpha by 'consensus' design. J. Mol. Biol. 365, 1201-16.

Acknowledgments

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