Engineering substrate specificity in TRIM-11 disaggregases

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Introduction

As of **2020** Parkinson's Disease (PD) Affects

10 MIL. [1] Globally

1 MIL. [1] U.S.

COST$

$52 BIL./yr

Symptoms of Parkinson's:

Movement[2]

- Temors & Stiffening
- SLOW Bradykinesia

Non-Movement[3]

- Issues with Attention/ Memory
- Sleep Disorders

Problem:

Disease Pathology

⬆ Inc. of intracellular Lewy Bodies

clusters of alpha-synuclein (α-syn) = α-syn

α-syn Aggregation:

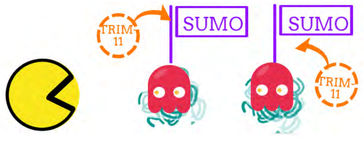
Monomer → Oligomer → Fibril

Oligomer most toxic form of aggregated proteins

Solution:

TRIM-11: Ring Finger | B-Box | Coiled Coil | PRY | SPRY

Recognizes Misfolded Proteins and marks with SUMO for degregation



Possesses ATP-independent disaggregase activity

Hypothesis

We hypothesize that Tripartite Containing Motif-11 (TRIM-11) can be engineered to target substrates, specifically α-syn, and dissolve the protein aggregates.

Results

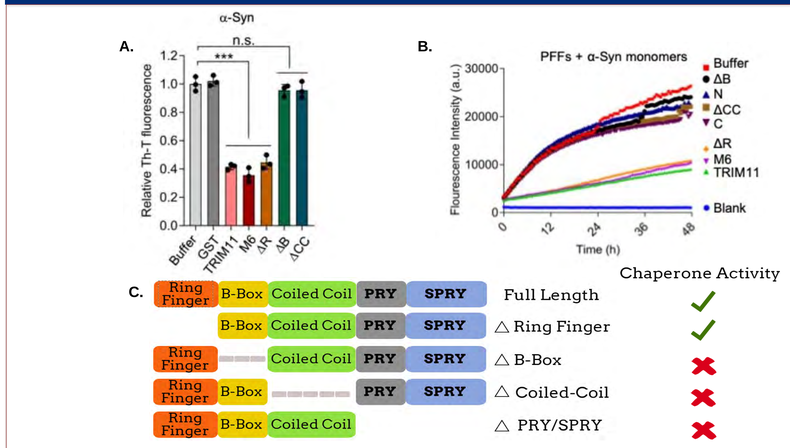


Figure 1: Structural Determinants of TRIM-11's chaperone and disaggregase activity.
A. Thioflavin assay on α-syn monomers marked with either glutathione S-transferase (GST) or the other 6xHis-tagged TRIM-11 proteins including M6 point mutations (at C16R, C19R, C92A, H95A, C111A, and C114A) and 2EA (E12A and E13A), in deletion mutations at the coiled-coil domain (CC) including ΔR (1-55), ΔB (88-127), ΔCC (128-207), and buffer.
B. Fluorescence assay on preformed fibrils (PFFs) and α-syn monomers with deletions in ΔB, N (287-468) indicating N-terminal RBCC end, ΔCC, C (1-286) indicating PRY-SPRY C-terminal end, ΔR, M6, whole TRIM-11, and a buffer. **C)** Summary of results indicating functional chaperone activity solely in full length TRIM-11 and with ring finger deletion.

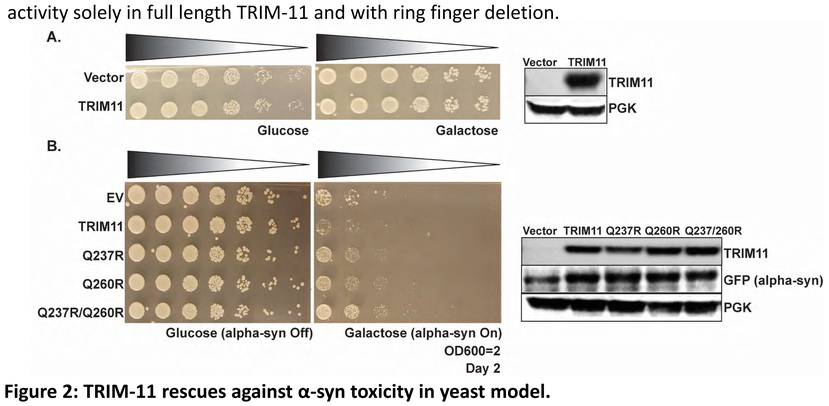


Figure 2: TRIM-11 rescues against α-syn toxicity in yeast model.
A. A yeast growth assay was performed on W303 yeast transformed with galactose inducible TRIM-11 or empty vector to discern if TRIM-11's expression was toxic. Results under galactose selection demonstrate that the expression of TRIM11 is not toxic and the yeast grow normally. Immunoblotting of the yeast lysate showed robust expression of TRIM-11 in galactose containing media. PGK is shown as a loading control. **B.** Wild-type TRIM-11 and engineered variants were transformed into W303 yeast with integrated galactose inducible expression of α-syn and they were spotted onto plates to measure growth. Results demonstrated that arginine point mutations (Q237R and Q260R) can potentiate TRIM-11 rescue ability against toxic α-syn and the effect is additive (Q237/260R). The corresponding immunoblot demonstrates expression of TRIM-11 as well as α-syn in galactose containing media. PGK is used as a loading control.

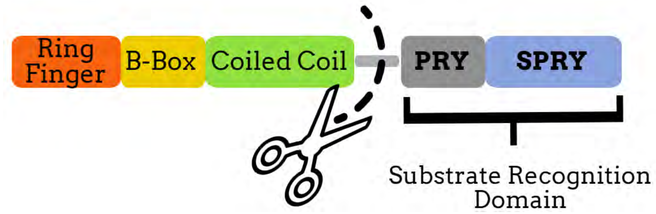
Conclusion/ Future Directions

What did we learn?

- TRIM-11 can be engineered to have improved chaperone/disaggregase activity
- Positive point mutations may have an accumulated effect on disaggregase activity

What's next?

- Can we get TRIM-11 to bind with a specific substrate?



- Replace with small chain fragment of an Antibody (Ab) specific to aggregation species

References

1. "Statistics." *Parkinson's Foundation*, www.parkinson.org/Understanding-Parkinsons/Statistics.
2."Movement Symptoms." *Parkinson's Foundation*, www.parkinson.org/Understanding-Parkinsons/Movement-Symptoms.
3."Non-Movement Symptoms." *Parkinson's Foundation*, www.parkinson.org/Understanding-Parkinsons/Non-Movement-Symptoms.
4. *G. Zhu, S. Ghaisas, W. Prall, L. Chen, L. Guo, E. Luna, K.L. Mack, M.P. Torrente, K.C. Luk, J. Shorter, and X. Yang,. (2020). TRIM11 prevents and reverses neurodegenerative-linked protein fibrillization. Cell Rep. In press.*
5. All figures generated from Biorender.com and Piktochart

Acknowledgments

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