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RATIONAL *IN SILICO* DESIGN OF A PEPTIDE BINDER THAT INHIBITS CERT1

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The Ceramide Transfer Protein 1 (CERT1) plays a pivotal role in sphingolipid metabolism by facilitating the transport of ceramide from the endoplasmic reticulum (ER) to the Golgi apparatus, where it is converted into sphingomyelin. Dysregulation of this process is implicated in neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and hereditary intellectual disabilities. Aberrant ceramide homeostasis in neurons can lead to the accumulation of toxic lipid intermediates, triggering neuronal stress, inflammation, and apoptosis – key pathological mechanisms in neurodegeneration. Specifically, elevated ceramide levels in the brain are associated with Alzheimer's disease progression, contributing to amyloid plaque formation and neurofibrillary tangle pathology.

Developing a peptide binder-inhibitor targeting CERT1 is a promising therapeutic strategy for modulating lipid homeostasis in neurons. Inhibiting CERT1 can reduce ceramide transport, leading to its accumulation in the ER, which activates controlled signaling pathways that counteract neurodegenerative processes such as oxidative stress and inflammation. Peptide-based inhibitors offer advantages due to their high binding specificity and potential for modification to enhance blood-brain barrier penetration, making them ideal candidates for neurodegenerative disease therapies. To develop a peptide binder-inhibitor for CERT1 in the context of neurodegenerative diseases, advanced computational tools based on artificial intelligence were used. Initially, the structure of CERT1, particularly its START domain responsible for ceramide binding, was optimized using AlphaFold 2. This deep learning model provided high-accuracy 3D structural predictions, capturing the conformational flexibility critical for inhibitor design in neuronal systems.

Subsequently, the search for an optimal peptide binder was conducted using ColabBinder, an adapted version of ColabFold tailored for peptide binder design. ColabBinder iteratively screened peptide sequences of 15–30 amino acids, optimizing their interactions with the CERT1 active site by prioritizing hydrophobic and electrostatic contacts to ensure high-affinity binding while minimizing off-target effects critical for neuronal applications.

The resulting peptide binder is characterized by the following metrics:

- pLDDT (predicted Local Distance Difference Test) = 74.8 % – indicating medium local structural accuracy;
- pAE (Predicted Aligned Error) = 2.9 Å – reflecting low global alignment error, confirming the reliability of the binder-CERT1 complex model.

Peptide sequence: EALEPIKKIRQRNKPYPEC.

The peptide binder for CERT1 shows moderate structural accuracy but needs optimization. Refining the backbone and side chains using AlphaFold-Multimer or Rosetta could boost pLDDT above 90 %. Assessing ADMET properties via tools like SwissADME is crucial for pharmacokinetic viability. Modifying the peptide with non-natural amino acids or cyclization could enhance stability and bioavailability.

Alternatively, transitioning to small-molecule inhibitors via pharmacophore modeling could improve drug-like properties and blood-brain barrier penetration. This would address peptide limitations for neurodegenerative applications. These optimizations could make the inhibitor more effective for drug development.