Development and Synthesis of Utrophin Actin Binding Domain 1 (ABD1)

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Introduction

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive geneCc disease resulCng in the absence or dysfuncCon of the muscle protein dystrophin. Dystrophin is proposed to dissipate mechanical stress placed on the muscle cell membrane (sarcolemma) during muscle contracCon. In paCents with DMD this mechanical stress is leH unchecked producing lesions within the cell membrane which leads to cell death and muscle deterioraCon.

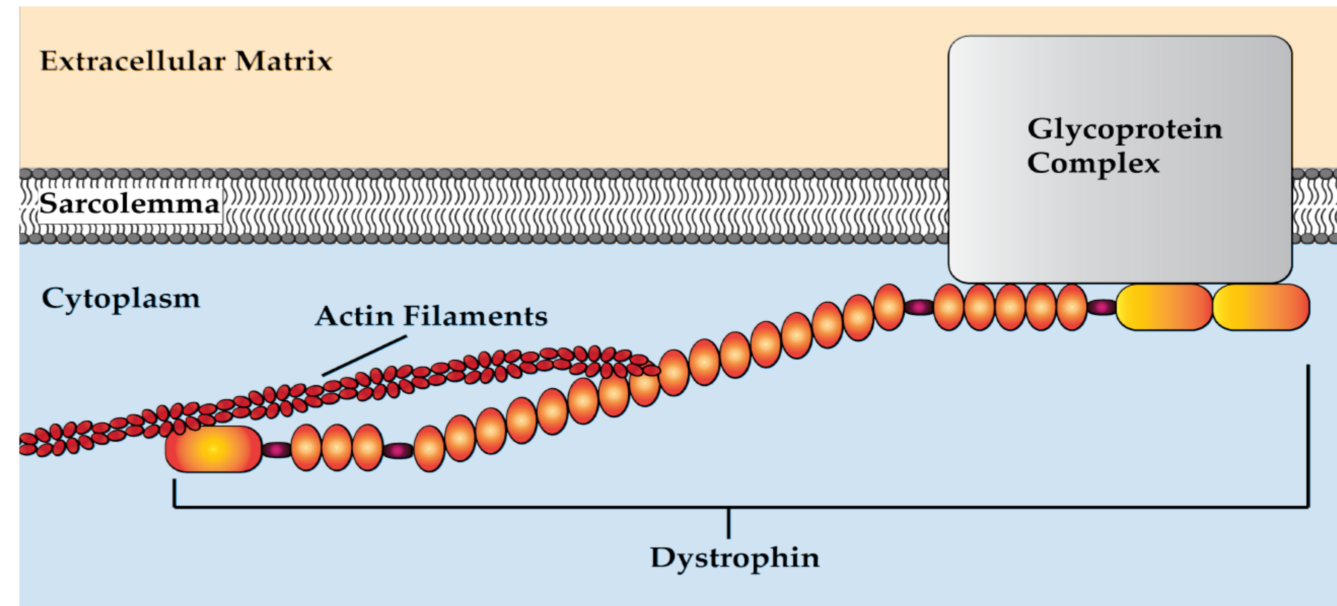

Figure 1: Dystrophin in Muscle cells. Dystrophin connects the sarcolemma to the acCn filaments and is criCcal in dissipaCng stress placed on muscle.

In paCents with DMD it has been observed that the expression of dystrophin's fetal homolog, utrophin, is upregulated. However, utrophin is unable to fully compensate for the lack of dystrophin resulCng in paCent mortality in mid to late twenCes.

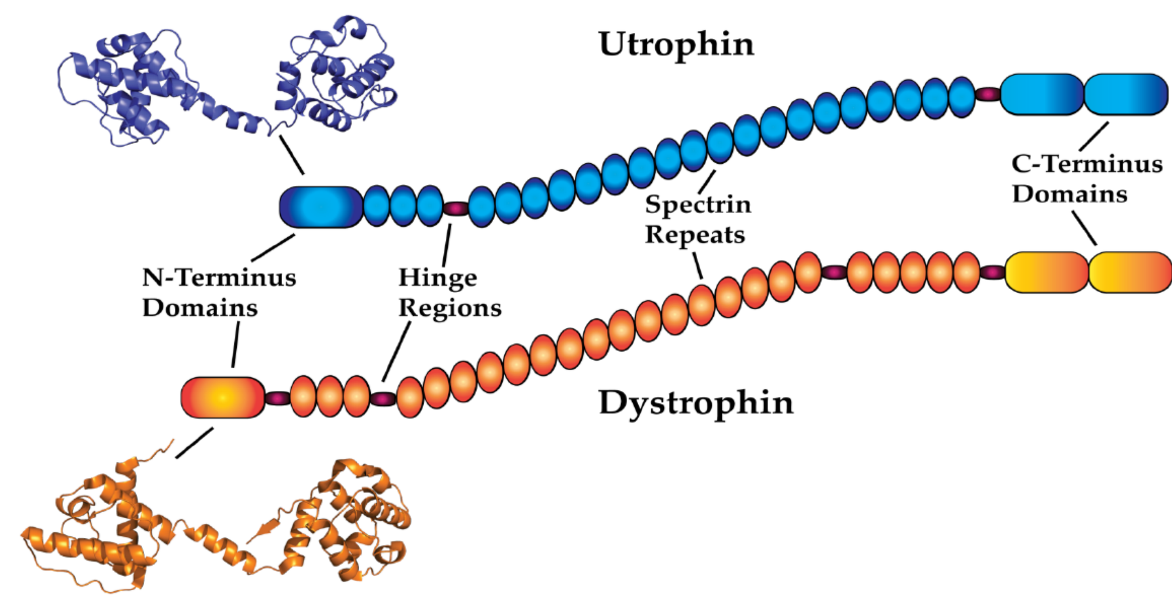

Figure 2: Structural Comparison of Utrophin and Dystrophin. Utrophin and Dystrophin share 85% homology in their acCn binding domains.

We seek to make clear the characterisCc differences between dystrophin and utrophin beginning with their first acCn binding domains (ABD1). We hypothesize that the difference in funcCon between dystrophin and utrophin lies within these characterisCcs. This project aims to design an expression vector containing utrophin ABD1.

Methods

The Utrophin ABD1 amino acid sequence (Universal Protein Resource) was used in the design of the plasmid. The sequence was codon opCmized for expression in *E. coli* and inserted into pUC57 plasmid using restricCon enzymes. The gene segment was amplified using polymerase chain reacCon and inserted into an expression vector containing components necessary for effecCve protein expression and purificaCon in *E. coli* cells.

Plasmid Design

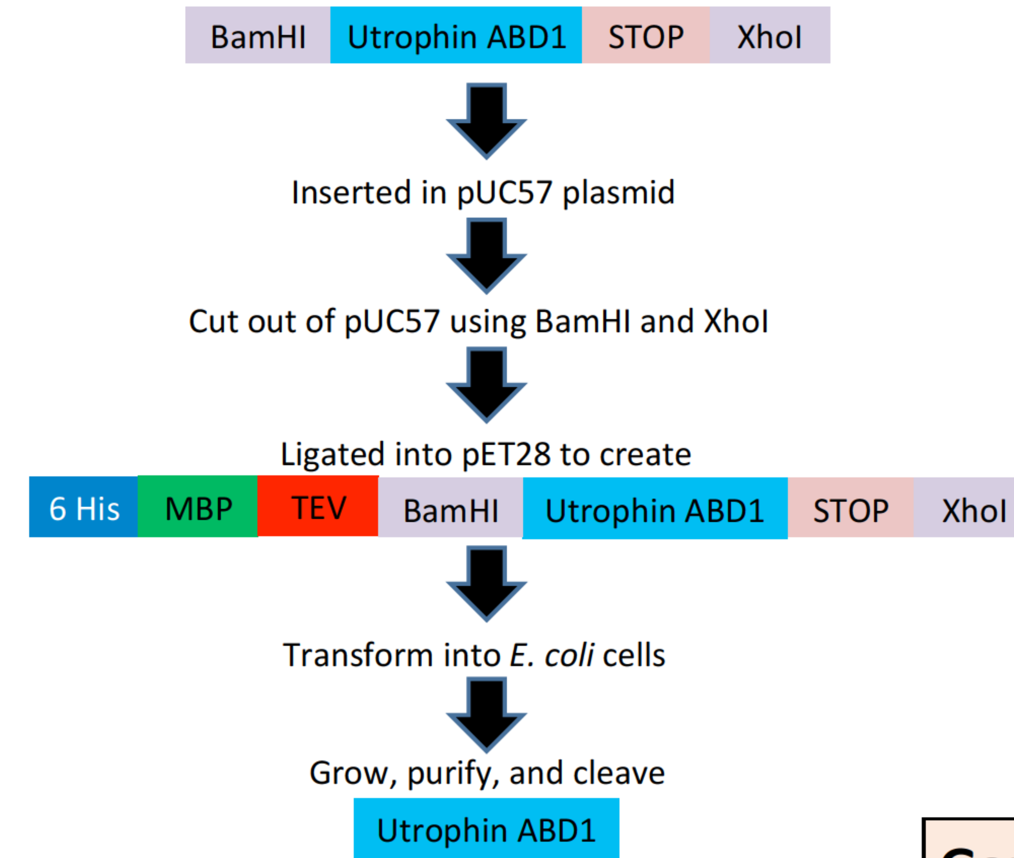

Figure 3: Map of Gene Development . The development and synthesis of utrophin ABD1 proceeded through a series of steps to obtain pure Utrophin ABD1. Each step was purposeful and specifically designed to obtain pure utrophin ABD1.

Table 1: Codon Optimization Provides Enhanced Sequencing . Example of codon opCmizaCon and the specificity when transiConing between organisms.

Gene	TTA	GTA
Amino Acid	Leucine	Valine
Codon Optimized	CTG	GTC

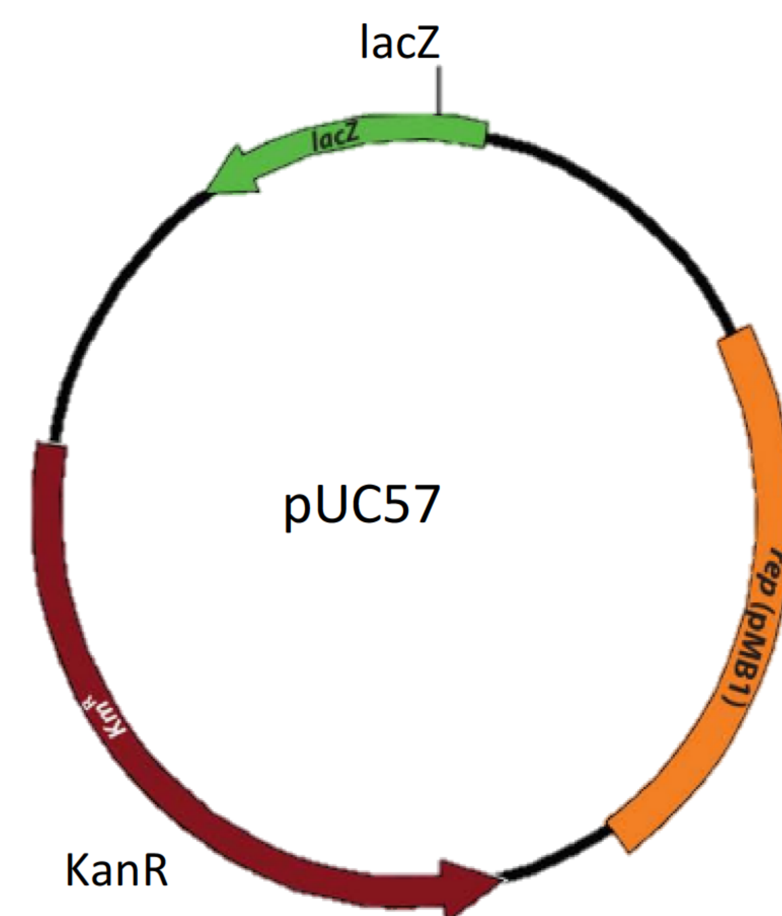

Figure 4: pUC5ti *E. coli* Plasmid. *E. coli* plasmid for inserCon of utrophin ABD1 containing selectable and screenable markers for visual analysis. These markers include a visual indicator and bacterial resistance.

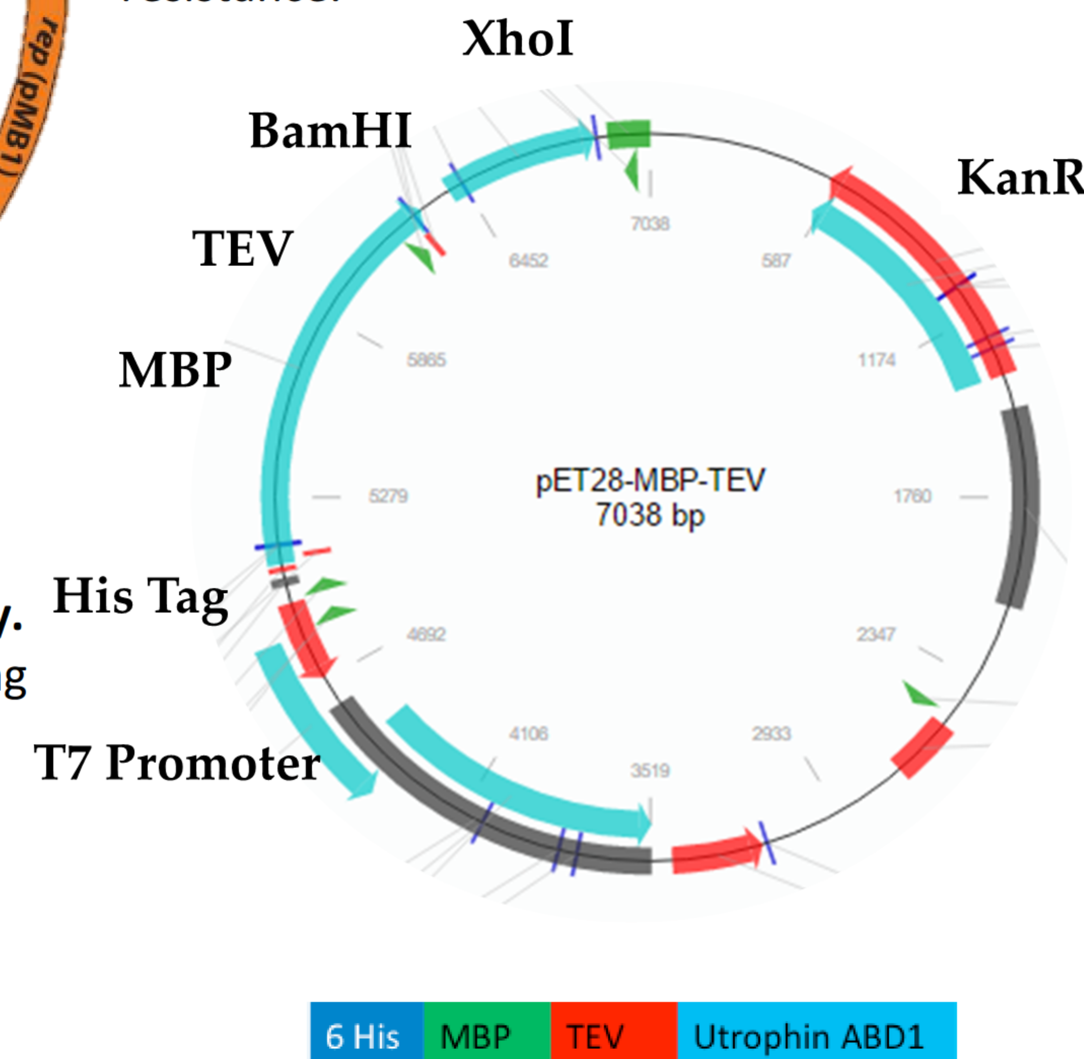

Figure 5: Expression Vector for Nickel Affinity Chromatography. The expression vector containing all necessary components for nickel column chromatography and the resulCng construct for purificaCon.

Protein Purification

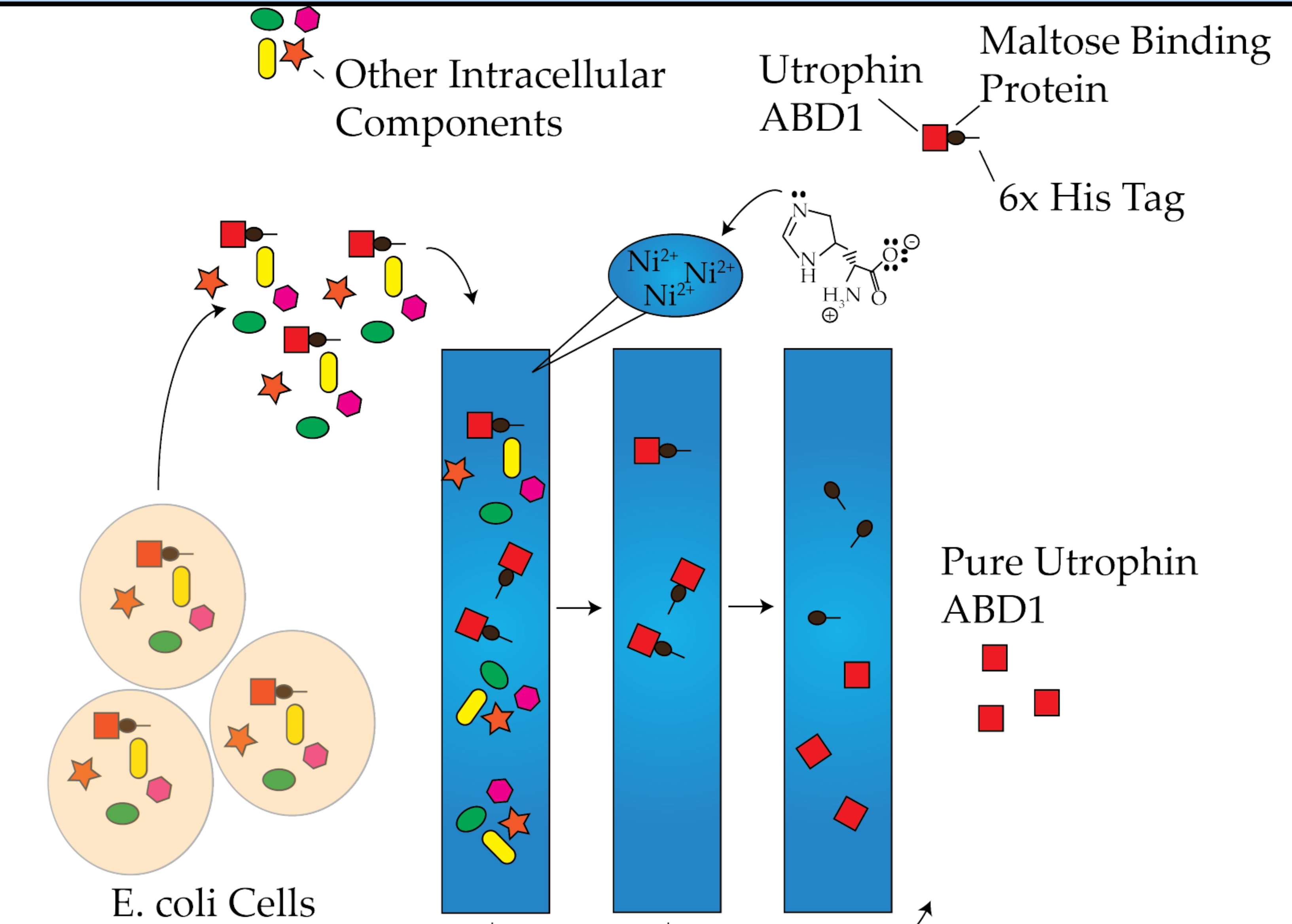

Figure 5: High Yield in Purity From Nickel Chromatography. The Cght binding of the lone pair electrons on hisCdine to the nickel enables the selected release of utrophin ABD1.

Conclusion

Duchenne Muscular Dystrophy is characterized by the failure to dissipate mechanical stress in the muscle. In order to create a potenCal therapeuCc target, utrophin ABD1 was developed and synthesized for future parameterizaCon of the first acCn binding domain. The construct was designed using the *E. coli*, codon opCmized gene flanked by two restricCon enzymes for excision of the the construct from the pUC57 plasmid into the expression vector. The expression vector added a hisCdine tag, maltose binding protein, and TEV protease for high yield purificaCon.

References

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