



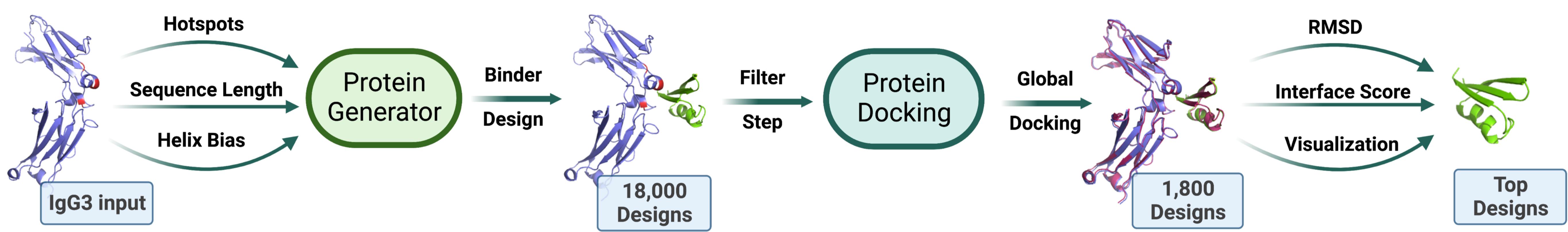
# De novo Immunoglobulin G3 binder design with ProteinGenerator

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## Abstract

Staphylococcal Protein A (Protein A) is a virulence factor that does not bind to the constant domain (Fc) of Immunoglobulin G3 (IgG3), yet tightly binds to the Fc region of IgG1, IgG2, and IgG4.<sup>1,2</sup> The lack of binding to IgG3 leads to limitations regarding the use of Protein A in antibody purification for therapeutic developments, since no Protein A scaffold can currently be used for IgG3 purification.<sup>2</sup> The aim of this project is to design a novel protein with high binding affinity to the IgG3 Fc domain using ProteinGenerator. Rosetta calculations, including RMSD, total score, and interface score, as well as PyMOL visualization were used to determine the most successful models. The selected top designs will be tested for expression and binding affinity to IgG3, which will further inform the functionality of ProteinGenerator for protein binder design.



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Fig 1. Computational workflow to generate de novo protein designs, filter through results, and validate top designs.

## Protein Design

Directory	Hotspots (residue #)	Sequence Length	Helix Bias	Generated Designs
High Helix	15, 74, 75, 81	40 - 60	0.05	6043
Many Hotspots	15, 74, 75, 81, 197, 198	40 - 75	0.03	5945
Long Sequence	15, 74, 75, 81	40 - 75	0.01	5951

Fig 2: ProteinGenerator binder design with different combinations of parameters

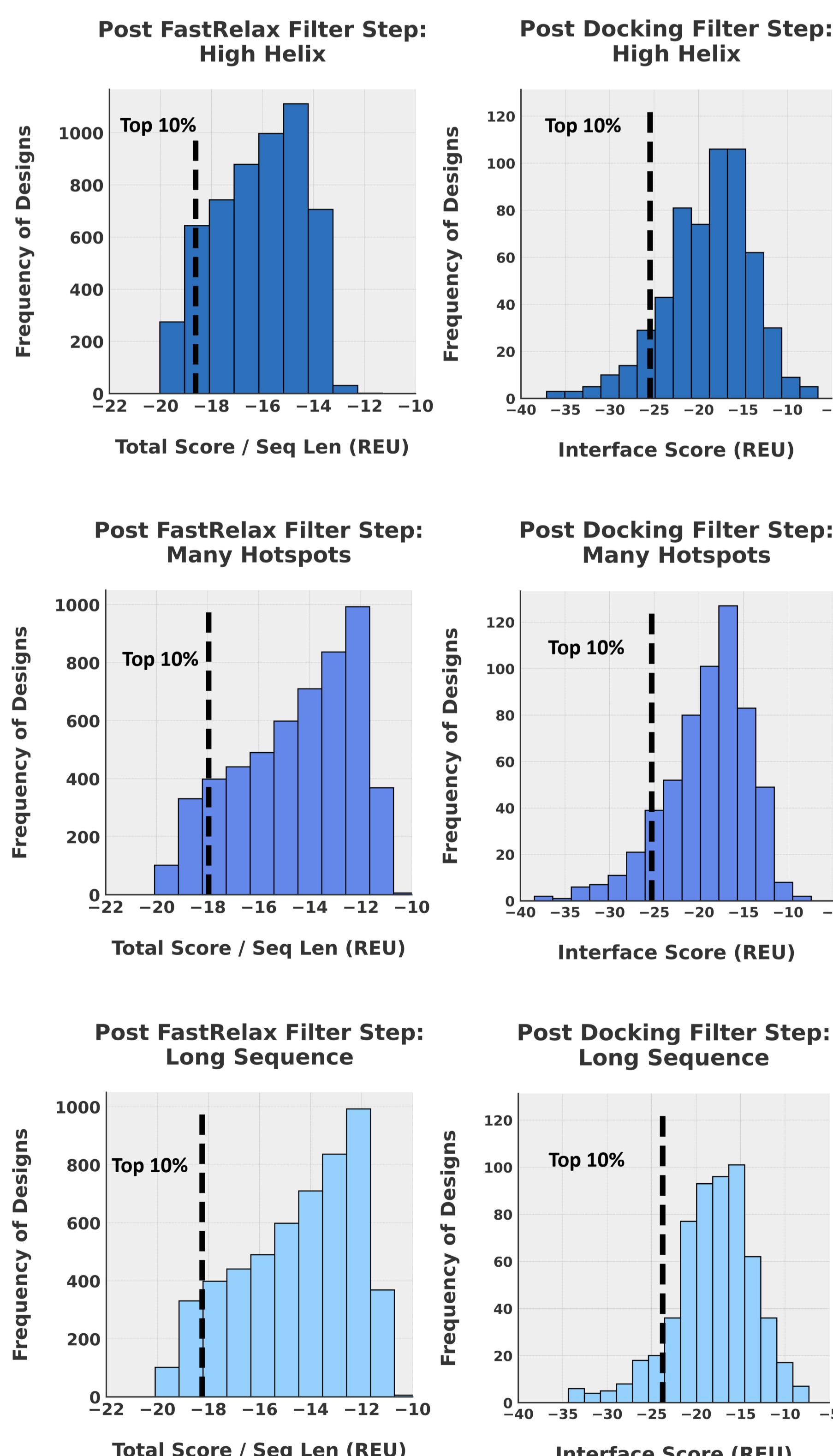


Fig 3: Major filter steps visualized. The top 10% best averaged scoring relaxed designs were taken for Protein Docking. Best designs were determined out of the top 10% Protein-Docking interface score models.

## Results

### Protein-Protein Docking

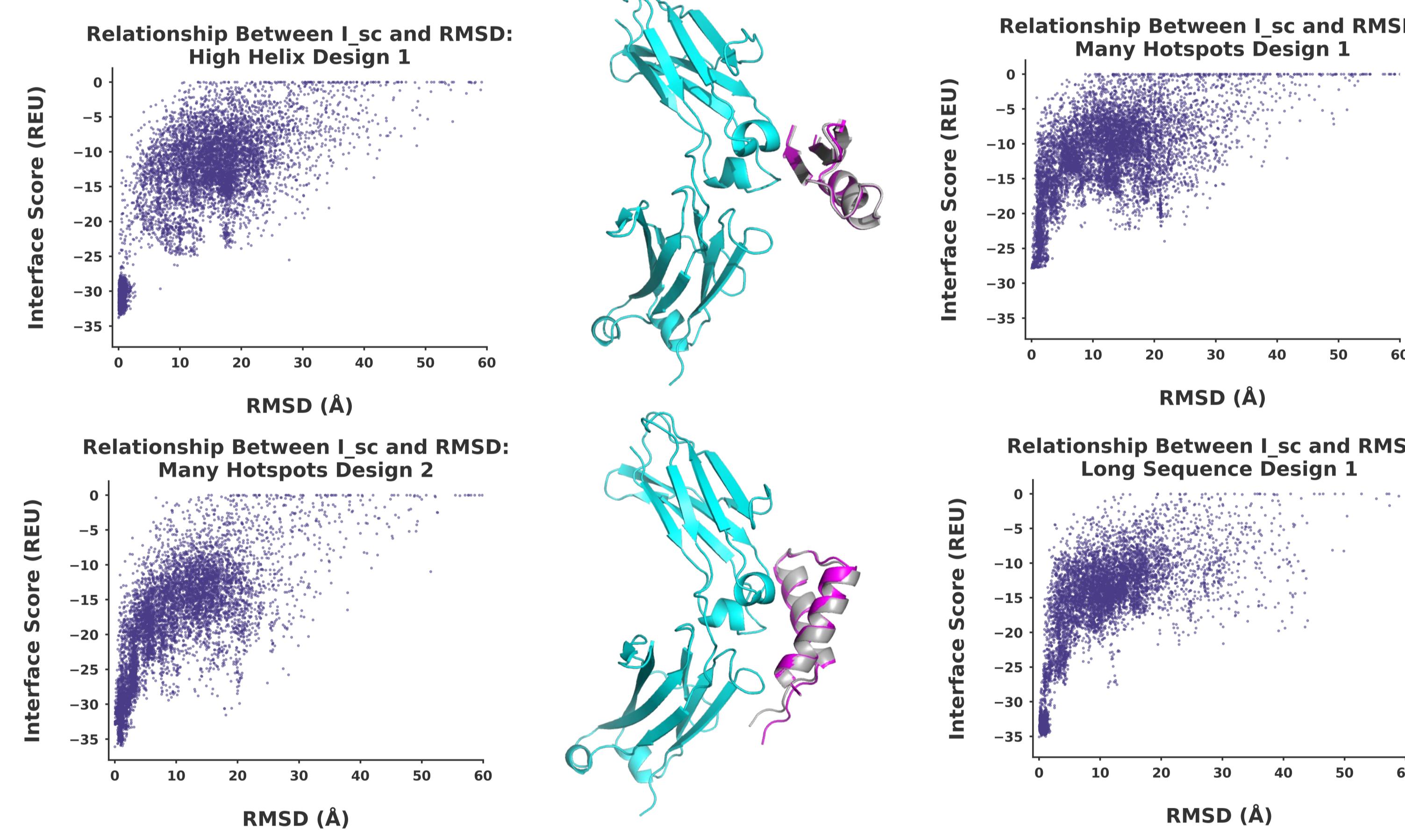


Fig 4. Funnel plots with corresponding design and top scoring structures indicate binding of design with IgG3.



Fig 5. Well folded model with low indication of binding on left. High scoring design with poor topology on right.

## Summary

- ProteinGenerator output 18,000 IgG3 binder designs which were filtered by average score for Protein Docking
- The top 15 designs were then selected manually by PyMOL visualization after interface score and RMSD filtering
- In our number of designs generated, many had to be rejected due to poor folding despite scoring highly.
- Combining ProteinGenerator with Rosetta Docking creates a promising workflow for de novo binder design

## Outlook

- Testing protein expression and IgG3 binding of top 15 selected designs
- Using experimental data to benchmark and validate our workflow

## Acknowledgements

I would like to thank Dr. Clara T. Schoeder and Johannes A. Klier for their mentorship during this project and the NSF and RosettaCommons for funding this research.

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## References

- <sup>1</sup> Deis, et al. (2015). Proceedings of the National Academy of Sciences of the United States of America, 112(29), 9028-9033.  
<sup>2</sup> Shah, et al. (2017). Molecular immunology, 92, 28-37.