PREVENTING CELL-TO-CELL TRANMISSION OF DISORDERED PROTO-FIBRILS OF α-SYNUCLEIN

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ABSTRACT

The cell-to-cell transmission of the oligomeric proto-fibrils of αSynuclein (αS) is believed to be a key driver in the progression of neurodegeneration including Parkinson's Disease. Here, we present a computational protocol for the design of inhibitors preventing the disordered C-terminal region of αS from interacting with its neuronal cell surface receptor, LAG3. We begin with the structural characterization of the binding of the disordered αS with LAG3 using information-driven docking from NMR derived constraints. Using this docked complex, we use partial diffusion and inverse folding to generate and design binders to LAG3. We perform exhaustive validation *in silico* using molecular dynamics and the MM/PBSA-IE method. 12 binders were evaulated *in vitro* of which several show pM-nM affinity binding (K_D), as measured by BLI. The best performing binders effectively downregulate neurodegeneration in mammalian cells and the lead candidates will be further validated in animal models to show functional activity of preventing cell-to-cell transmission of αS.

INTRODUCTION

The disordered proto-fibrils of α-synuclein (αS) are major pathogenic agents in several neurodegenerative diseases, including Parkinson's disease. These proto-fibrils between neuronal and glial cells exhibit prion-like behaviour, rendering them highly cytotoxic as they induce progressive neuronal loss, a hallmark of Parkinson's disease [Dawson (2016)]. Further mounting evidence has shown that pre-formed amyloid fibrils serve as the key pathological entities with a wide range of distinct pathological activities, including neuron-to-neuron transmission and propagation, disruption of protein quality control, induction of neuroinflammation, and cell death. Neuronal cell surface receptors such as Lymphocyte Activation Gene 3 (LAG3) can preferentially bind amyloid-like proto-fibrils (*aka* pre-formed fibrils) over monomers of αS. Various biophysical experiments reveal mechanistic insights of the sub nanomolar affinity interaction of the C-terminal disordered peptide residues of αS (residues 118-140) with the Domain 1 of LAG3 (L3D1). In this work, we present a strategy for designing antagonist binders of the cell-to-cell transmission of αS by competitively inhibiting its interaction with L3D1.

RESULTS AND DISCUSSION

We begin with computationally characterizing the binding modes of the C-terminal αS peptide (residues 118-140) with L3D1 by identifying the key residue-residue contacts from 1H NMR chemical shifts and 2D HSQC spectra [Zhang et al. (2021; 2023)]. We used a flat bottom harmonic potential (as restraints) to rigidly dock the disordered αS peptide (starting conformations taken from PDBID: 2N0A, 1A) to the AF2 predicted structure of L3D1. The rigidly docked poses were subjected to flexible interface refinement and explicit-solvent molecular dynamics simulations in water using HADDOCK [Koukos & Bonvin (2020)]. For all starting conformations from PDB 2N0A, we generate 5000 docked poses (with restraints applied). The docked poses were analyzed for binding affinity (Figure 1B) and the best αS-L3D1 complex mimicing the reported NMR contacts was chosen as a template for the design of antagonists.

We generate new binder backbones using RFDiffusion [Watson et al. (2023)]. We generated 10,000 backbones by partially diffusing the αS coordinates using 30 steps and adding potentials to maintain contacts with key residues on L3D1 (identified from residue-wise MM/PBSA from MD trajectories).

The noise scale was set to a value of 0.25 to enable diversity in the backbones generated. We further applied a radius of gyration potential to prevent the generation of unphysical structures (Figure 1C). We generated 10 sequences using ProteinMPNN [Dauparas et al. (2022)] for every backbone generated and validated the designs using AF2 with initial guess [Bennett et al. (2023)] resulting in about 100,000 predictions. Of which, 49 designs passed the following criteria: binder pLDDT > 80, inter-chain pAE < 10 and a binder RMSD < 1 $\mathring{A}$. We then performed molecular dynamics simulations (100 ns) of these 49 candidates and ranked them based on i) MM/PBSA-IE binding affinity (ΔG) and the fraction of L3D1 contacts satisfied with those experimentally derived (Figure 1D,E). 12 lead candidates were shortlisted for *in vitro* validation.

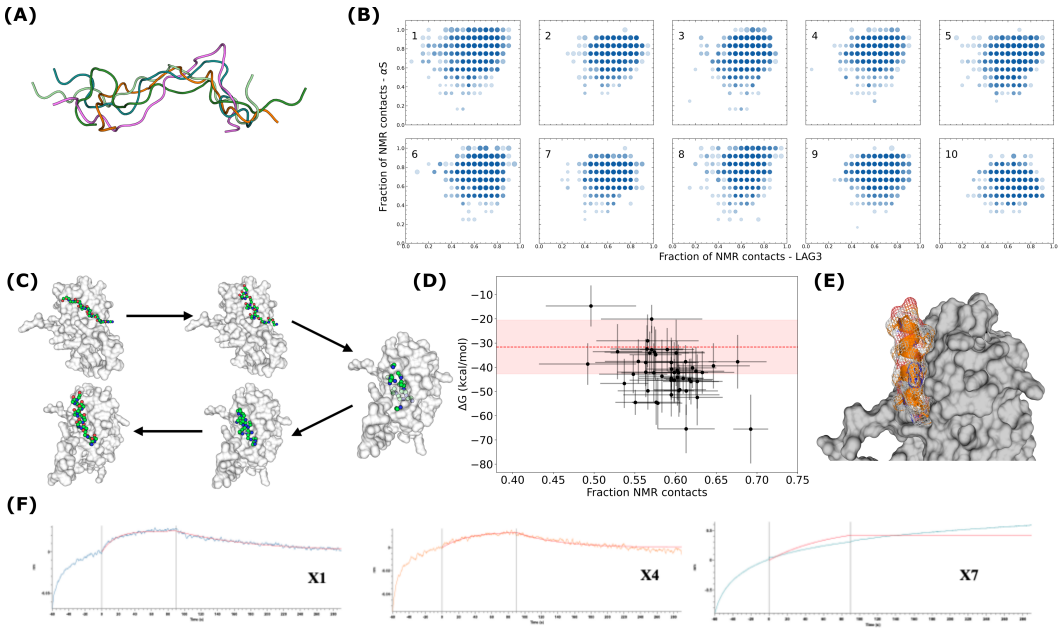

Figure 1: **Computational Design and *in vitro* screening of LAG3 binder antagonists. A** Visual representation of the disordered nature of the C-terminal peptide of αS (residues 118-140) from the full-length proto-fibrillar assembly (PDB ID: 2N0A). **B** The disordered C-terminus of α-Synuclein (αS) is docked to its neuronal-cell surface receptor (LAG3) using NMR-derived contact residues as restraints. The fraction of contacts made by the docked poses are compared to the experimental values. The size denotes the binding free energy (larger is better) and the shade represents the cluster population (darker is populated). **C** Partial diffusion of 30 steps was performed to design the backbone of the inhibitors starting from the top docked poses identified in the previous step. **D** 49 anti-LAG3 inhibtors passing AF2 derived metrics were validated by molecular dynamics. The ΔG of binding is calculated by the MM/PBSA-IE method on a 100 ns trajectory. The binding affinity of the C-terminus αS (MM/PBSA-IE) with L3D1 is highlighted as red band. **E** Visualization of one of the best performing designed anti-LAG3 binders (in orange) bound to LAG3 (in gray). **F** BLI binding curves for a subset of the experimentally validated binders show affinity in the pM-nM range.

Our preliminary data confirm that the binders effectively downregulate neurodegeneration in mammalian cells, specifically reducing necrosis, early apoptosis, and late apoptosis. Biolayer interferometry reveals strong fibril binding, with binder X4 showing the best results (pM affinity, Figure 1F). Furthermore, receptor-based blocking via LAG3 binding and neuroblastoma-based models indicate the binders are ready for final screening. Future studies will expand cell cytotoxicity assessments using Parkinson's disease models to validate the binder efficacy in disease-relevant conditions. In-vivo animal studies will be conducted to confirm the reduction of Parkinson's-like symptoms, ensuring translational potential.

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