

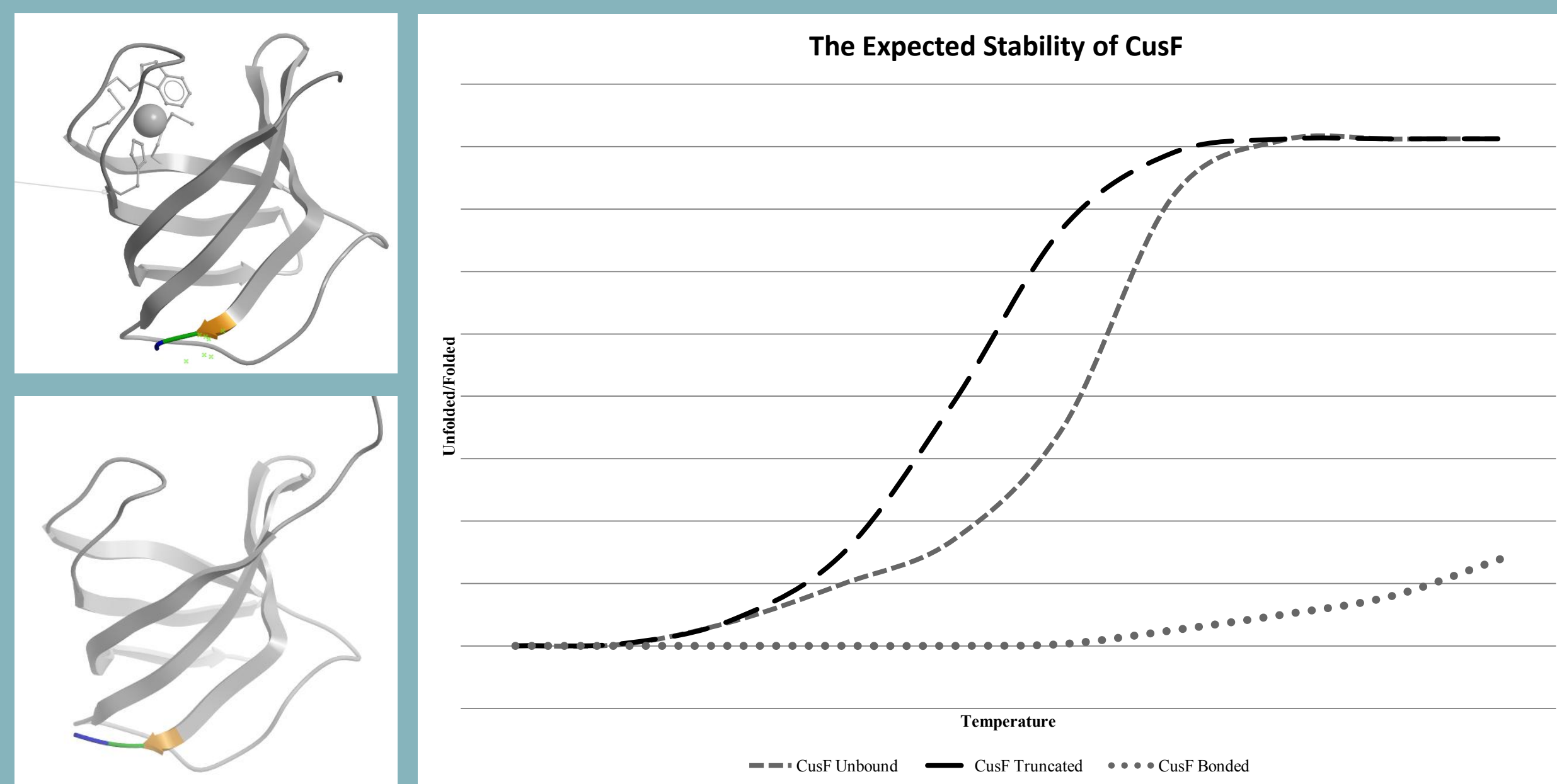
Investigating the stability of the copper binding protein CusF by C-terminal truncation via site-directed mutagenesis



Bianca Teminello, Michael Collazo, Blake Gillespie

Abstract

The bacterium *Escherichia coli* produce the protein CusF which helps the cell regulate copper and silver concentrations in the cell. When the protein is unbound to a metal ion, it will unfold in high temperatures. However, when CusF is bound to the metal atom it will not unfold. By introducing C-terminus truncation by induced mutagenesis, it is hoped that the bound protein will be unfold at lower temperatures allowing its free energy to be measured.



Mutagenesis

Truncations were made by altering the location of the stop codon. These alterations resulted in a shorter C-terminal end of the protein, which may result in the desired destabilization of CusF.

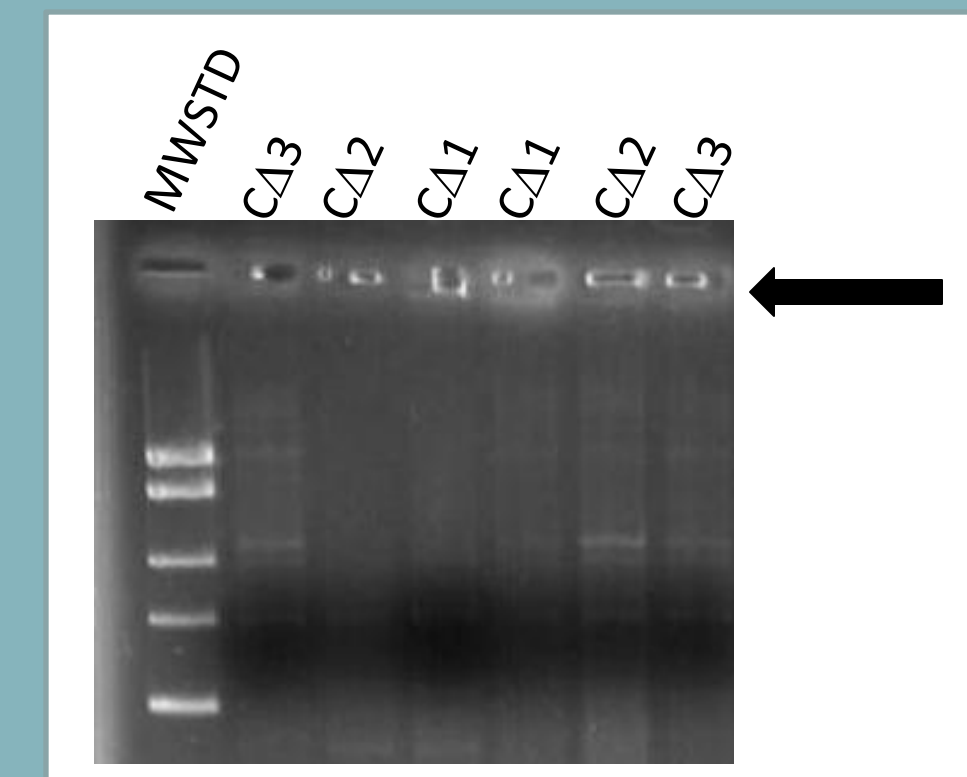
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(TEMPLATE STRAND C-TERMINAL END) (ORIGINAL STOP CODON LOCATION)
AGT.GAA...TTA.TTA.CAG.GAT.ATT.AAA.GTC.AGC.CAG.TAA.CAG.SGG.GAC.CAT...
RET.E...L.L.Q.D.I.R.V.S.G.-.Q.G.D.H...

FIRST TRUNCATION
(PRIMER OVER) GAT.ATT.AAA.GTC.AGC.CAG.TAA.CAG.SGG.GAC.C
AGT.GAA...TTA.TTA.CAG.GAT.ATT.AAA.GTC.AGC.CAG.TAA.CAG.SGG.GAC.CAT...
RET.E...L.L.Q.D.I.R.V.S.-.-

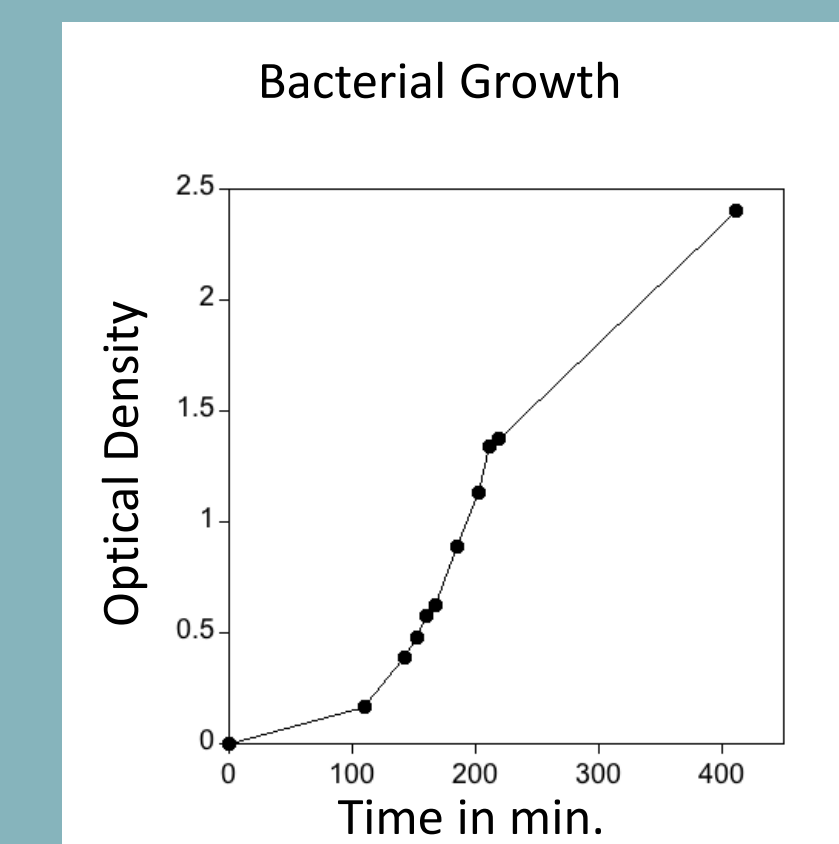
SECOND TRUNCATION
(PRIMER OVER) CAG.GAT.ATT.AAA.GTC.CAG.TAA.CAG.SGG
AGT.GAA...TTA.TTA.CAG.GAT.ATT.AAA.GTC.CAG.TAA.CAG.SGG.GAC.CAT...
RET.E...L.L.Q.D.I.R.V.-.-

THIRD TRUNCATION
(PRIMER OVER) G.GAT.ATT.AAA.TAA.AGC.CAG.TAA.CAG.SGG.GAC.C
AGT.GAA...TTA.TTA.CAG.GAT.ATT.AAA.GTC.AGC.CAG.TAA.CAG.SGG.GAC.CAT...
RET.E...L.L.Q.D.I.R.V.-.-
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Successful PCR products were evident in the agarose gel. After PCR, DPN1 was added to destroy the template DNA, leaving only non-methylated PCR products.



E. coli cells containing the modified plasmid, which imparted a resistance to the antibiotic ampicillin. The cells were placed in to a nutrient broth containing ampicillin, to prevent the growth of foreign bacteria, and cultured.



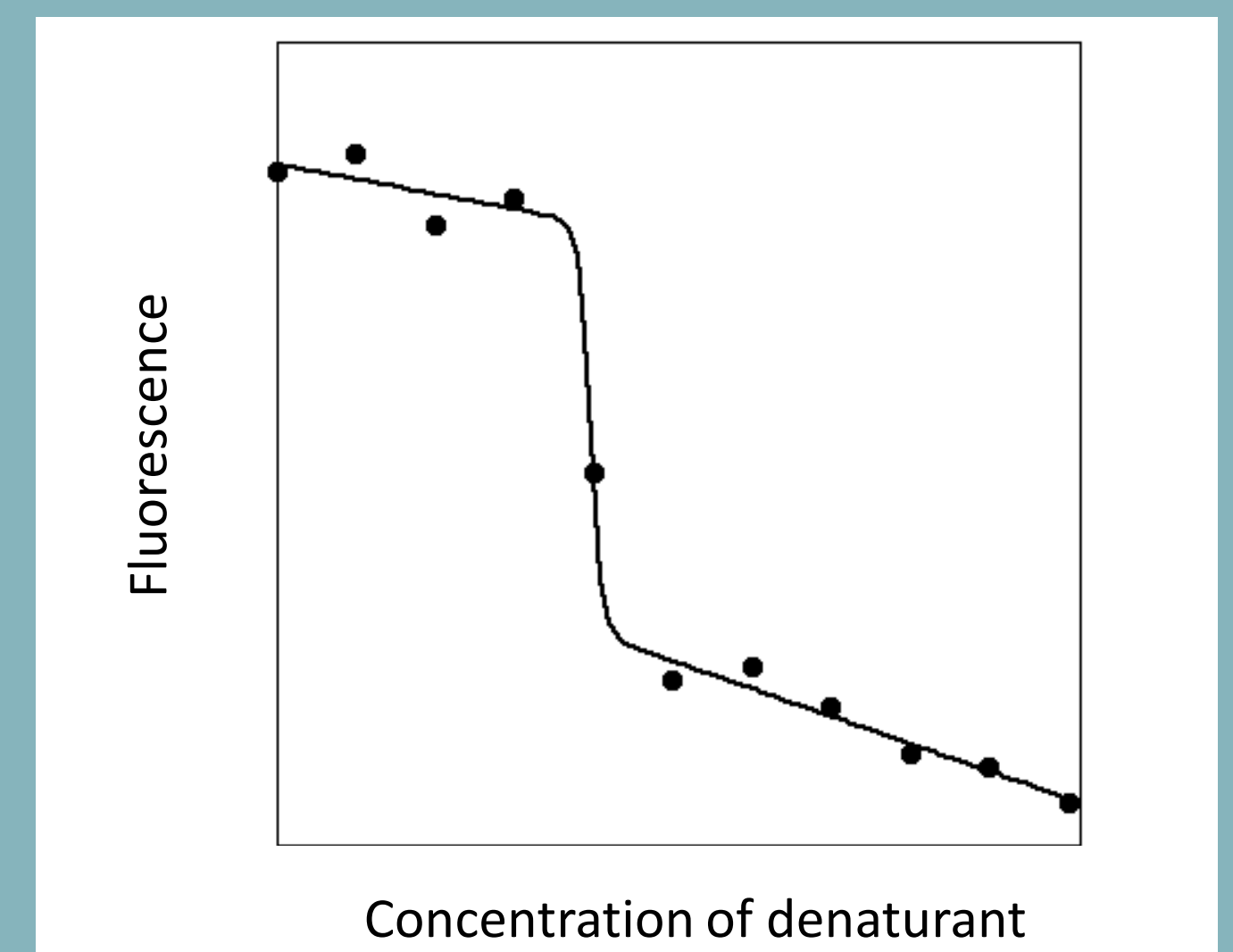
This graph shows the logarithmic growth of the *E. Coli* cells. The cells were induced with IPTG at an optical density of 0.5 to produce CusF in excess.

Characterization

The protein was characterized based on its fluorescence. The folded state of CusF will fluoresce with greater intensity than the unfolded state, allowing the ratio of folded and unfolded states to be measured. The data was curve fitted to the equation $K = e^{-(\Delta G/RT)}$, where K is the equilibrium constant and ΔG is the measurement of free energy. Guanidine salt was used as a chemical denaturant instead of thermal denaturation.

We were able to calculate that for the wild type CusF protein (unbonded) $\Delta G = -35$ kcal/mol.

Additional measurements at different denaturant concentrations will be needed to produce a more precise value.



Methods

In order to destabilize the protein once bound to the copper, several mutations were introduced at the C-terminus end. Using PCR, the mutated DNA was reproduced.

Mutagenesis

- Using primers with our desired mutations, the plasmids were reproduced using PCR.
- After, DPN1 was used to destroy the template strand, leaving only the recombinant DNA
- Plasmid uptake was caused by heat shock. The *E. Coli* were then cultured overnight.

Protein Purification

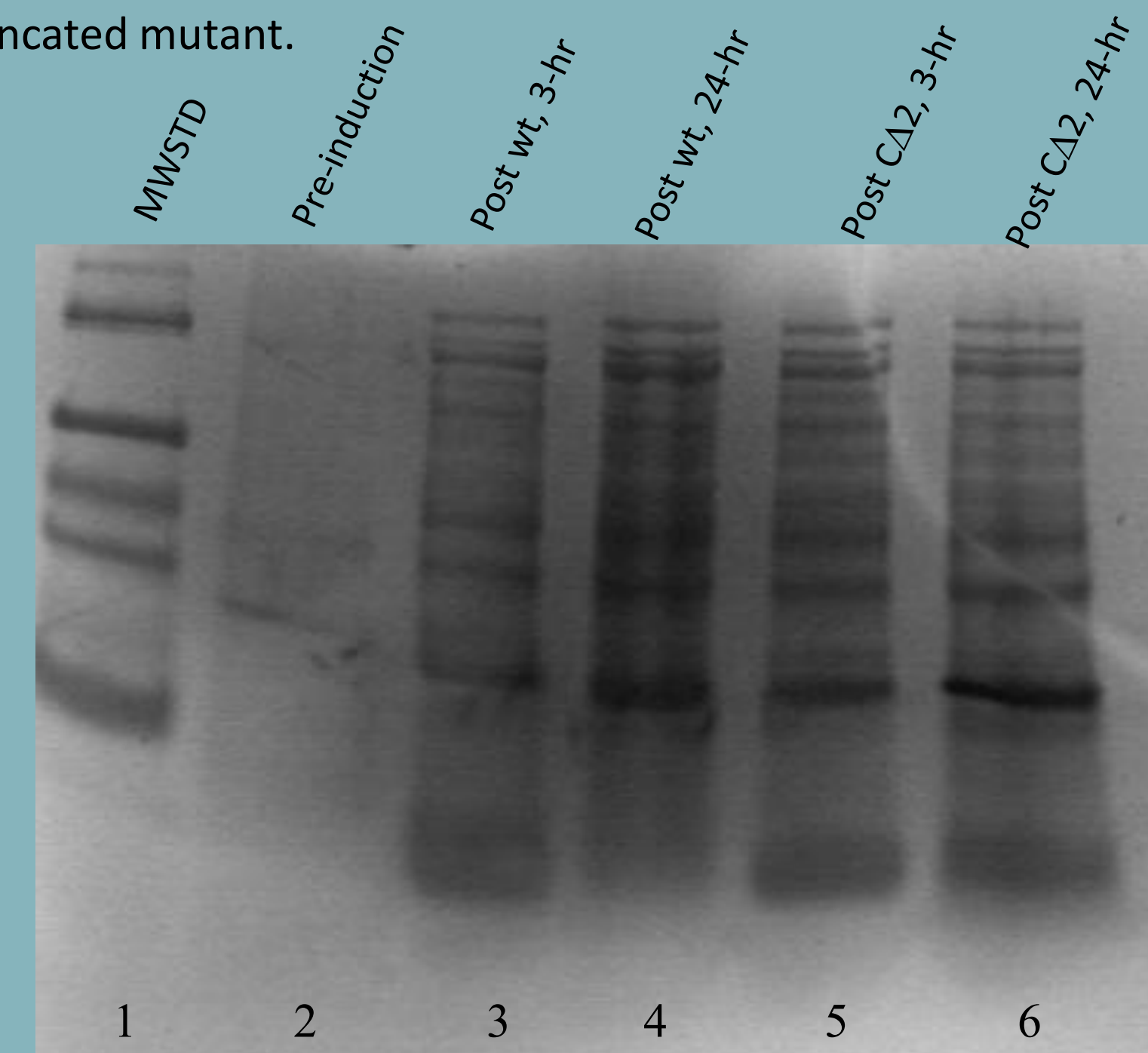
- IPTG was used to cause the *E. Coli* to over express the desired CusF mutants.
- The cells were broken by freezing and thawing, and the lysate was acidified, causing contaminants to precipitate out.
- The protein was separated by ion exchange chromatography.

Characterization of the Protein

- A gradient of 8M Guanidine was used to chemically denature the mutant proteins.
- Tryptophan was used as a monitor of the folded state of the protein.
- Free energy of folding was extracted from chemical denaturation curves by fitting the data to a two state model of folding.

Protein Purification

The figure to the right shows the presence of CusF in post induction cells of both wild type and the C42 truncated mutant.



After the presence of the protein CusF was confirmed by the gel, we attempted to extract the protein using ion exchange chromatography. However, the protein was lost in the purification process. Stock protein was used in the characterization process.

Conclusion

Although the truncated proteins could not be extracted from the mutated *E. coli* cells, we expect that the truncated proteins generated will be less stable than the wild type of CusF. In order to observe and calculate the free energy of CusF in the presence of copper ions, it will be necessary to successfully extract the truncated proteins from the mutated cells and characterize them to confirm that they are less stable.

In continuing research, greater variations in the concentration of denaturant, thermal denaturation and different truncated proteins could provide further insight into the stability of CusF.

It was evident that a greater number of thermal cycles in PCR (~30) yielded the greatest results, when producing the truncated DNA.

Acknowledgments & References

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