

# Drugs through Bugs: Expression of Naglu-PTD4 in *Spodoptera frugiperda*

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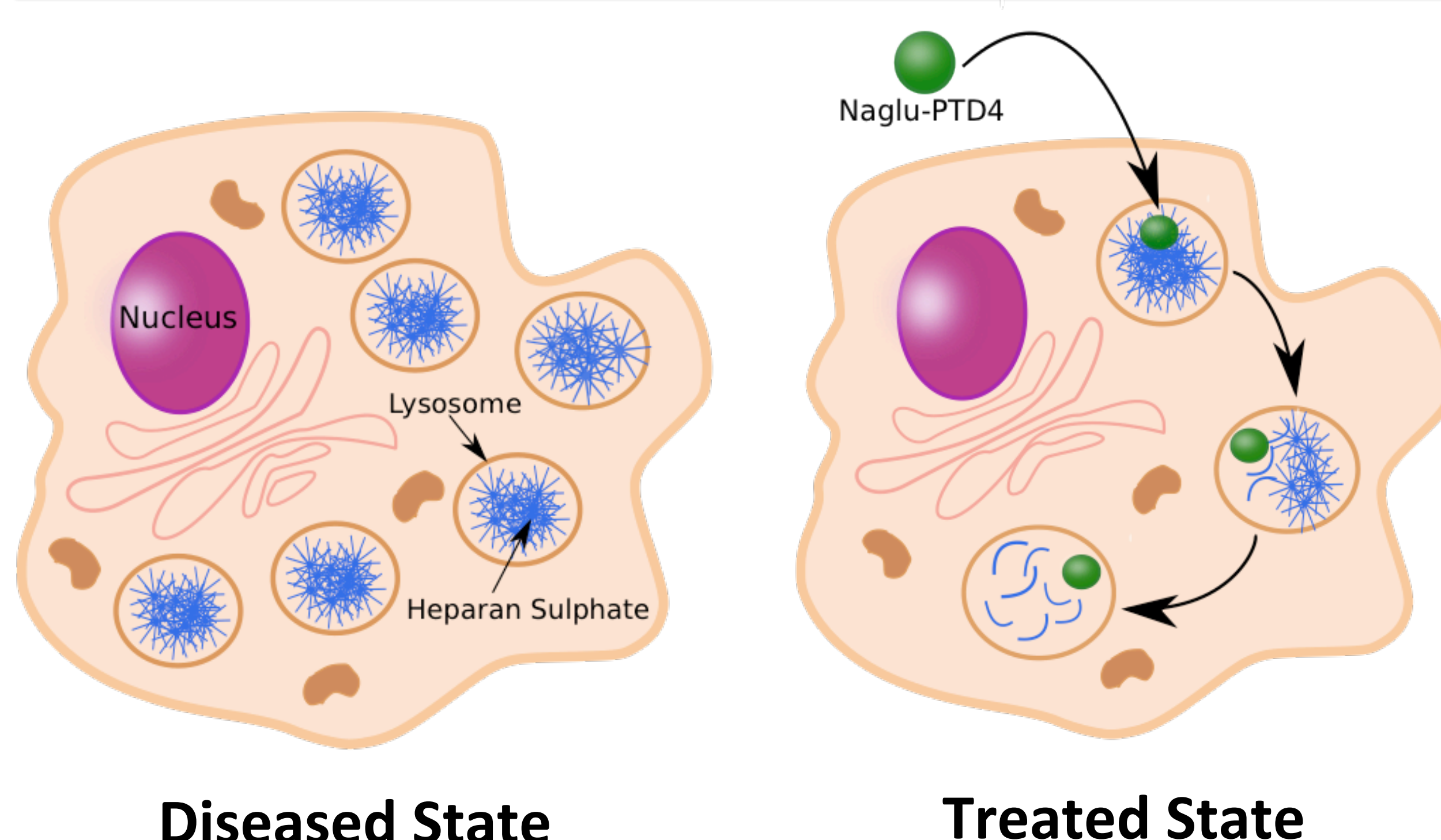
## Objective

Establish an efficient system for the large-scale production of human recombinant Naglu-PTD4 that has the potential to be successfully used for enzyme replacement therapy for Mucopolysaccharidosis IIIB.

## Background

$\alpha$ -N-acetylglucosaminidase (Naglu) is a lysosomal enzyme involved in the break down of heparan sulphate in the cell. A deficiency in Naglu results in a lysosomal storage disorder known as Mucopolysaccharidosis (MPS) III type B. This disease is characterized by progressive neuronal degeneration primarily of the central nervous system (CNS). Enzyme replacement therapy, which is an effective treatment for lysosomal storage disorders, has shown limited success in treating MPS IIIB because the exogenous functional Naglu is unable to cross the blood-brain barrier and treat the afflicted cells of the CNS.

PTD4 is a synthetic protein transduction domain derived from HIV type I. Previous research has shown that PTD4 is able to deliver biologically active enzymes *in vivo* across the blood-brain barrier in mice. The fusion of PTD4 to Naglu has promising implications for improving enzyme replacement therapy for MPS IIIB.



## Previous Work in the Choy Lab

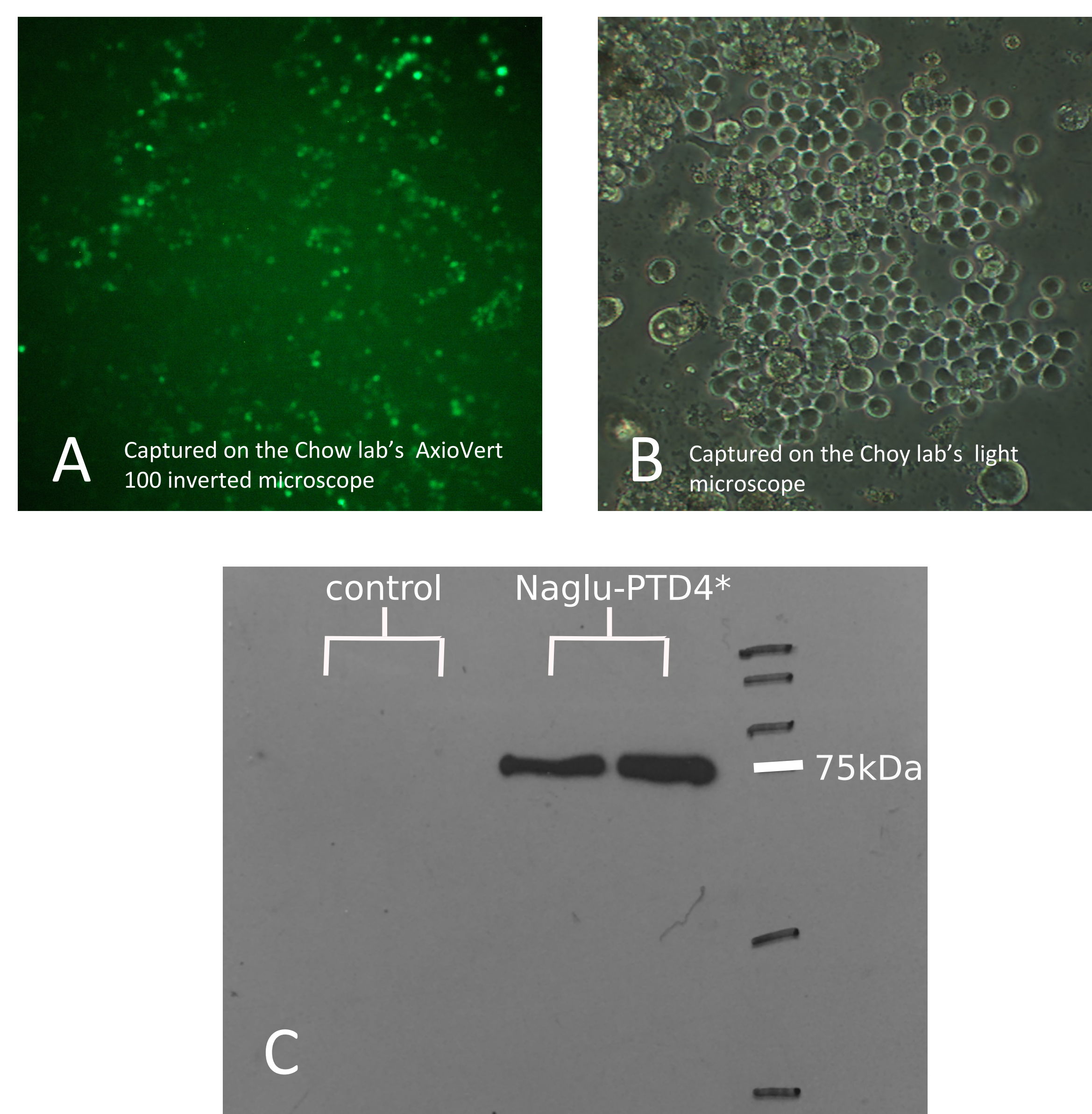
The previous Naglu-PTD4 gene construct (Jantzen *et al.*, 2013) was improved by introducing a stop codon after the PTD4 sequence.

The improved Naglu-PTD4 construct was ligated into a pIZT/V5-His vector then used to transform *Escherichia coli*



## Transfection and Selection of Sf9 insect cells

A non-liposomal transfection reagent was used to introduce the Naglu-PTD4 plasmid into *Spodoptera frugiperda* (Sf9) insect cells. Transient expression of the Naglu-PTD4 plasmid by Sf9 insect cells was detected via GFP fluorescence 48 hours post-transfection (A). Stable expression of Naglu-PTD4 was achieved by selecting the transfected Sf9 insect cells with Zeocin™ (B).



## Acknowledgements

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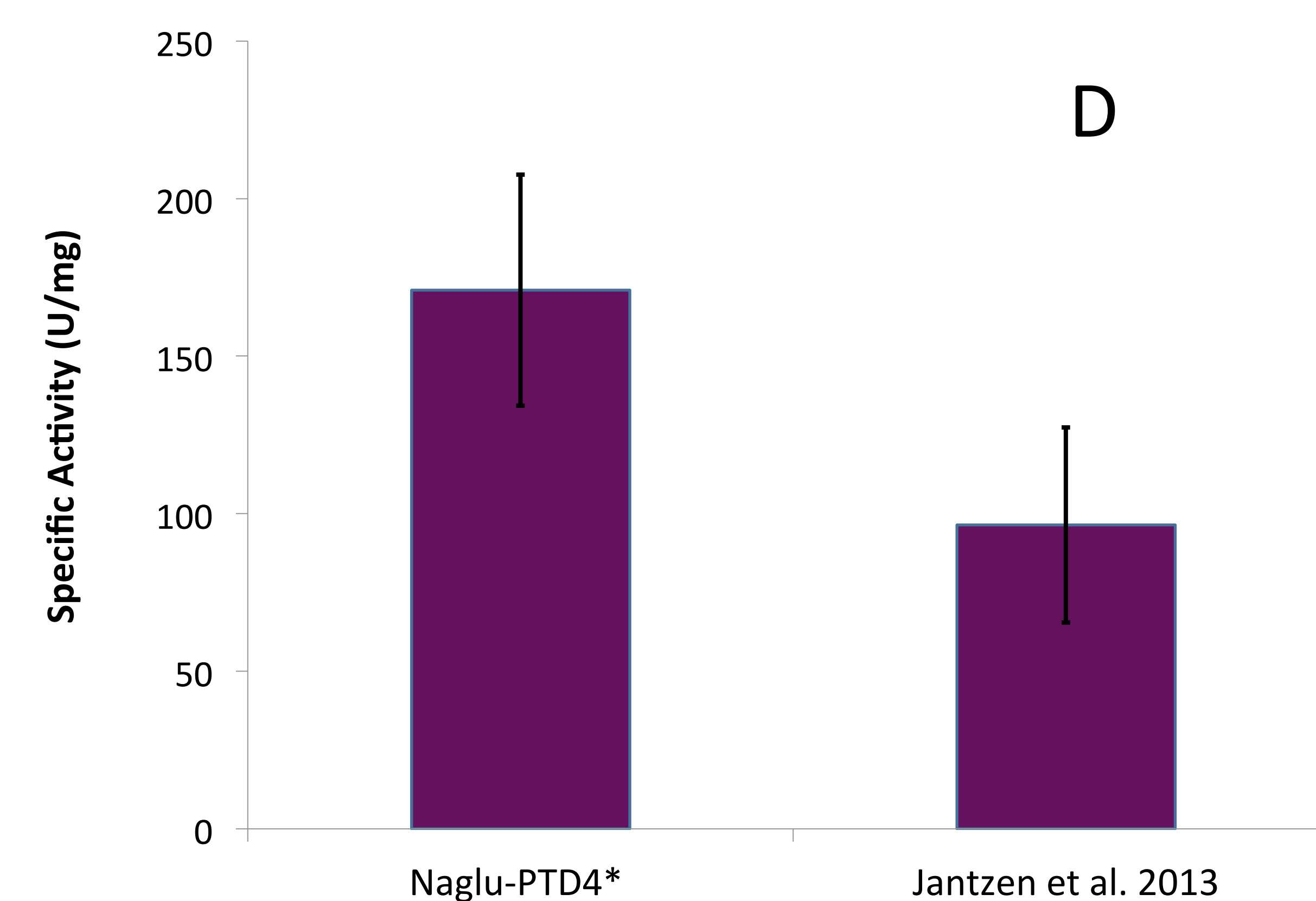
## References

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- Jantzen, R.R.; Truelson, S.N.; Choy, F.Y.M. *Process Biochem.* 2013, 48, 1197-1202.
- Schwarze, S.R.; Ho, A.; Vocero-Akbani, A.; Dowdy, S.F. *Science.* 1999, 285, 1569-1572.
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All diagrams and pictures in this presentation were captured or created by the author.

## Sf9 Production of Naglu-PTD4

Media samples were collected from stably selected Sf9 cell lines and tested for the evidence of active Naglu-PTD4 enzyme. The presence of Naglu-PTD4 enzyme was confirmed by immunoblotting. Results showed a band with an approximate molecular weight of 75-80 kDa, which corresponds to the molecular weight of Naglu (C). The specific activity of Naglu-PTD4 was determined by conducting a fluorogenic activity assay and Bradford protein assay. These results were compared to previous studies that expressed Naglu-PTD4 in Sf9 insect cells (D).



## Conclusion

Active Naglu-PTD4 enzyme was stably expressed in Sf9 insect cells with a specific activity that was comparable to previous studies ( $p=0.07$ ).

## Future Work

- Purify Naglu-PTD4 enzyme using high performance column chromatography
- Conduct uptake studies with MPS IIIB fibroblasts
- Conduct blood-brain barrier penetration studies with MPS IIIB mice