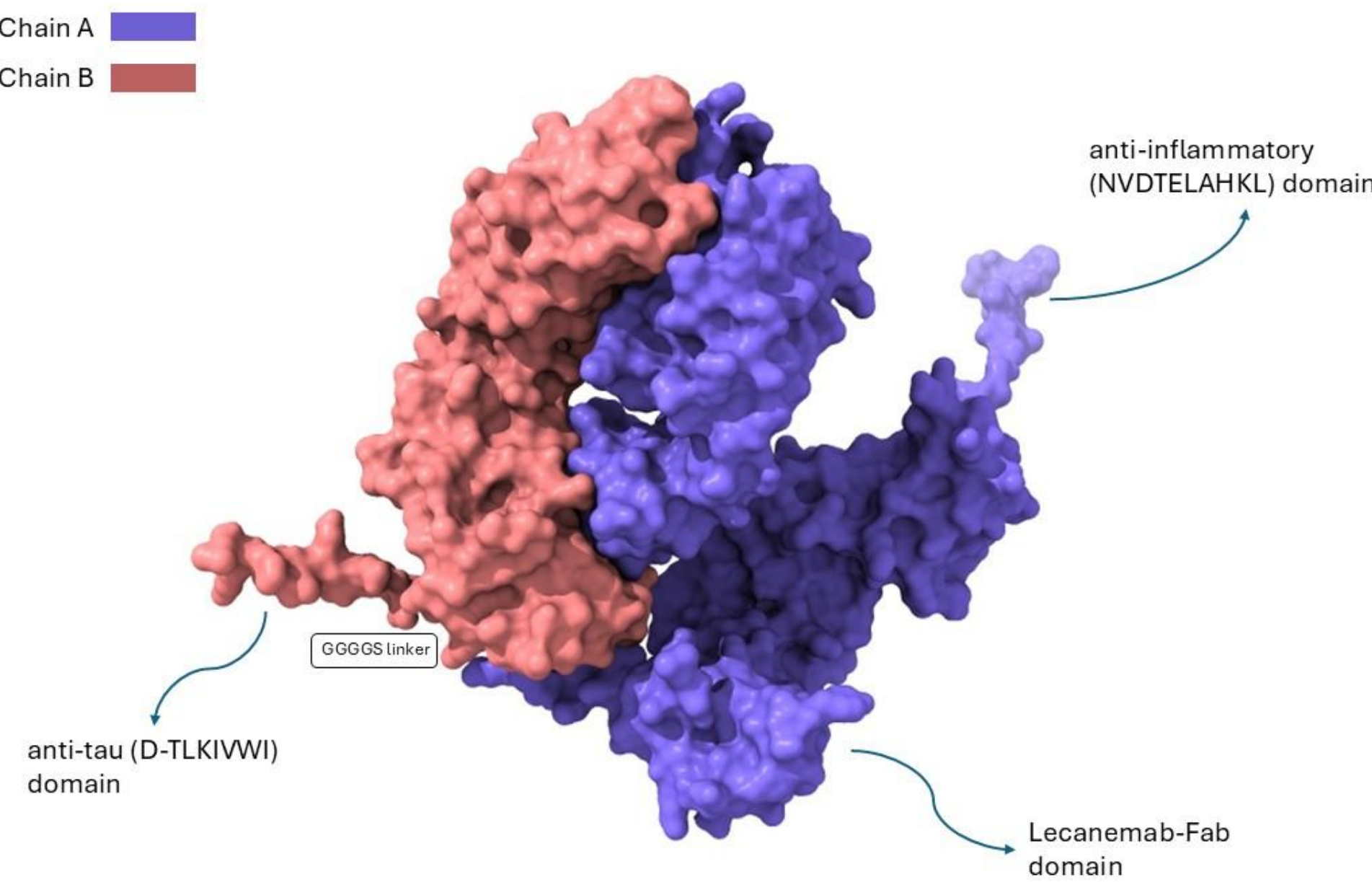


Engineering A Tri-specific Lecanemab-fab Antibody Targeting Amyloid, Tau Aggregation, And Neuroinflammation

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Moaz Elsayed Sayed Ahmed, undergraduate MPharm Candidate, Undergraduate Researcher, School of Life and Medical Sciences, University of Hertfordshire, New Administrative Capital, Cairo, Egypt

This study presents a **structurally robust tri-specific Lecanemab-Fab scaffold** that simultaneously targets **Aβ42, Tau, and TREM2**. Engineered using **AlphaFold2** and **HDOCK**, the scaffold (BSA **2,265 Å²**) demonstrated strong predicted binding to **Aβ42 (−265.48)** and **TREM2 (−329.45)**, highlighting a novel multi-target strategy to address neuroinflammation and potentially mitigate **ARIA-associated complications** in Alzheimer’s disease.

INTRODUCTION	METHODS	RESULTS	RESULTS																								
The Therapeutic Gap: - AD involves a complex interplay of amyloid-beta (Aβ) plaques, Tau aggregates, and chronic neuroinflammation. - Current FDA approved therapies like lecanemab target Aβ alone, leaving Tau unaddressed and posing risks for amyloid related imaging abnormalities (ARIA). The Innovation: Tri-specific Multi-target Scaffold - Aims: To engage the Amyloid-Tau-Neuroinflammation axis simultaneously using a novel tri-specific Lecanemab-Fab scaffold. - Mechanism: Integration of anti-tau (D-TLKIVWI) and anti-inflammatory (NVDTELAHKL) peptides to provide comprehensive therapy and potentially mitigate ARIA through TREM2 engagement. Study Objective: The goal of this research was to design, engineer, and validate the structural robustness and multi-target binding efficacy of this tri-specific scaffold using an integrated computational chemistry workflow	STAGE 1: Structure Prediction Lecanemab-Fab & Engineering The primary antibody structure was modeled and engineered using AlphaFold2_multimer_v3 to ensure accurate assembly of the fragment antigen-binding (Fab) domains. Peptide Domain Acquisition The anti-inflammatory peptide (NVDTELAHKL) was predicted via AlphaFold2, while the anti-tau peptide (D-TLKIVWI) was retrieved from the Protein Data Bank (PDB ID: 9B41). STAGE 2: Scaffold Engineering Linker Integration The selected peptide domains were computationally connected to the Fab heavy and light chains to create the tri-specific architecture. Flexible GGGGS Linker Mechanism Flexible glycine-serine (GGGGS) linkers were utilized to ensure overall structural stability and preserve the accessibility of each binding domain. STAGE 3: Target Preparation Amyloid-beta (Aβ42) Modeling The structure for the Aβ42 target was generated and optimized using AlphaFold2 prediction. Receptor Retrieval from PDB High-resolution structures for Tau (PDB 9B40) and the TREM2 Ig-like domain (PDB 5UD7) were retrieved from the Protein Data Bank for docking simulations. STAGE 4: Docking & In Silico Validation Structural Processing and Primary Docking All prepared structures were processed using AutoDock 1.5.7, followed by primary target engagement simulations executed with the HDOCK computational engine. Cross-Platform Validation Binding reliability was confirmed through cross-platform redocking in ClusPro to ensure concordance across different algorithms. Interface Analysis & Visualization Final binding validation and interface visualization were conducted using ChimeraX and PDBePISA to analyze surface area and contact residues.	Chain A Chain B  anti-inflammatory (NVDTELAHKL) domain anti-tau (D-TLKIVWI) domain GGGGS linker Lecanemab-Fab domain Figure 1: Predictive 3D structural Modeling of the Tri-specific Scaffold via AlphaFold2_multimer_v3 <table><tr><th>Domain</th><th>Target</th><th>Rank</th><th>Docking Score</th><th>Confidence Score</th><th>Ligand RMSD (Å)</th></tr><tr><td>Lecanemab-Fab</td><td>Aβ42</td><td>1</td><td>-242.77</td><td>0.8648</td><td>37.19</td></tr><tr><td>D-TLKIVWI (D-peptide)</td><td>Tau</td><td>1</td><td>-429.62</td><td>0.9963</td><td>150.36</td></tr><tr><td>NVDTELAHKL</td><td>TREM2 Ig-like receptor</td><td>1</td><td>-187.54</td><td>0.6794</td><td>44.43</td></tr></table> Figure 2: Functional Validation and Binding Affinity Analysis of Individuals domains	Domain	Target	Rank	Docking Score	Confidence Score	Ligand RMSD (Å)	Lecanemab-Fab	Aβ42	1	-242.77	0.8648	37.19	D-TLKIVWI (D-peptide)	Tau	1	-429.62	0.9963	150.36	NVDTELAHKL	TREM2 Ig-like receptor	1	-187.54	0.6794	44.43	•Stable scaffold: Structural integrity was supported by 554 hydrogen bonds, 3,592 contacts, and a BSA of 2,265 Å². •Multi-target binding: Strong predicted binding to Aβ42 (−265.48; 0.9097 confidence) and TREM2 (−329.45; 0.9731 confidence). •Protocol validation: Aβ42 docking showed high agreement between HDOCK and ClusPro (BSA 2,133–2,245 Å²; ΔG ≈ −19 to −18 kcal/mol). •Target variability: TREM2 docking showed greater cross-platform variability, reflecting the complexity of neuroinflammatory target modeling.
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			CONCLUSIONS This study demonstrates the feasibility of a structurally robust tri-specific scaffold targeting the amyloid-tau-neuroinflammation axis. Strong predicted engagement with Aβ42 and TREM2 supports its potential to address ARIA-associated mechanisms. While these computational findings provide a solid foundation, experimental validation is required. 																								