Mechanisms of Gene Expression

Team Project 3 and 4

Project developed by: Carolina Santos, Diogo Ramos and Lara Inocêncio

28 de março de 2025

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of amyloid beta (Aβ) plaques (aggregates) and tau tangles in the brain. The preprint article [1] we chose on the last Team Project 2 focuses on evaluating lysosomal exocytosis and how this mechanism relates with amyloid beta (Aβ) secretion. It makes use of a valuable human induced pluripotent stem cell (hiPSC) derived model for studying neurodegenerative diseases like AD. The authors found that lysosomal exocytosis is a key pathway for Aβ secretion from neurons. Briefly, Aβ accumulates primarily in lysosomes and is released into the extracellular space through exocytosis, contributing to the formation of Aβ aggregates characteristic of AD. In this manner, lysosomal exocytosis is a potential target for AD therapies, as blocking this pathway could reduce the spread of toxic amyloid aggregates.

Rab27b is a small GTPase that interacts with Munc13-4 to regulate lysosomal secretion. These proteins are involved in vesicle trafficking and lysosomal exocytosis and its silencing significantly reduces Aβ secretion [1]. Even though there are several Rab proteins that are involved in lysosomal exocytosis, the work of Tsang *et. al.* [1], demonstrated that Rab27b displays higher localized expression in the central nervous system (CNS) and so it is more prone to be involved in AD progression. The authors also found that by silencing only these two proteins the secretion of Aβ can be reduced.

Knocking out the genes that code for these proteins could be an option to block the Aβ secretion to the extracellular space, preventing its accumulation. However, as these proteins play important roles in vesicle priming and exocytosis, the knocking out of these genes could affect synaptic transmission and possibly exosome release. This could lead to cognitive or motor deficits, because changes in neurotransmitters release my impact on learning, memory or motor function. The knocking out of these genes could also reduce exosome secretion that could potentially affect neurodegeneration or neuroinflammation. Besides that, knocking genes that code for a protein in humans is a complex scientific challenge and would require significant research and development. The process would be highly experimental and currently not feasible as a routine therapy. Therefore, it is essential to develop alternative therapeutic strategies that are easier to implement in a clinical context.

Hypothesis

Considering what was previously mentioned regarding that Rab27b forms a complex with Munc13-4 that induces lysosomal exocytosis and thus amyloid beta (Aβ) secretion, we hypothesize that by preventing the interaction between these proteins with a small peptide, it could be possible to reduce lysosomal exocytosis and consequent Aβ secretion and aggregation, creating a novel therapeutic approach for AD by slowing disease progression.

Tasks

Task 1: Identification of the specific interaction sites between Rab27b and Munc13-4

With this task we aim to identify the region with the binding/interaction site of Rab27b and Munc13-4 so we can investigate how to block/prevent the interaction between the two proteins.

A crystal structure of Rab27b is already published in Protein Data Bank (PDB) (PDB DOI: https://doi.org/10.2210/pdb2F7S/pdb). However, the protein structure of Munc13-4 is not yet available and can be predicted using protein structure prediction methodologies, which may be complemented by other computational biology approaches, such as protein-protein docking methods. For protein structure prediction both comparative modelling methodologies [2] and advanced deep learning generative machine learning models like AlphaFold3 (https://alphafoldserver.com/) or Chai-1 (https://www.chaidiscovery.com/) can be tested and their results will be evaluated to select the most reliable models. For molecular visualization, PyMOL and ChimeraX programs will be used.

Task 2: Design small peptides to selectively and simultaneously inhibit Rab27b and Munc13-4 interaction in neurons

By investigating the interaction site between the two proteins, that was obtained in task 1, we aim to design small peptides that will interact with Rab27b or Munc13-4 and that would inhibit their interaction. After designing the small peptides for both proteins, we will characterize its interactions using Surface Plasmon Resonance [3] that will allow us to study the binding interaction in real-time. With this technique we aim to measure the binding affinity, kinetics, and concentration of interactions between our proteins and the designed peptides.

Task 3: *In vitro* validation of the designed peptides in lysosomal exocytosis and Aβ secretion using an hiPSC-derived AD model

Using the hiPSC-derived AD model previously used by Tsang et. al. [1], we will test the design of the small peptides obtained in task 2 and evaluate its effect in Aβ secretion. We will test the peptides design for Rab27 alone, test the peptides design for Munc13-4 alone and finally test them both together and observe if any of these combinations lead to a reduced Aβ secretion.

Finally, we will compare our experimental results with the ones obtained using gene silencing techniques by Tsang et. al. [1].

Contingency plan

- ✓ If none of the peptides show significant inhibition of Rab27b-Munc13-4 interaction, other approaches can be used to selectively inhibit the complex interaction: CRISPR-mediated mutagenesis, small molecule inhibitors and short hairpin RNA or silencing RNA.
- ✓ If we are unable to significantly reduce Aβ secretion with the tested inhibition approaches, we will try to implement them to other complexes of Munc13-4 and rab proteins, also involved in lysosomal exocytosis, since Munc13-4 has also been shown to interact with other rab proteins like rab27a [4].

Proof-of-concept

As a proof-of-concept, we used AlphaFold3 to predict the structure and interactions between human Rab27b and Munc13-4 and visualized the results using PyMOL as shown in the figures below. We were able to observe the interaction between these two proteins (figure 1B) and identified the possible location of the interaction site.

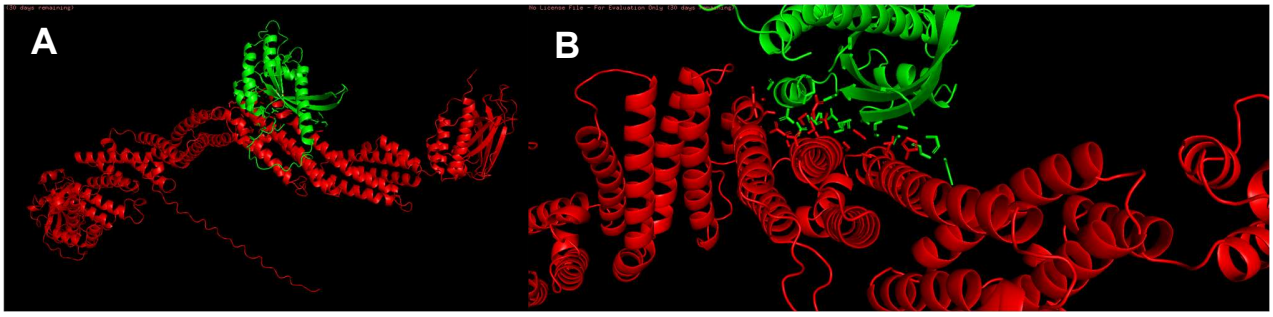
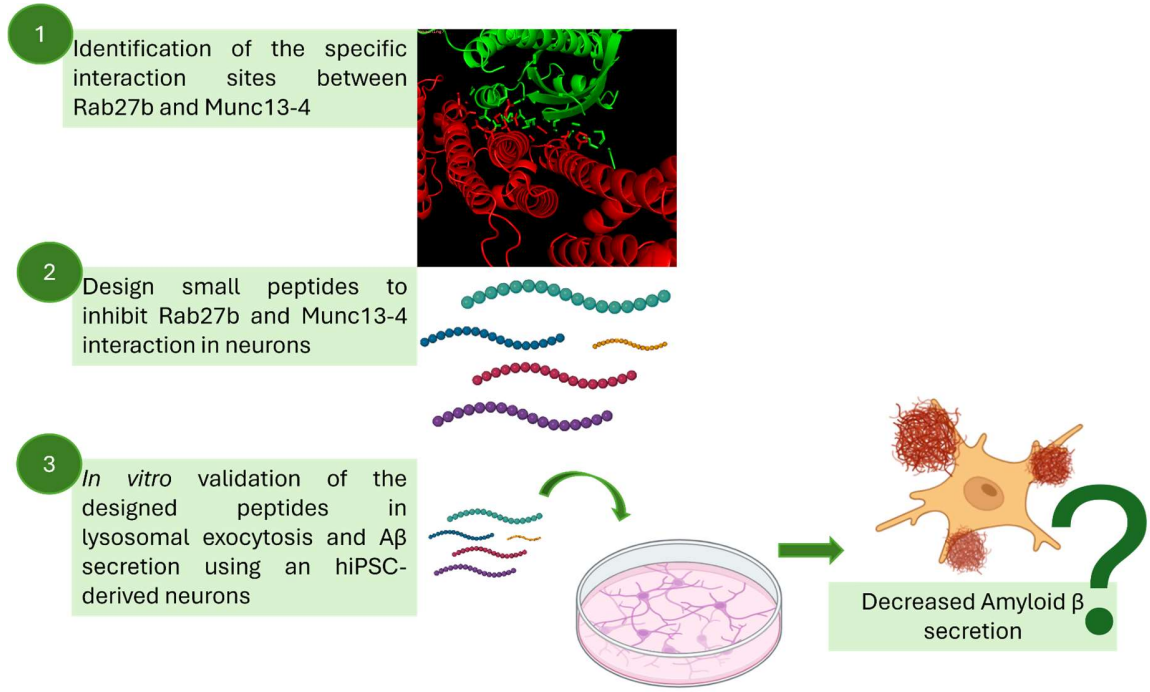


Figure 1 - Predicted AlphaFold 3 interaction between Rab27b and Munc13-4 visualized in Pymol. **(A)** Overall view of the two proteins' interaction. **(B)** Detailed view of the interaction site.

Workflow



References

[1] Adrianna R Tsang, Manoj Reddy Medapati, Claudia Seah, Jordan M Krupa, Stephen H. Pasternak, Amyloid beta is released by lysosomal exocytosis from hiPSC-derived neurons, bioRxiv 2025.03.01.640950, doi: 10.1101/2025.03.01.640950

[2] Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol*. 1993;234(3):779-815. doi:10.1006/jmbi.1993.1626

[3] Zidane F, Zeder-Lutz G, Altschuh D, et al. Surface plasmon resonance analysis of the binding mechanism of pharmacological and peptidic inhibitors to human somatic angiotensin I-converting enzyme. *Biochemistry*. 2013;52(48):8722-8731. doi:10.1021/bi4006144

[4] Neeft M, Wieffer M, de Jong AS, et al. Munc13-4 is an effector of rab27a and controls secretion of lysosomes in hematopoietic cells. *Mol Biol Cell*. 2005;16(2):731-741. doi:10.1091/mbc.e04-10-0923