A Novel Approach for Alzheimer's Disease Treatment: Inhibition and Degradation of Beta-Amyloid 42 Aggregates Using a Nasal Ultrasonic Nebulizer for Peptide Delivery

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Abstract

This study proposes an innovative therapeutic approach for Alzheimer's disease (AD) targeting the aggregation and clearance of the neurotoxic amyloid-beta 42 (Aβ42) peptide. Utilizing computational design tools such as AlphaFold 2, we identified and optimized functional peptides capable of binding the hydrophobic C-terminal region of Aβ42 to prevent aggregation and promote degradation via the ubiquitin-proteasome system (UPS). This non-invasive strategy envisions peptide administration through a nasal ultrasonic nebulizer, which could bypass the blood-brain barrier (BBB) and deliver peptides directly to the brain. The concept focuses on the potential of combining targeted peptide design with a delivery system that enables rapid, brain-specific absorption, presenting a novel direction for AD therapy.

1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders globally, characterized by progressive memory decline, cognitive impairment, and behavioral changes. Its incidence increases sharply with age, and the lack of a fundamental treatment imposes a significant social and economic burden. Substantial research has been conducted to understand the pathological mechanisms underlying AD, and various hypotheses have been proposed regarding the causes of the disease. The major contributing factors to AD's pathogenesis include the early accumulation of beta-amyloid (Aβ) peptides and, at later stages, the abnormal behavior of tau proteins. Additionally, complex factors such as inflammation, vascular issues, and genetic mutations may play critical roles.

The amyloid hypothesis is the most widely recognized theory for the early causes of AD. According to this hypothesis, one of the primary pathological characteristics of AD is the

accumulation of beta-amyloid (Aβ) peptides. These peptides are derived from amyloid precursor protein (APP) by the sequential cleavage of β- and γ-secretase, with the Aβ42 form exhibiting particularly high toxicity and a propensity to aggregate as plaques outside neurons. Aβ42, with its hydrophobic nature, tends to form oligomers and fibrils, which induce neurotoxicity. Such accumulation impairs synaptic function and triggers neuroinflammatory responses, ultimately leading to neuronal death. Although Aβ accumulation is generally accepted to play a significant role in the early stages of AD, low success rates in clinical trials targeting amyloid have raised questions about the sole role of this hypothesis in AD.

The second major hypothesis, relevant in the later stages of AD, focuses on the hyperphosphorylation and aggregation of tau proteins. Tau proteins normally stabilize microtubules and maintain the structure and function of neurons. However, in AD, tau proteins become hyperphosphorylated, accumulating as neurofibrillary tangles (NFTs). This accumulation leads to structural collapse in neurons, disrupting neural connections and eventually causing cell death. Tau pathology generally follows amyloid plaque accumulation and is closely associated with disease progression and severe cognitive impairment. The amyloid and tau hypotheses are believed to act in a complementary manner, suggesting that these mechanisms may work together in the pathological progression of AD.

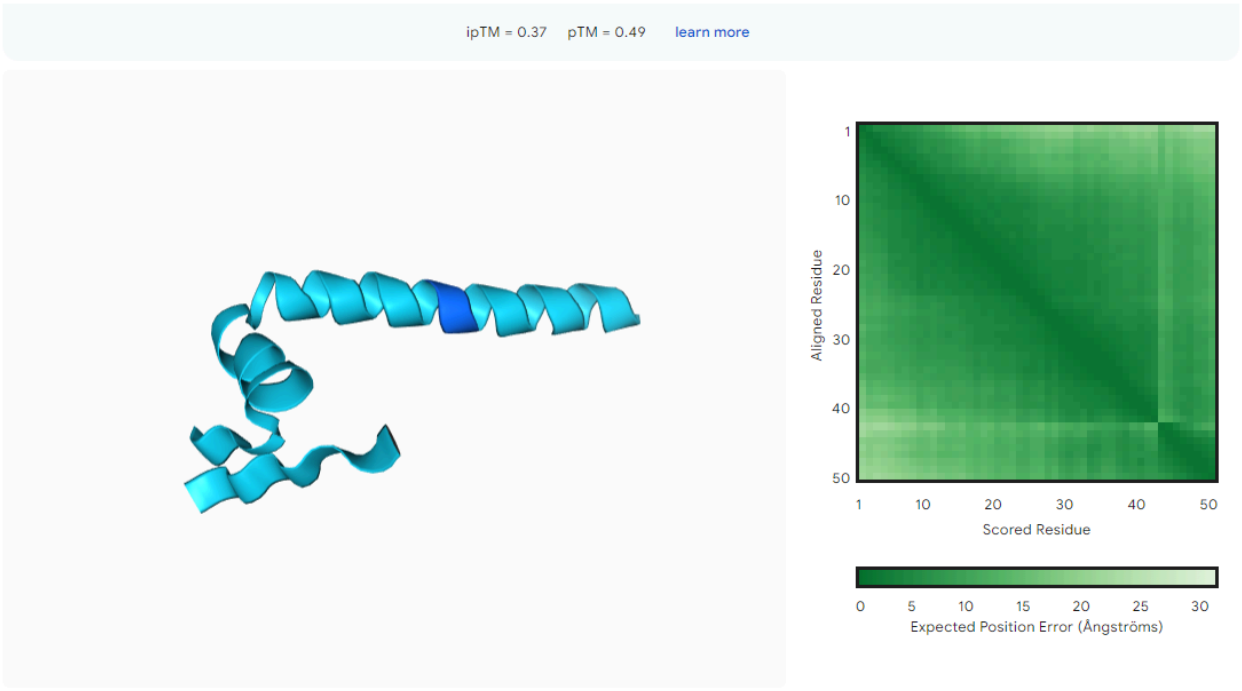
Beyond amyloid and tau, other factors can contribute to the disease's progression. Inflammatory responses are associated with activated microglia, which respond to amyloid plaques and tau accumulation. Microglia usually serve as immune surveillance cells in the brain, but excessive activation in AD exacerbates inflammation, leading to neuronal damage. Genetic factors also play a crucial role, as specific alleles like APOE ε4 increase the risk of AD. Vascular issues can further accelerate AD progression by impairing the supply of oxygen and nutrients to the brain.

In this study, we aim to apply two novel ideas based on current research in Alzheimer's treatment. First, instead of relying on intravenous delivery to cross the blood-brain barrier (BBB), we propose intranasal administration to bypass the BBB, inspired by nasal delivery methods seen in certain psychoactive drugs. Second, we focus on the hydrophobic C-terminal region of Aβ42, hypothesizing that a peptide designed to bind here could prevent Aβ42 aggregation. By incorporating an E3 ligase domain into the peptide, we aim to induce Aβ42 degradation through the ubiquitin-proteasome system. To achieve these goals, we utilize an ultrasonic nebulizer for effective intranasal delivery.

2. Concept and Methodology

2.1 Peptide Design Using AlphaFold 2

In exploring peptides that could effectively bind to the C-terminal region of Aβ42(1), we referenced existing research on neuroprotective peptides derived from microtubule-stabilizing proteins, known to inhibit amyloid-beta aggregation. Among these, the NAP peptide (NAPVSIPQ)(2), with a molecular weight of approximately 900 Da, is recognized for its neuroprotective properties and amyloid-beta aggregation inhibitory effect. Subsequently, we analyzed several modified forms of the NAP peptide with AlphaFold 2 to assess their affinity with Aβ42. From these analyses, the QPISVAVN peptide showed the strongest binding affinity to the C-terminal region of Aβ42.(Figure 1)



Information

Type	Copies	Sequence
Protein	1	DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA
Protein	1	QPISVAVN

Figure 1. Interaction between Aβ42 (top) and the QPISVAVN peptide (bottom) as determined using AlphaFold 2

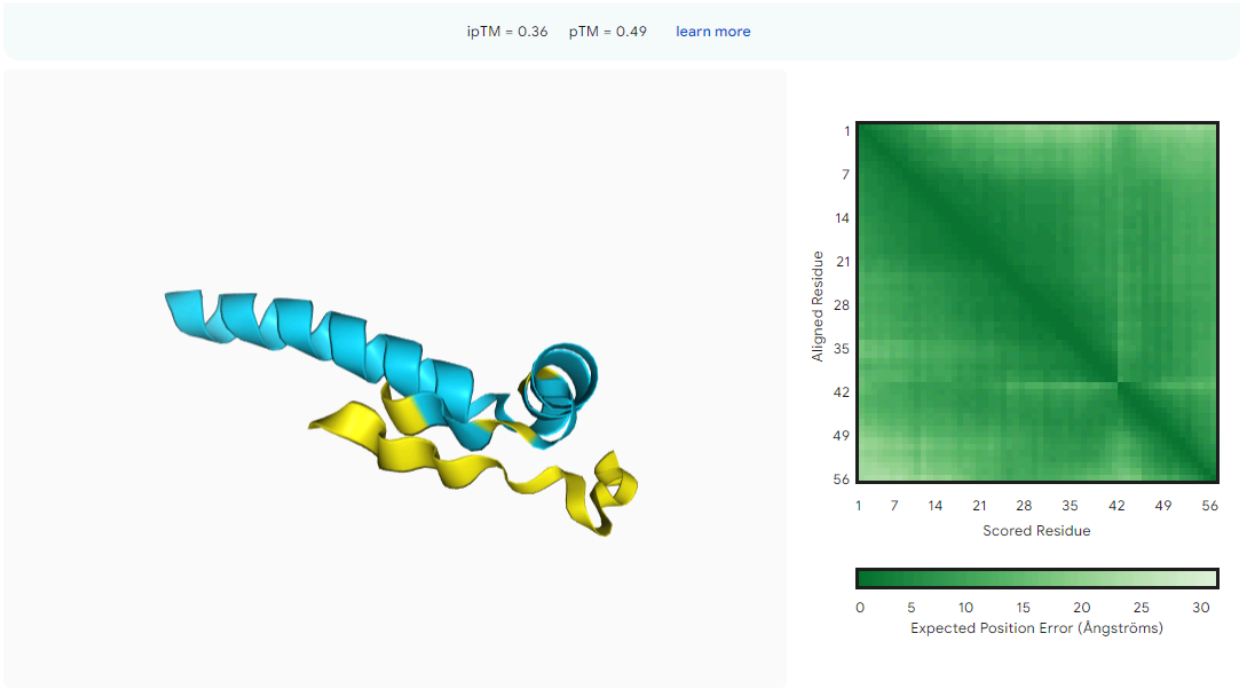
To induce degradation after binding the peptide to amyloid-beta 42, we attached E3 ligase domains (PEST, KENQ(H), DSGS, and RALLN) from the ubiquitin-proteasome system. For the linker, we used the CGGC sequence, designed to form an S-S bond between cysteines, allowing the QPISVAVN sequence to remain in a “clipped” form before reacting with amyloid-beta 42.

I then confirmed the interactions of the four peptide sequences

1. QPISVAVNCGGCPEST
2. QPISVAVNCGGCKENQ(H)
3. QPISVAVNCGGCDSGS

4. QPISVAVNCGGCRALLN

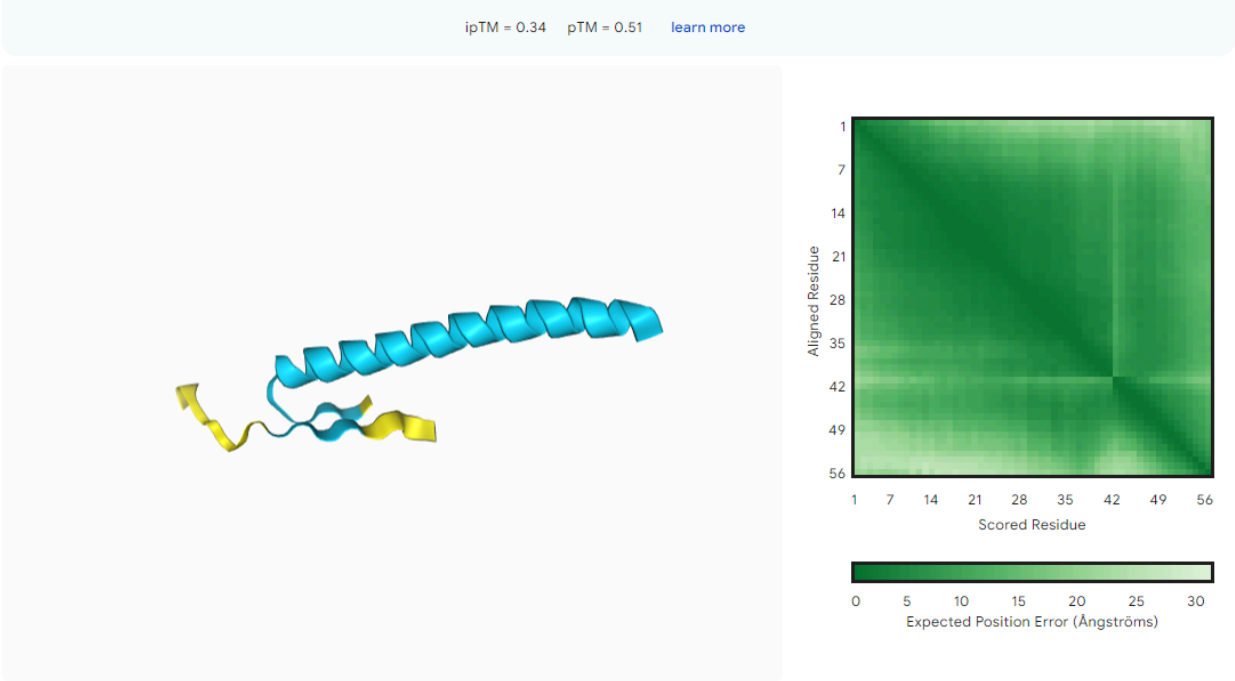
with amyloid-beta 42 using AlphaFold 2.



Information

Type	Copies	Sequence
Protein	1	DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA
Protein	1	QPISVAVNCG GCPEST

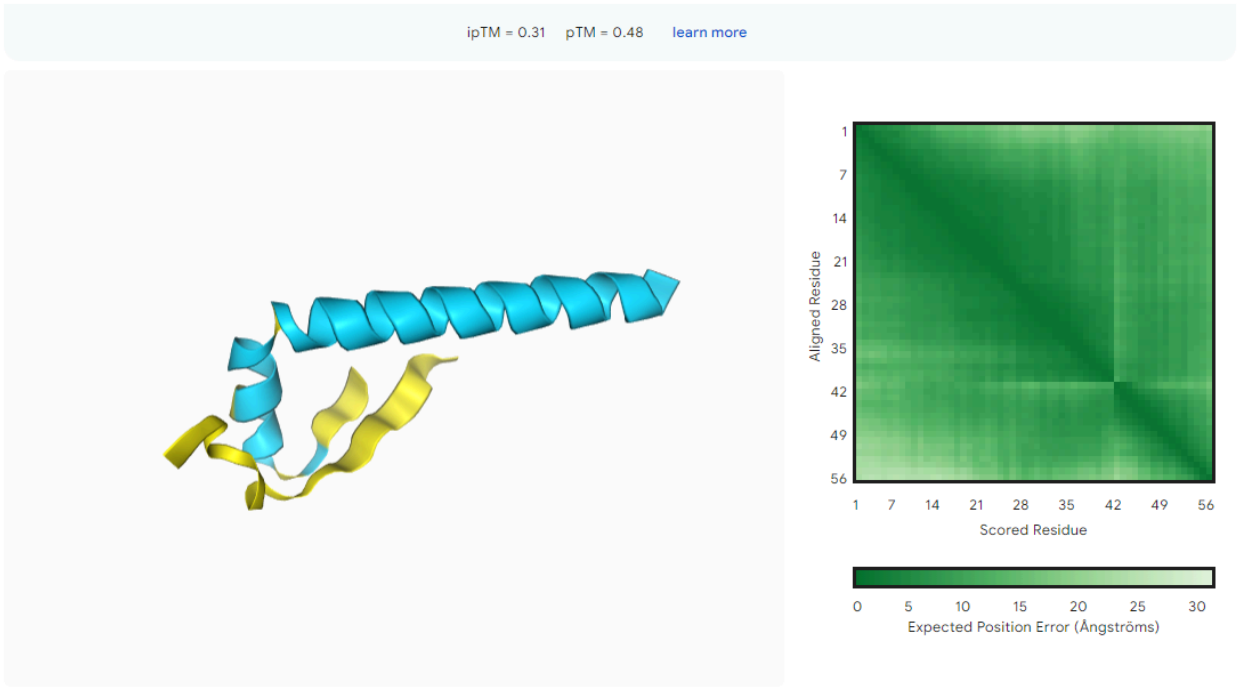
Figure 2. Interaction between Aβ42 (top) and the QPISVAVNCGGCPEST peptide (bottom) as determined using AlphaFold 2



Information

Type	Copies	Sequence
Protein	1	DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA
Protein	1	QPISVAVNCG GCKENQ

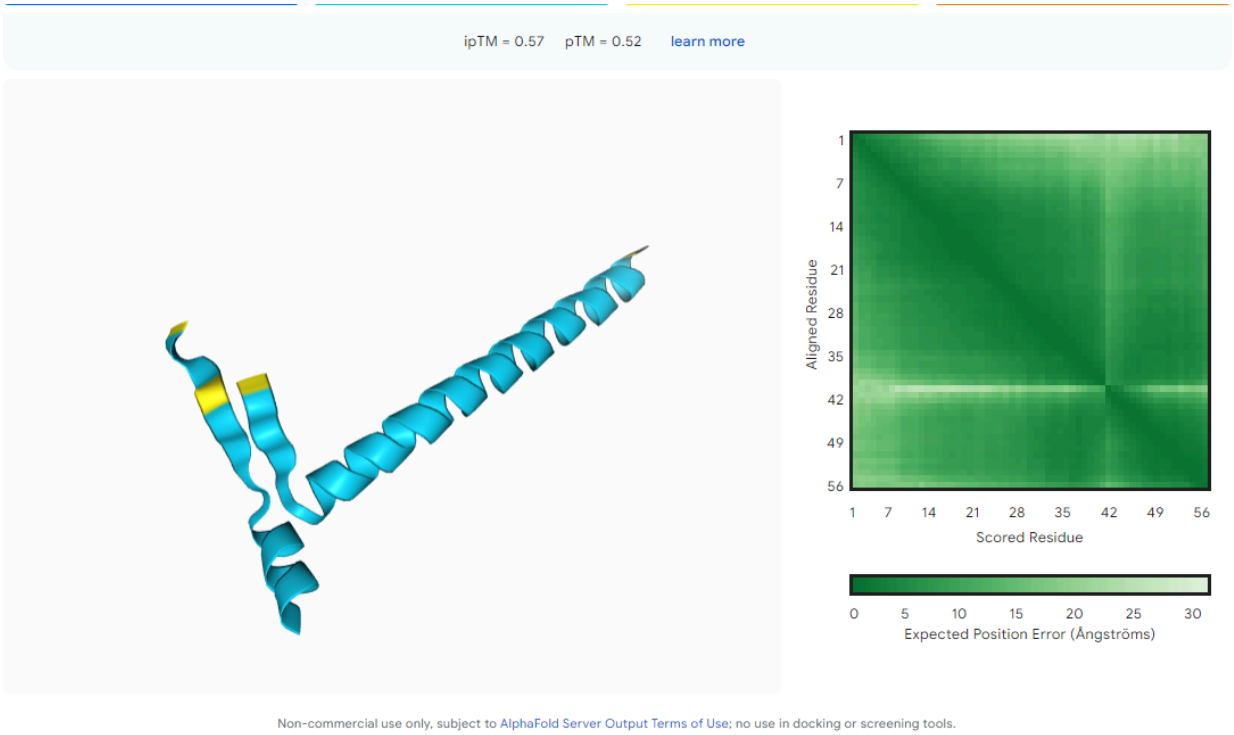
Figure 3. Interaction between Aβ42 (top) and the QPISVAVNCGGCKENQ(H) peptide (bottom) as determined using AlphaFold 2.



Information

Type	Copies	Sequence
Protein	1	DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA
Protein	1	QPISVAVNCG GCDSGS

Figure 4. Interaction between Aβ42 (top) and the QPISVAVNCGGCDSGS peptide (bottom) as determined using AlphaFold 2.



Information

Type	Copies	Sequence
Protein	1	DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA
Protein	1	QPISVAVNCG GCRALLN

Figure 5. Interaction between Aβ42 (right/top) and the QPISVAVNCGGCRALLN peptide (left/bottom) as determined using AlphaFold 2.

2.2 Ultrasonic Nebulizer Formulation

To ensure stability and controlled release, we propose formulating these peptides in a liposomal suspension suitable for nasal administration. This preparation would use lecithin and cholesterol in a thin-film hydration process, with ultrasonic treatment to achieve uniform particle size and enhance bioavailability. A nasal ultrasonic nebulizer could then deliver the aerosolized formulation to the brain via the olfactory and trigeminal pathways, potentially allowing for efficient and targeted brain uptake.

2. To enhance stability and intracellular delivery, I aim to encapsulate the peptide in liposomes suitable for nasal administration. A mixture was prepared with 20% aqueous solution and 80%

PEG 3350, soy lecithin, and cholesterol, then formulated using a sonicator to encapsulate the aqueous solution within small liposomes. The detailed method and composition are as follows:

A. Dissolve 10-14 mg of synthesized peptide in PBS to prepare a solution with a concentration of 1 mg/mL, making a total volume of 10 mL.
B. Prepare 25 mL of PBS containing PEG 3350 at a concentration of 50%.
C. Prepare 10 mL of soy lecithin.
D. Prepare 5 mL of cholesterol.

Add B, C, and D to a 50 mL conical tube and mix well. Then, add solution A to the mixture. Shake the tube thoroughly to ensure complete mixing.

Place the conical tube in a sonicator set to 40 kHz and sonicate for 3 minutes. After sonication, allow the tube to rest on ice for 10 minutes. Repeat this process 5 times to ensure the formation of fine liposomes.

The prepared 5 mL solution will be used as a formulation in an ultrasonic nebulizer for the experiment.

3. Proposed Mechanism of Action

The designed peptides are intended to bind selectively to the C-terminal region of Aβ42, preventing further aggregation. Upon binding, the attached degradation motifs are theorized to activate the UPS, promoting the clearance of Aβ42 aggregates. This dual-action approach, which combines aggregation inhibition with targeted degradation, may provide a comprehensive strategy for reducing Aβ42 levels in the brain, addressing a primary pathological feature of AD.

4. Discussion

This theoretical model presents a potential alternative to existing AD therapies by directly targeting Aβ42 aggregates through BBB-free delivery and using a design that maximizes both binding affinity and degradative capacity. If validated, this approach could bypass the challenges of antibody-based therapies, providing a more accessible and non-invasive option for AD

treatment. While the peptide design and delivery system are supported by in silico evidence, further experimental research is required to validate the practical efficacy of this approach.

5. Conclusion

This work outlines a novel conceptual framework for AD therapy, suggesting that specifically designed peptides can be effectively delivered to the brain using a nasal ultrasonic nebulizer. Through targeted inhibition and degradation of Aβ42, this approach has the potential to overcome significant barriers in AD treatment and contribute to a more effective therapeutic solution.

However, this idea and formulation have not yet undergone in vitro, in vivo, or clinical testing. I am currently conducting several experiments through a one-person enterprise. As an independent researcher, I will strive to secure funding and carry out these experiments, though the timeline remains uncertain. If any pharmaceutical company develops a final product based on this formulation, it is hoped that the cost of a single-use dose will be set at no more than five dollars.

Keywords: Alzheimer's disease, amyloid-beta 42, peptide therapy, nasal delivery, ultrasonic nebulizer, aggregation inhibition, ubiquitin-proteasome system

6. Reference

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