AI-Driven Discovery of Novel Molecular Tools for Intraoperative Nerve Visualization

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Overview/Abstract

Nerve sparing surgery is a type of surgery where the surgeon tries to avoid cutting or damaging nerves near other tissues that are being removed. Normal, conventional methods, like using fluorescent dyes to highlight and visualize important tissues and bodily structures are not sufficient when it comes to highlighting and detailing surrounding, peripheral nerves. The specific peripheral nerves we are concerned with express the voltage-gated sodium Nav1.7 channel, which is a very selective biomarker in the peripheral nervous system. We are interested in designing miniprotein binders targeting Nav1.7, using state-of-the-art machine learning diffusion models, and verifying if the generated designs are effective and have an affinity to bind with the channel with molecular dynamics simulations. These binders are being designed and tested with the intention of developing fluorophores that mark nerves that express the Nav1.7 sodium channel, since none exist yet, as well as therapeutics.

Nav1.7 Sodium Ion Channel

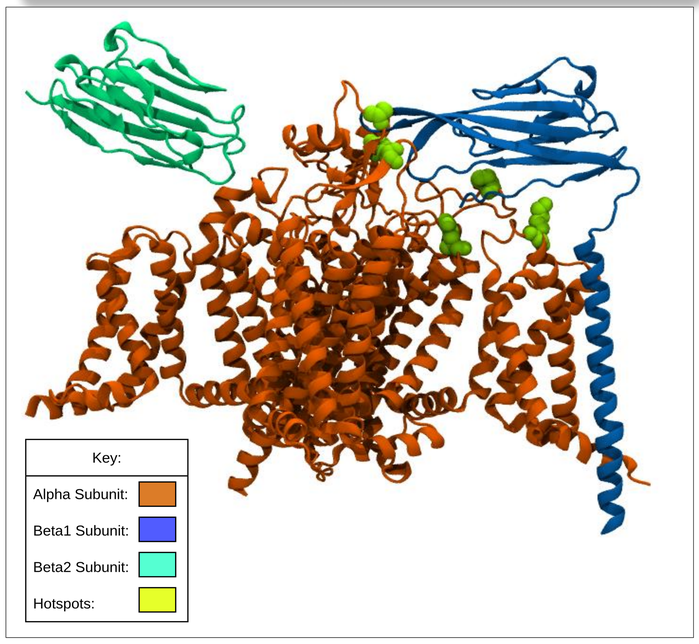


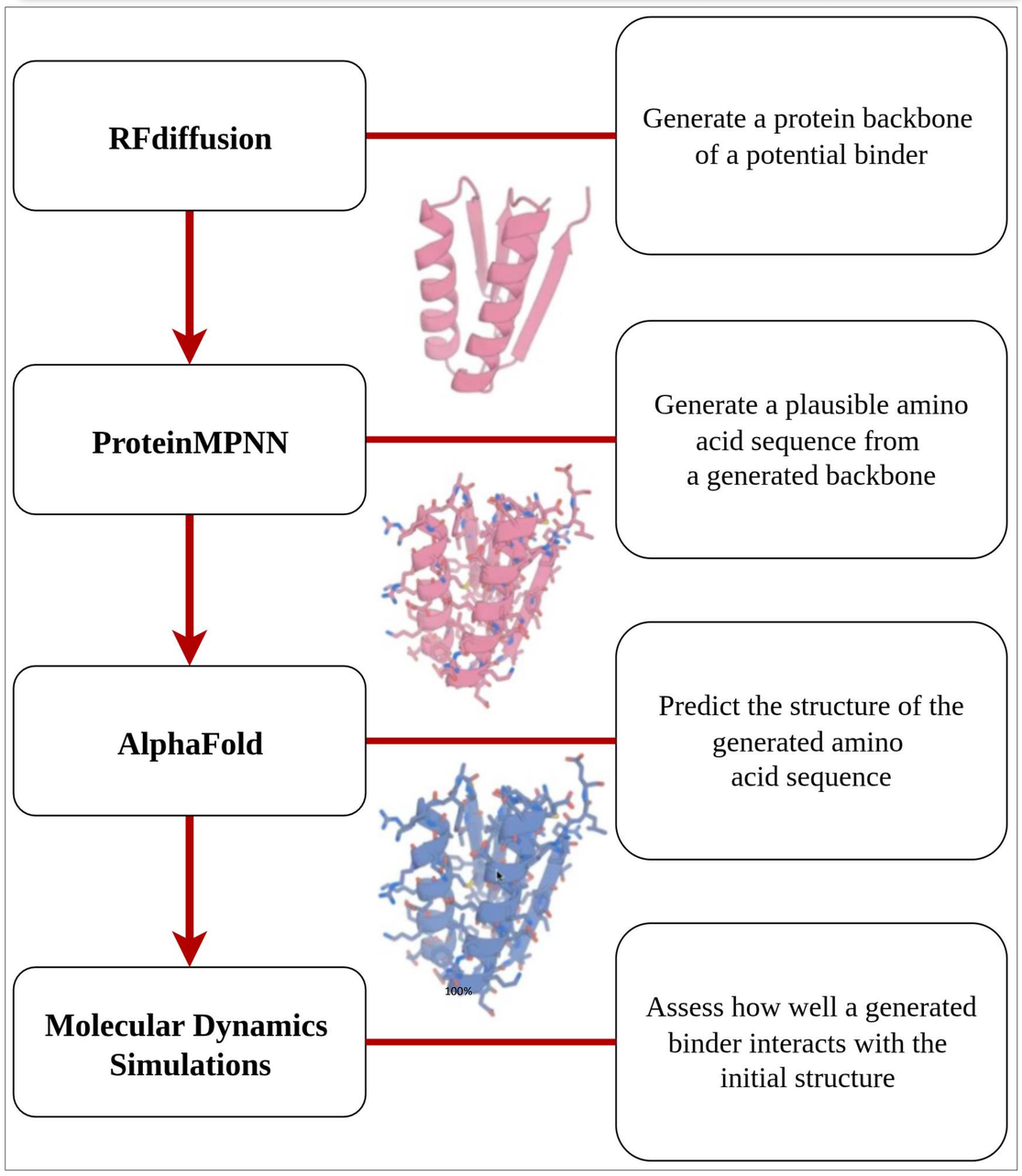
Figure 1: Render of Nav1.7 Complex

The Nav1.7 channel is a voltage-gated sodium channel (VGSC) expressed in motor and sensory neurons in the peripheral nervous system. VGSCs start and spread action potentials in excitable cells. The Nav1.7 complex is made up of a single polypeptide channel (the alpha subunit), and is modulated by 1-2 Beta subunits. The beta subunits essentially control how much the channel opens and closes, and how much of the channel is expressed and visible. Beta subunits of VGSCs have a big impact on the regulation of neurons firing. Mutations in genes encoding VGSCs beta subunits are linked to pathophysiology and channelopathies that manifest as certain diseases. Such diseases impact the body's nervous system, like epilepsy, cardiac arrhythmia, Parkinson's, and much more. Along with creating miniprotein binders for surgical dye purposes, there is a therapeutic motivation for developing miniprotein binders as well. The diseases caused by mutations provide a therapeutic motivation for creating de novo miniprotein binders that prevent mutated beta subunit from binding with the alpha subunit, essentially neutralizing the interaction between the alpha and beta subunit without changing the overall function of the complex. In the above figure, we have identified "hotspots" where the Beta1 subunit interacts the most with the alpha subunit. These hotspots are present at residues 279, 328, 1220, 1682, and 1722 on the alpha subunit.

Methods and Approach

To design and validate novel protein structures, we implemented a multi-step computational workflow combining generative AI with molecular modeling and simulation tools. Backbone generation was performed using RFdiffusion, a diffusion-based model capable of producing diverse, physically plausible protein topologies conditioned on a 6J8G protein structural motif. We used ProteinMPNN, a deep learning model, to generate amino acid sequences optimized to fold into the designed backbones with high biochemical plausibility and stability. AlphaFold predicted the resulting 3D structures, which were compared to the original backbones using RMSD and confidence metrics to assess structural fidelity. Structures were visualized and analyzed in VMD to evaluate folding motifs, key interactions, and overall consistency. To assess dynamic behavior, we ran molecular dynamics (MD) simulations using GROMACS. Soluble proteins were solvated in cubic water boxes (22.5 Å buffer), neutralized, and ionized (0.15 M K^+/Cl^-). Membrane proteins were embedded in POPC bilayers and equilibrated using CHARMM-GUI's default protocol. Simulations used the CHARMM force field with PME for long-range electrostatics and a 10–12 Å force-switch for van der Waals interactions. Each system underwent 5,000 steps of steepest descent energy minimization, followed by 125 ps of NVT equilibration. An extended NPT simulation (~150 ns) was conducted on the 6J8G alpha subunit with its AlphaFold beta subunit complex to examine binding poses and conformational adaptability in a solvated, pressure-regulated environment. These simulations provided insight into the structural robustness and thermodynamic behavior of the designed proteins, complementing static predictions.

Machine Learning Pipeline



Binder 3D Structures

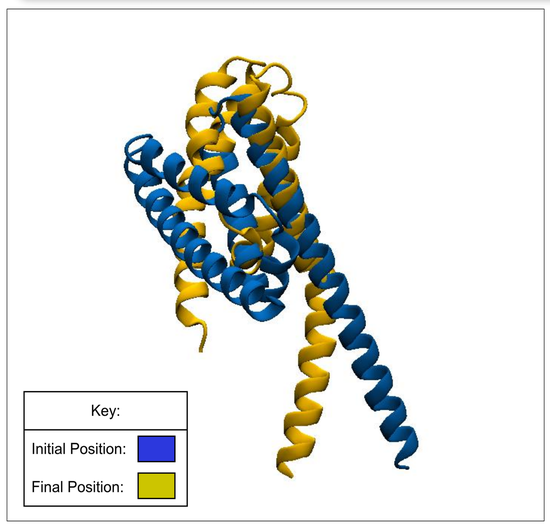


Figure 2: Generated Binder 1 Initial and Final Positions

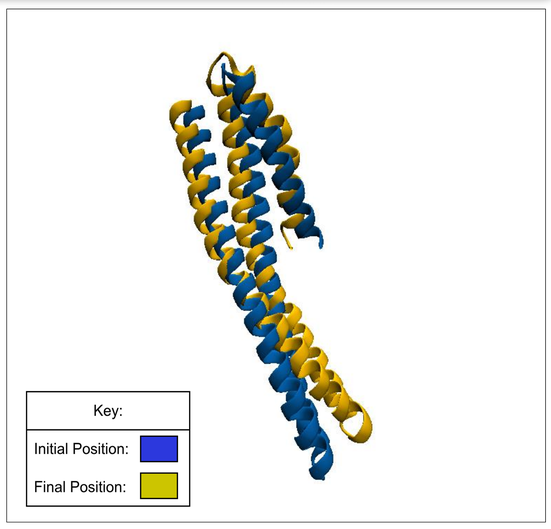


Figure 3: Generated Binder 2 Initial and Final Positions

Upon initial viewing, we can observe that these generated binders' initial and final positions from running molecular dynamics simulations are very different.

Plots of RMSD vs. Time

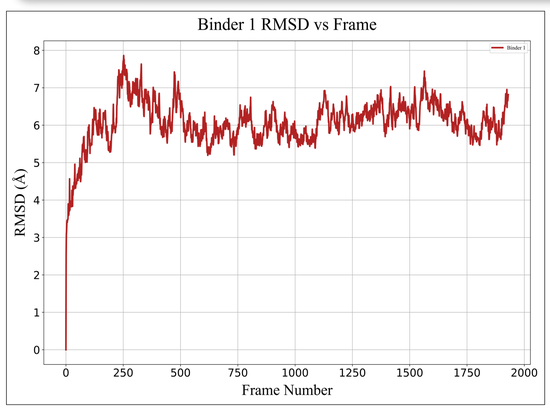


Figure 4: Plot of Binder 1 RMSD per frame position

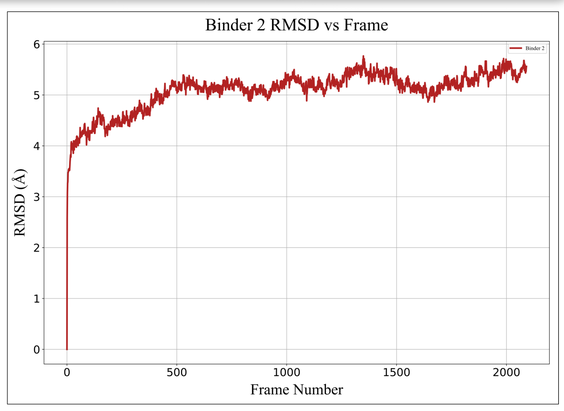


Figure 5: Plot of Binder 2 RMSD per frame position

Results/Discussion

To evaluate the conformational stability of our designed Nav1.7-targeting miniprotein binders, we conducted molecular dynamics (MD) simulations and analyzed structural changes over time. As shown in Figure 2,3, each image compares the initial (blue) and final (yellow) conformations of the same protein after simulation. The minimal displacement between the two chains indicates that the overall protein fold is preserved throughout the trajectory, suggesting good structural integrity under solvated, physiological conditions. To quantify these structural fluctuations, we plotted root mean square deviation (RMSD) over time (Figure 4,5). Both trajectories exhibit a rapid increase in RMSD during the early equilibration phase (first ~200 frames), followed by stabilization between approximately 4–6 Å for the remainder of the simulation. This range is consistent with moderate structural flexibility expected in small, solvated proteins and does not indicate denaturation or significant unfolding. Taken together, the close alignment of initial and final structures (Figure 2,3) and the plateauing RMSD values (Figure 4,5) confirm that the binders maintain their core structural features over time. These results demonstrate the dynamic stability of the designs and support their suitability for downstream development as fluorophore-conjugated nerve-targeting tools or therapeutic candidates.

Conclusions and Future Work

Our study lays the groundwork in designing a robust pipeline combining generative deep learning models, structure prediction, and molecular dynamics to design and validate novel protein binders for the Nav1.7 sodium channel. The designed miniproteins exhibit relatively decent structural fidelity and stability over extended MD simulations, supporting their potential as viable candidates for fluorophore conjugation or therapeutic development. However, much can be improved, as the generated miniproteins experienced a lot of structural displacement throughout MD simulations. Continuations of this work should focus on:

- ❑ **Experimental validation** of binding affinity using biochemical assays
- ❑ **Model Optimization:** tuning of each machine learning model in the pipeline (i.e. varying parameters for RFdiffusion and ProteinMPNN)
- ❑ **Fluorophore conjugation** to test nerve-labeling efficiency in vitro and in vivo.
- ❑ **Binder optimization** through iterative rounds of sequence redesign guided by binding energy calculations and enhanced sampling MD techniques.

The integration of AI and simulation-based design offers vast possibilities for scalable and efficient framework for developing novel targeting molecules for neurological and clinical applications.

References

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Acknowledgments

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