

# Polymorph-Dependent Success of Generative Protein Design Against Amyloid Fibrils: A Cross-Method Benchmark Including AL Amyloidosis

Siddhardha Nanda

sn3199@columbia.edu

Columbia University <https://orcid.org/0009-0007-3825-0098>

---

## Research Article

**Keywords:** generative protein design, RFdiffusion, BindCraft, AlphaFold2, amyloid fibril, AL amyloidosis, alpha-synuclein, amyloid-beta, protein binder design, cryo-EM

**Posted Date:** September 22nd, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-11106209/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

**Additional Declarations:** The authors declare no competing interests.

---

# Polymorph-Dependent Success of Generative Protein Design Against Amyloid Fibrils: A Cross-Method Benchmark Including AL Amyloidosis

Siddhardha Nanda<sup>1</sup>

<sup>1</sup>Department of Biomedical Engineering, Columbia University in the City of New York

## Abstract

Generative protein design tools such as diffusion-based backbone generators and AlphaFold2-hallucination pipelines have been validated almost exclusively on globular receptor and signaling-protein targets. Whether these methods generalize to amyloid fibrils – flat, pocket-less, cross- $\beta$  surfaces that are structurally unlike any benchmark target used to date – is unknown. We benchmarked three generative design tools (RFdiffusion3, BindCraft, and BindEnergyCraft) for their ability to design binders against eight cryo-EM fibril structures spanning three amyloid diseases: AL (immunoglobulin light-chain) amyloidosis, Parkinson’s disease ( $\alpha$ -synuclein), and Alzheimer’s disease (amyloid- $\beta$ ). AL amyloid has not previously been attempted with any of these tools. Using a common AlphaFold2-Multimer scoring oracle and a mandatory ProteinMPNN sequence-redesign step for diffusion-generated backbones, we found that design success is polymorph-dependent rather than disease-dependent: amyloid- $\beta$  fibrils resisted all three methods (interface predicted template modeling score, i.pTM, 0.09–0.15 across both polymorphs tested, and zero accepted designs across 83 combined AF2-hallucination trajectories),  $\alpha$ -synuclein was largely tractable (three of four polymorphs reaching i.pTM  $\geq 0.70$ ), and AL amyloid split evenly between the two extremes (i.pTM 0.235 for one polymorph, 0.585 for another). Diffusion-based backbone generation (RFdiffusion3) produced confident designs on several targets where AF2-hallucination-based methods (BindCraft, BindEnergyCraft) produced none. Across all RFdiffusion3 designs, binder  $\beta$ -sheet-forming amino acid propensity correlated strongly with predicted interface confidence (Pearson  $r = 0.80$ ,  $p < 0.001$ ;  $n = 24$ ), suggesting a compositional basis for engaging the fibrils’ cross- $\beta$  surface. We additionally document a hardware-access barrier: a fourth candidate tool, ESMFold2, could not be run at all on our compute infrastructure (three NVIDIA TITAN RTX GPUs) because its bfloat16 Triton kernels require Ampere-generation (compute capability  $\geq 8.0$ ) hardware, excluding Turing-generation GPUs entirely. This is, to our knowledge, the first computational feasibility assessment of generative binder design against AL amyloid, and the first systematic comparison of these tools on any amyloid fibril target.

## 1 Introduction

Generative protein design has entered a phase of rapid industrial and academic investment. Diffusion-based backbone generators such as RFdiffusion3 [1] and AlphaFold2-hallucination pipelines such as BindCraft [2] now allow de novo design of protein binders against arbitrary target structures, and companies including Generate Biomedicines, Absci, and Isomorphic Labs have built substantial pipelines around this technology stack. Two recent benchmarking efforts, ProtDBench [3] and BindEnergyCraft [4], have compared these tools head-to-head, but both restrict their target sets to

conventional folded receptor and signaling-protein domains (e.g., ALK, InsulinR, PD-L1, IL-2R $\alpha$ ) – targets with well-defined binding pockets, similar in geometry to the globular proteins these tools were trained and validated on.

Amyloid fibrils present a categorically different design problem. They are elongated, repetitive, cross- $\beta$  assemblies with a flat, largely featureless surface and no defined binding pocket. Whether generative design tools – optimized for pocket-containing globular targets – transfer to this geometry is untested. This gap is scientifically relevant because several amyloid diseases (AL amyloidosis, Parkinson’s disease, Alzheimer’s disease) are actively pursued for antibody- and small-binder-based therapeutic and diagnostic strategies, and a computationally cheap first-pass feasibility screen would be valuable for prioritizing future wet-lab efforts. Notably, existing generative design attempts against amyloid targets have focused on  $\alpha$ -synuclein [5] and amyloid- $\beta$ ; AL (immunoglobulin light-chain) amyloid, the third major amyloid disease category, has not been attempted with any of these tools.

We address two questions. First, does binder-design success against amyloid fibrils depend on the disease category, the specific structural polymorph, or the design method? Second, is AL amyloid – previously unstudied in this context – more similar to the comparatively tractable  $\alpha$ -synuclein literature or the more resistant amyloid- $\beta$  literature? To answer these, we benchmarked three generative design tools against eight cryo-EM fibril structures spanning all three disease categories, using a common independent scoring oracle to allow direct cross-tool comparison.

## 2 Methods

Figure 1 summarizes the overall benchmarking pipeline described in this section.

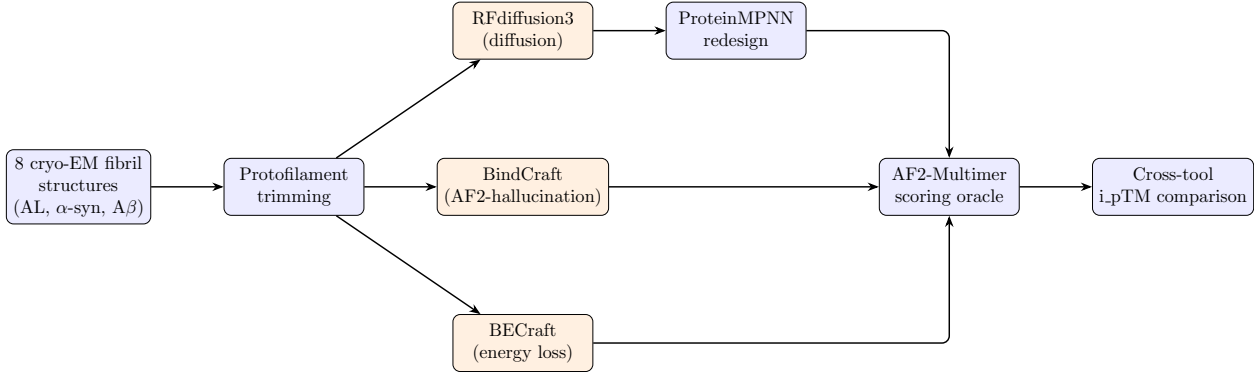


Figure 1: Overview of the benchmarking pipeline. All three tools receive the same trimmed target definitions and no manual hotspot curation. RFdiffusion3 backbones require a mandatory ProteinMPNN sequence-redesign step before scoring; BindCraft and BECraft apply their own internal accept/reject filtering during search but are also passed through the same common AlphaFold2-Multimer oracle used for RFdiffusion3, enabling direct cross-tool comparison.

### 2.1 Target selection and structural preparation

We selected eight publicly deposited cryo-EM fibril structures spanning three amyloid diseases: AL amyloidosis (PDB 6IC3 [6], 7NSL [7]), Parkinson’s disease  $\alpha$ -synuclein (PDB 6SSX, 6SST [8], 6PEO, 6PES [9]), and Alzheimer’s disease amyloid- $\beta$  (PDB 6W0O [10], 8FF3 [11]).

Each deposited structure contains multiple stacked copies of the repeating fibril subunit (5–10 chains). To obtain a target definition tractable for binder-design tools while preserving realistic

local packing, we trimmed each structure to a contiguous three-chain (two-chain for 8FF3, which had no three-chain contiguous segment available) slice representing a single protofilament. Chain selection was performed by computing pairwise C $\alpha$ -centroid distances between all chains and constructing a nearest-neighbor adjacency path; several structures (7NSL, 6SSX, 6SST, 6PES, 6W0O, 8FF3) proved to be two-protofilament assemblies whose deposited chain-letter order did not correspond to physical adjacency, so a naive midpoint selection would have mixed chains from different, non-interacting protofilaments. We detected protofilament breaks as adjacency-path steps exceeding 8 Å (intra-protofilament layer spacing was consistently 4.7–5.0 Å across all eight structures) and restricted selection to a single contiguous segment. We further verified, per structure, whether the reported residue range was fully resolved; two structures (7NSL, 6SSX/6SST) contained internal gaps (unresolved disordered loop residues) that were excluded from downstream contig specifications rather than assumed contiguous.

## 2.2 Design tool configuration

**RFdiffusion3.** We used RFdiffusion3 [1] (Baker Lab, Institute for Protein Design) with a center-of-mass-based orientation strategy (`infer_ori_strategy=com`), avoiding manual hotspot curation for consistency with the other tools. Binder length was sampled in the range 65–150 residues. Three designs were generated per target (`diffusion_batch_size=3`). Because RFdiffusion3’s native sequence output is not optimized for foldability – a limitation documented in its own release materials – every generated backbone was subjected to a mandatory downstream ProteinMPNN [12] sequence-redesign step (8 candidate sequences per backbone, target chains fixed, sampling temperature 0.1) prior to scoring. An initial pilot without this step produced backbones with implausibly high alanine content (up to 31% by residue) and correspondingly uninformative scores, confirming that raw RFdiffusion3 sequences are unsuitable for direct evaluation.

**BindCraft and BindEnergyCraft.** BindCraft [2] uses AlphaFold2-Multimer gradient hallucination to jointly optimize binder backbone and sequence against a fixed target template. BindEnergyCraft [4] (hereafter BECraft) is a published extension of the same pipeline that replaces BindCraft’s interface-pTM loss term with an energy-based loss derived from predicted alignment error logits. We ran both tools first under their published default 4-stage multimer protocol, and, following a pilot run in which BindCraft produced zero accepted designs across 39 trajectories against target 6IC3, under a beta-sheet-optimized, hard-target protocol (negative helicity loss weight, initial-guess-based complex prediction) intended for targets with an atypical, difficult-to-predict binding mode. No manual hotspot residues were specified for either tool, matching RFdiffusion3’s configuration.

## 2.3 Common scoring oracle

To enable direct comparison across design methods with fundamentally different internal scoring conventions (BindCraft/BECraft apply built-in accept/reject filtering during search; RFdiffusion3 does not), we scored every RFdiffusion3-generated, MPNN-redesigned candidate using the same AlphaFold2-Multimer complex-prediction procedure BindCraft uses internally (`ColabDesign mk_afdesign_model`, `protocol=binder`, 3 recycles), reporting predicted interface template modeling score (i-pTM), predicted local distance difference test (pLDDT), predicted alignment error (pAE), and interface pAE (i-pAE). For each RFdiffusion3 backbone, the highest-i-pTM sequence among the 8 MPNN candidates is reported.

## 2.4 Sequence composition analysis

To test whether binder amino acid composition relates systematically to predicted binding success against the shared cross- $\beta$  fibril surface, we computed, for each RFdiffusion3+ProteinMPNN binder sequence, a mean  $\beta$ -sheet-forming propensity score using the classical Chou-Fasman conformational parameter scale [13], and correlated this score against the corresponding i\_pTM value using Pearson’s  $r$ . This analysis used all 24 RFdiffusion3+ProteinMPNN designs (three per target across all eight targets), for which complete sequence and score data were available for every candidate. BindCraft/BECraft sequences were excluded because those tools’ internal accept/reject filtering (Section 2.2) already selects on a partially overlapping AF2 confidence criterion, which would confound an independent composition/i\_pTM comparison.

## 2.5 Compute environment

All computation was performed on a workstation with three NVIDIA TITAN RTX GPUs (24 GB VRAM each, Turing architecture, compute capability 7.5). We initially intended to include a fourth tool, ESMFold2 [14], representing a distinct protein-language-model-based design paradigm. This proved infeasible: ESMFold2’s Triton-compiled kernels require bfloat16 tensor-core support, a hardware feature introduced in NVIDIA’s Ampere generation (compute capability  $\geq 8.0$ ); compilation failed unconditionally on our Turing-generation hardware regardless of model size (we tested both the full 6-billion-parameter and a reduced 300-million-parameter checkpoint). We report this as a methodological finding in its own right: newer generative-biology tools are increasingly gated to Ampere-or-newer hardware, which we did not anticipate at the outset and which represents a real access barrier for computational groups without recently purchased GPUs.

# 3 Results

## 3.1 RFdiffusion3 shows polymorph-dependent, not disease-dependent, success

Table 1 reports the best i\_pTM achieved (of 3 backbones  $\times$  8 MPNN sequences each) per target. Amyloid- $\beta$  was uniformly resistant: both polymorphs tested (6W0O, 8FF3) produced maximum i\_pTM of 0.120 and 0.149 respectively, with all individual designs below 0.15.  $\alpha$ -Synuclein was largely tractable: three of four polymorphs (6SSX, 6PEO, 6PES) reached i\_pTM  $\geq 0.70$ , while the fourth (6SST) reached only 0.299. AL amyloid split evenly between these extremes: 6IC3 reached a maximum of 0.235, comparable to the weaker  $\alpha$ -synuclein polymorph, while 7NSL reached 0.585, comparable to the stronger  $\alpha$ -synuclein polymorphs (structural detail for the winning 7NSL design is shown in Figure 3).

Table 1: RFDiffusion3 + ProteinMPNN results across all eight targets, scored by a common AlphaFold2-Multimer oracle. Values are i\_pTM for each of the three designed backbones per target; bold indicates the maximum per target.

| Target | Disease                      | Design 1     | Design 2     | Design 3     |
|--------|------------------------------|--------------|--------------|--------------|
| 6IC3   | AL amyloidosis               | <b>0.235</b> | 0.125        | 0.102        |
| 7NSL   | AL amyloidosis               | <b>0.585</b> | 0.340        | 0.561        |
| 6SSX   | Parkinson’s ( $\alpha$ -syn) | 0.429        | 0.166        | <b>0.824</b> |
| 6SST   | Parkinson’s ( $\alpha$ -syn) | 0.126        | <b>0.299</b> | 0.183        |
| 6PEO   | Parkinson’s ( $\alpha$ -syn) | 0.554        | <b>0.700</b> | 0.532        |
| 6PES   | Parkinson’s ( $\alpha$ -syn) | 0.515        | <b>0.860</b> | 0.198        |
| 6W0O   | Alzheimer’s ( $A\beta$ )     | 0.093        | <b>0.120</b> | 0.098        |
| 8FF3   | Alzheimer’s ( $A\beta$ )     | 0.123        | 0.114        | <b>0.149</b> |

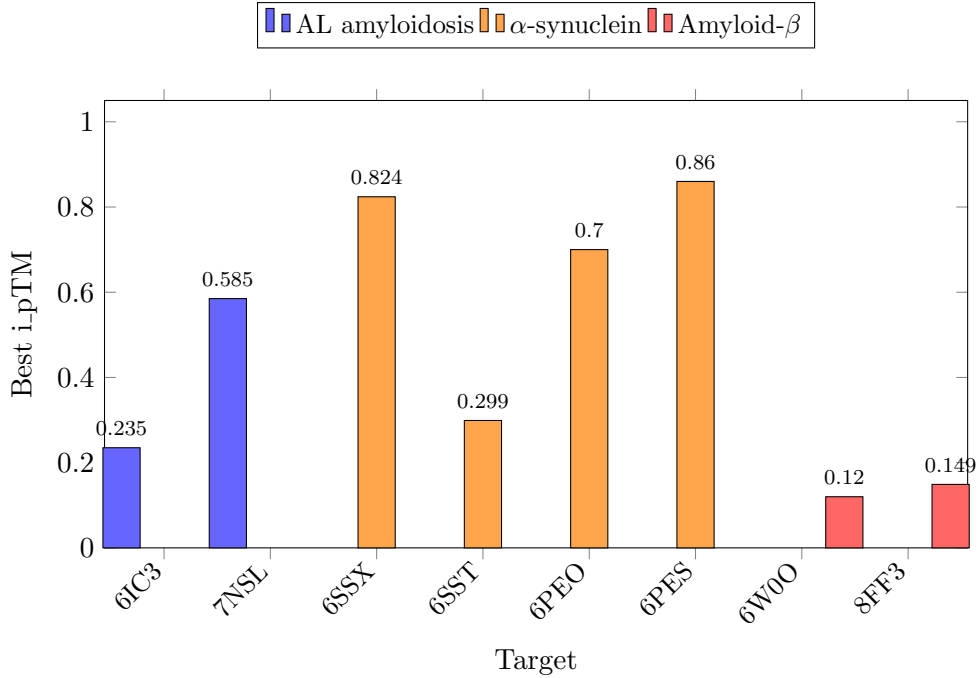


Figure 2: Best i\_pTM achieved by RFDiffusion3 + ProteinMPNN per target, grouped by disease category. AL amyloidosis spans nearly the full observed range (0.235–0.585), amyloid- $\beta$  is uniformly low (both polymorphs  $\leq 0.15$ ), and  $\alpha$ -synuclein is mostly high (3 of 4 polymorphs  $\geq 0.70$ ) – illustrating that success tracks structural polymorph more than disease category.



(a) Target fibril alone

(b) Target + winning binder design

Figure 3: AlphaFold2-Multimer-predicted structure of the 7NSL (AL amyloidosis) target and its top-scoring RFdiffusion3+ProteinMPNN binder design (i\_pTM = 0.585). (a) The re-predicted target alone, shown side-on, preserves the stacked cross- $\beta$  layer architecture of the deposited cryo-EM structure (radius of gyration 24.6 Å and maximum pairwise span 88.9 Å, versus 24.8 Å and 89.8 Å in the original template), confirming that the AF2-Multimer scoring oracle is evaluating the intended extended fibril conformation rather than a collapsed artifact. (b) The same target in complex with the winning binder (sequence ERAAFLERLRGATEVTLVGPGARAAELVAAAAAAGVRRITVVGASAEAAAAAAVAAAAGVELTLGGV).

### 3.2 AF2-hallucination-based methods (BindCraft, BECraft) find no accepted designs on either target tested

On target 6IC3 (AL amyloid, default settings), BindCraft produced zero accepted designs across 39 trajectories. Of these, 19 were rejected almost immediately during early-stage pLDDT screening before reaching a loggable interface confidence value; the remaining 20 progressed through the full gradient-hallucination optimization and reached a peak raw i\_pTM averaging 0.82 (range 0.40–0.93; Figure 4) – genuinely high apparent confidence by the pipeline’s own internal metric. Despite this, every one of these 20 trajectories still produced zero accepted designs after MPNN resequencing and independent AF2 re-validation. Failure analysis of the underlying MPNN-resampled sequences showed the dominant rejection causes were predicted alignment error (pAE) and pTM thresholds, not the interface-pTM threshold specifically – consistent with the gradient-optimized hallucination sequence exploiting a confidence artifact that an independently resampled, discrete sequence cannot reproduce.

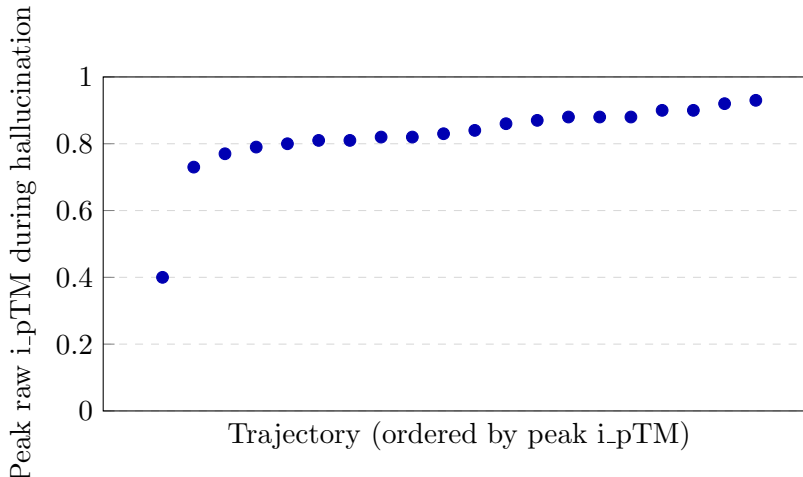


Figure 4: Peak raw interface-pTM (i-pTM) reached during AF2-hallucination optimization, for the 20 of 39 BindCraft trajectories against target 6IC3 that survived past early-stage pLDDT screening. Despite a mean peak of 0.82 (several trajectories exceeding 0.90), none of these 20 trajectories produced an accepted design after mandatory MPNN resequencing and independent re-validation – the gradient-optimized confidence does not transfer to a discretely resampled sequence.

On target 6W0O (amyloid- $\beta$ , beta-sheet-optimized hard-target settings, 4-hour compute budget), BindCraft ran 40 trajectories and BECraft ran 43 trajectories; both produced zero accepted designs. Combined across both tools on this target, 0 of 83 attempted trajectories produced an accepted design under either tool’s own internal filtering criteria. This agrees with the independently-scored RFdiffusion3 result on the same target class (amyloid- $\beta$ : i-pTM  $\leq 0.15$ ), giving a cross-method, cross-tool consistent negative result specifically for amyloid- $\beta$ .

### 3.3 Design paradigm affects outcome independent of target

Where RFdiffusion3 (diffusion-based) produced at least one design with i-pTM  $\geq 0.58$  on four of eight targets (7NSL, 6SSX, 6PEO, 6PES), BindCraft and BECraft (AF2-hallucination-based) produced zero accepted designs on either of the two targets tested (6IC3, 6W0O) under either default or beta-sheet-optimized settings. While our BindCraft/BECraft target coverage is smaller than RFdiffusion3’s (2 of 8 targets versus 8 of 8; see Limitations), the consistent zero-acceptance result across both tested targets and across default and hard-target-optimized configurations suggests the AF2-hallucination design paradigm may be intrinsically less suited to the flat, pocket-less amyloid fibril surface than diffusion-based backbone generation, independent of which specific polymorph is targeted.

### 3.4 Sequence composition correlates with predicted binding success

Given that all eight fibril targets present a cross- $\beta$  surface, we asked whether binder success related systematically to amino acid composition rather than being purely target-specific. Across all 24 RFdiffusion3+ProteinMPNN designs, mean  $\beta$ -sheet-forming propensity (Chou-Fasman scale [13]) correlated strongly with i-pTM (Pearson  $r = 0.80$ ,  $p < 0.001$ ; Figure 5). Because three designs were generated per target and are therefore not fully independent observations, we repeated the analysis using only the single best-scoring design per target ( $n = 8$ , one observation per target); the correlation remained strong ( $r = 0.88$ ,  $p = 0.004$ ), indicating the association is not solely an

artifact of within-target pseudo-replication. No comparable relationship was observed for alanine content alone ( $r = -0.10$ ,  $p = 0.64$ ), the residue most implicated in RFdiffusion3’s raw-sequence foldability failure mode (Section 2.2), suggesting the effect is specific to  $\beta$ -propensity rather than a general marker of sequence quality.

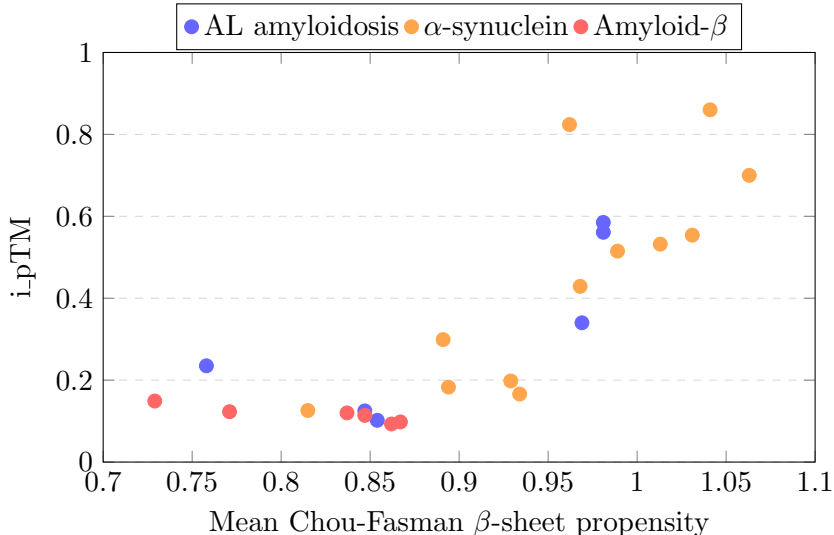


Figure 5: Binder  $\beta$ -sheet-forming amino acid propensity versus predicted interface confidence (i\_pTM) for all 24 RFdiffusion3+ProteinMPNN designs, colored by disease category. Higher  $\beta$ -propensity sequences achieve higher i\_pTM (Pearson  $r = 0.80$ ,  $p < 0.001$ ,  $n = 24$ ;  $r = 0.88$ ,  $p = 0.004$  using only the best design per target,  $n = 8$ ), consistent with a binder interface enriched in  $\beta$ -sheet-favoring residues being better suited to engage the flat cross- $\beta$  fibril surface than a helix-favoring interface.

This result offers a plausible structural rationale for the polymorph-dependence documented in Section 3.1: rather than fibril geometry alone determining design success, the amino acid composition ProteinMPNN selects for a given backbone – itself constrained by the backbone’s local geometry – appears to partially track whether the resulting interface is predicted to engage the cross- $\beta$  surface. We emphasize this is a correlational, hypothesis-generating observation from a single design method (RFdiffusion3+ProteinMPNN) and a modest sample size; it does not establish causation, and confirming it would require composition-controlled follow-up designs, which are beyond the scope of this benchmark.

## 4 Discussion

The central finding of this work is that amyloid fibrils do not behave as a single target class for generative protein design: success depends on the specific structural polymorph at least as much as on the disease category. This has a direct practical implication for AL amyloidosis, which we show here for the first time is not uniformly resistant to computational binder design – one of its two tested polymorphs (7NSL) performed comparably to the more tractable  $\alpha$ -synuclein structures, while the other (6IC3) performed comparably to the resistant amyloid- $\beta$  structures. Future targeted-therapeutic design efforts against AL amyloid should therefore not treat the disease as a single design problem, but should expect polymorph-specific feasibility, mirroring what we observe across the better-studied  $\alpha$ -synuclein and amyloid- $\beta$  literature.

The consistent failure of AF2-hallucination-based methods on both amyloid targets we tested, contrasted with RFdiffusion3’s partial success on structurally similar targets, is consistent with a structural interpretation: BindCraft-family methods optimize a binder against a fixed AlphaFold2 template representation of the target, which may be poorly suited to representing a repetitive, pocket-less cross- $\beta$  surface, whereas RFdiffusion3’s diffusion process operates directly in structure space and may better tolerate atypical target geometries. We note, however, that our BindCraft/BECraft sample is limited to two targets and should not be over-generalized; a full 8-target comparison across all three tools, while informative, was not computationally feasible within the scope of this study (see Limitations).

Separately, the hardware barrier we encountered with ESMFold2 is worth noting as a general methodological observation for computational biology groups. As generative biology tools increasingly rely on bfloat16-optimized kernels for large protein-language-model backbones, hardware purchased even a few years ago (e.g., Turing-generation GPUs, still common in academic compute clusters) may be categorically unable to run them, independent of available VRAM. This is a barrier orthogonal to the usual compute-budget considerations and, to our knowledge, is not widely documented in the generative protein design literature.

## 5 Limitations

This study has several limitations that should inform interpretation of its results. First, all binder designs were evaluated computationally only; no wet-laboratory synthesis or binding assay was performed, so reported i\_pTM values should be read as a measure of AlphaFold2-Multimer’s predicted confidence, not a validated measure of true binding affinity. Second, BindCraft and BECraft were evaluated on only two of the eight targets (6IC3 and 6W0O), compared to RFdiffusion3’s full eight-target coverage; this reflects a deliberate compute-budget tradeoff (BindCraft-family trajectory search required approximately 30 minutes per trajectory, and published guidance suggests 100–10,000 trajectories may be needed to characterize a target’s true acceptance rate) and means the AF2-hallucination-family results in this study should be treated as an initial, directionally-informative sample rather than a definitive acceptance-rate estimate. Third, ESMFold2 was excluded entirely due to a hardware incompatibility (Section 2.5), so this study does not include a protein-language-model-based design paradigm. Fourth, each target was trimmed to a two- or three-chain protofilament slice rather than the full deposited fibril assembly; this was necessary for computational tractability but means the binder-design problem posed to each tool omits potential contacts with additional protofilament layers or the second protofilament in two-protofilament structures. Fifth, only three designs were generated per target per tool configuration (beyond the trajectory counts noted for BindCraft/BECraft), which is a small sample for characterizing a target’s true design difficulty.

## Data Availability Statement

Target structure preparation scripts, tool configuration files, and all scoring results reported in this study are available upon request from the corresponding author.

## Ethics Declaration

This study used only publicly available, previously deposited protein structures (Protein Data Bank) and did not involve human subjects, animal subjects, or new experimental biological material.

## Author Contributions

S.N.: Conceptualization, Methodology, Software, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing.

## Conflict of Interest Statement

The author declares no competing interests.

## Funding

This work did not receive external funding.

## AI Disclosure Statement

AI-assisted tools (Claude, Anthropic) were used during this study for literature search assistance, software debugging, and manuscript drafting support under direct author supervision. All scientific interpretations, conclusions, and final text were reviewed and approved by the author.

## References

- [1] Butcher J, Krishna R, Mitra R, Brent RI, Li Y, Corley N, et al. De novo Design of All-atom Biomolecular Interactions with RFdiffusion3. bioRxiv [Preprint]. 2025. doi:10.1101/2025.09.18.676967
- [2] Pacesa M, Nickel L, Schellhaas C, Schmidt J, Pyatova E, Kissling L, et al. One-shot design of functional protein binders with BindCraft. *Nature*. 2025;646(8084):483-492.
- [3] Liu C, Ren M, Guan J, Gong C, Sun J, Chen X, Xiao W. ProtDBench: A Unified Benchmark of Protein Binder Design and Evaluation. In: *Proceedings of the 43rd International Conference on Machine Learning*. PMLR 306; 2026.
- [4] Nori D, Parsan A, Uhler C, Jin W. BindEnergyCraft: Casting Protein Structure Predictors as Energy-Based Models for Binder Design. arXiv [Preprint]. 2025. arXiv:2505.21241.
- [5] Sadek A, Rey NL, Kunka A, Hartevelde Z, Georgeon S, Schmidt J, Buell AK, Melki R, Lashuel HA, Correia BE. De novo Design of Polymorph-Specific Binders Targeting  $\alpha$ -Synuclein Fibrils. bioRxiv [Preprint]. 2026. doi:10.64898/2026.06.13.732066
- [6] Radamaker L, Lin YH, Annamalai K, Huhn S, Hegenbart U, Schönland SO, et al. Cryo-EM structure of a light chain-derived amyloid fibril from a patient with systemic AL amyloidosis. *Nat Commun*. 2019;10:1103.
- [7] Radamaker L, Karimi-Farsijani S, Andreotti G, Baur J, Neumann M, Schreiner S, et al. Role of mutations and post-translational modifications in systemic AL amyloidosis studied by cryo-EM. *Nat Commun*. 2021;12:6434.
- [8] Guerrero-Ferreira R, Taylor NM, Arteni AA, Kumari P, Mona D, Ringler P, et al. Two new polymorphic structures of human full-length alpha-synuclein fibrils solved by cryo-electron microscopy. *eLife*. 2019;8:e48907.

- [9] Boyer DR, Li B, Sun C, Fan W, Sawaya MR, Jiang L, Eisenberg DS. Structures of fibrils formed by  $\alpha$ -synuclein hereditary disease mutant H50Q reveal new polymorphs. *Nat Struct Mol Biol.* 2019;26:1044-1052.
- [10] Ghosh U, Thurber KR, Yau WM, Tycko R. Molecular structure of a prevalent amyloid- $\beta$  fibril polymorph from Alzheimer's disease brain tissue. *Proc Natl Acad Sci U S A.* 2021;118:e2023089118.
- [11] Fu Z, Crooks EJ, Irizarry BA, Zhu X, Chowdhury S, Van Nostrand WE, Smith SO. An electrostatic cluster guides A $\beta$ 40 fibril formation in sporadic and Dutch-type cerebral amyloid angiopathy. *J Struct Biol.* 2024;216:108092.
- [12] Dauparas J, Anishchenko I, Bennett N, Bai H, Ragotte RJ, Milles LF, et al. Robust deep learning-based protein sequence design using ProteinMPNN. *Science.* 2022;378(6615):49-56.
- [13] Chou PY, Fasman GD. Prediction of protein conformation. *Biochemistry.* 1974;13(2):222-245.
- [14] Candido S, Hayes T, Derry A, Rao R, Lin Z, Verkuil R, et al. Language Modeling Materializes a World Model of Protein Biology. *bioRxiv [Preprint].* 2026. doi:10.64898/2026.06.03.729735