



UNIVERSITÄT
LEIPZIG
Medizinische Fakultät

De novo Immunoglobulin G3 binder design with ProteinGenerator

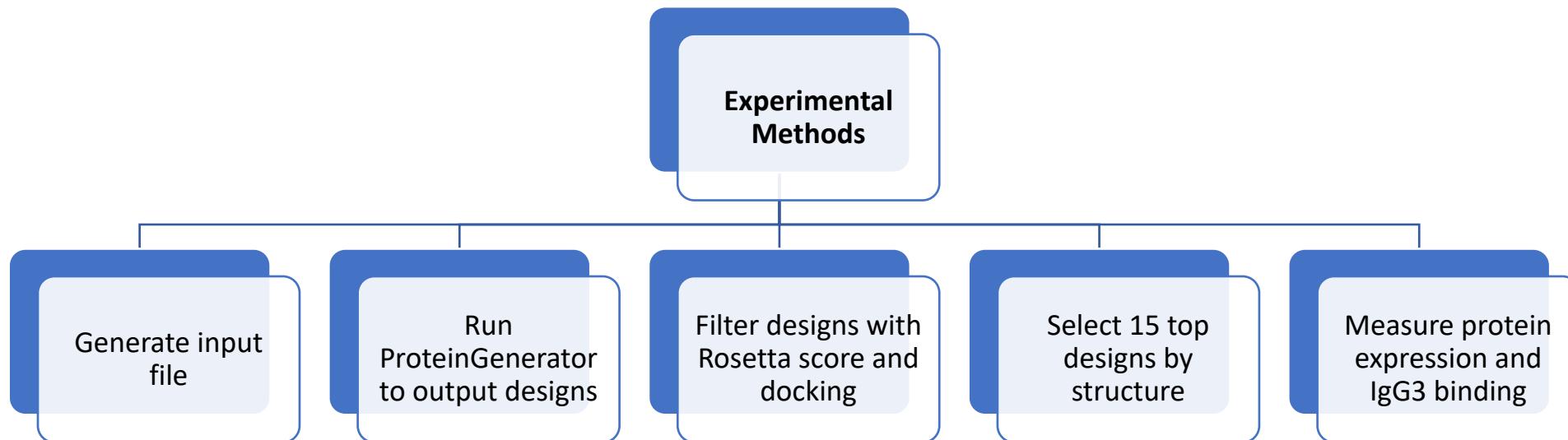
Erin Ahern

Advisor: Dr. Clara T. Schoeder

Mentor: Johannes Klier

Overview

Aim: Design a novel protein with binding affinity to Immunoglobulin G3 (IgG3) using ProteinGenerator



Background

Staphylococcal Protein A (Protein A):

- virulence factor from the bacterium *Staphylococcus aureus*
- binds with high affinity to the Fc region of host antibody¹

Immunoglobulin G3 (IgG3):

- third most abundant IgG subclass
- contains structural characteristics that differ from the other subclasses²

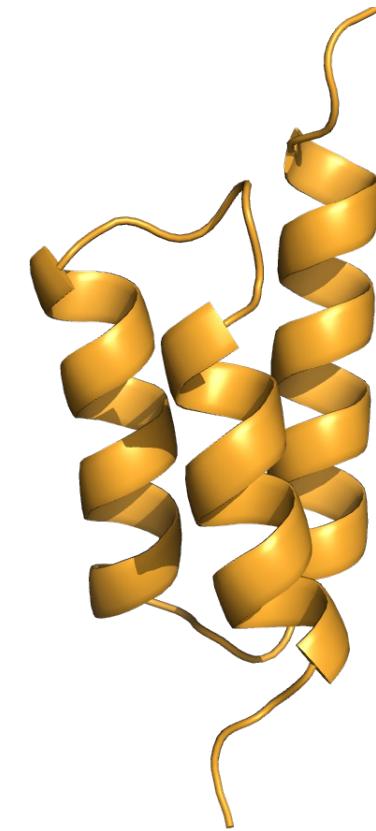


Fig 1. PyMol figure of 4WWI
Protein A domain that binds to IgG1

Background

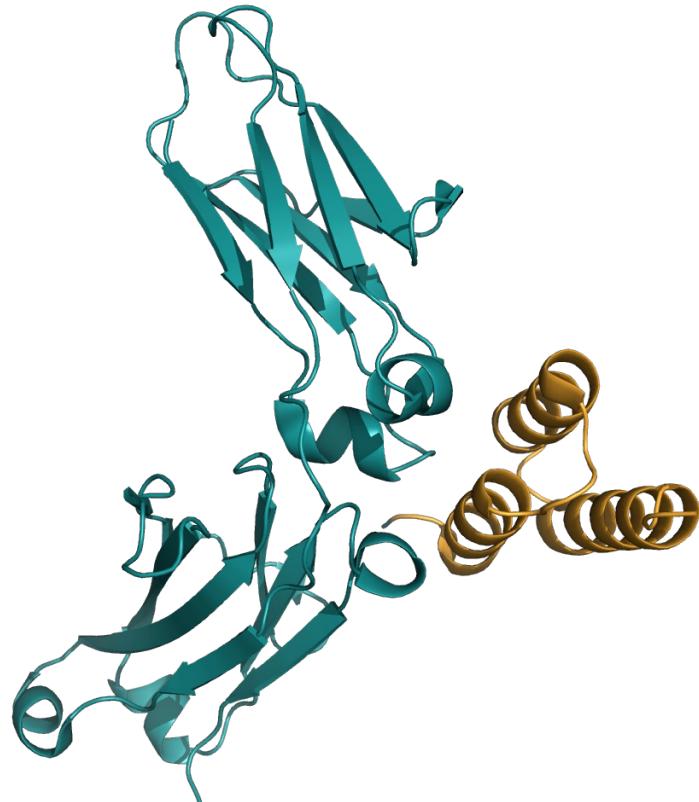
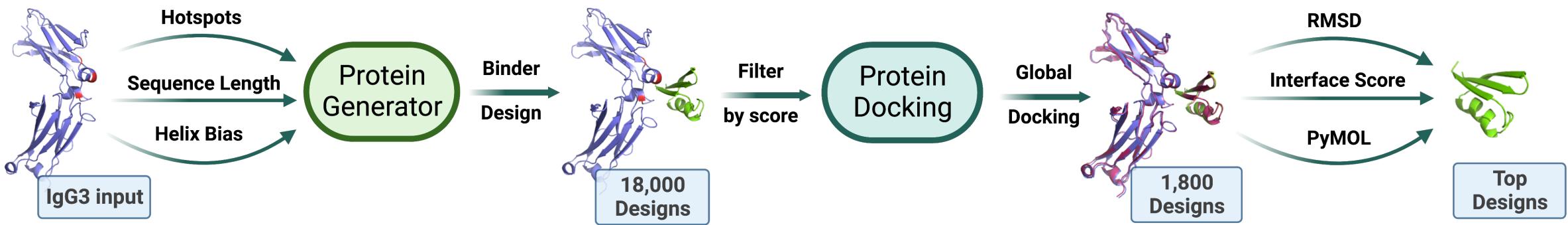


Fig 2. PyMol figure of 4WWI
Staphylococcal Protein A in complex with
IgG1 (domains C and D)

- Protein A tightly binds to the Fc region of IgG1, IgG2, and IgG4¹
- Does *not* successfully bind to the Fc region of IgG3¹

→ Limitations of how Protein A can be utilized for antibody purification

Computational Workflow



Pre-Processing

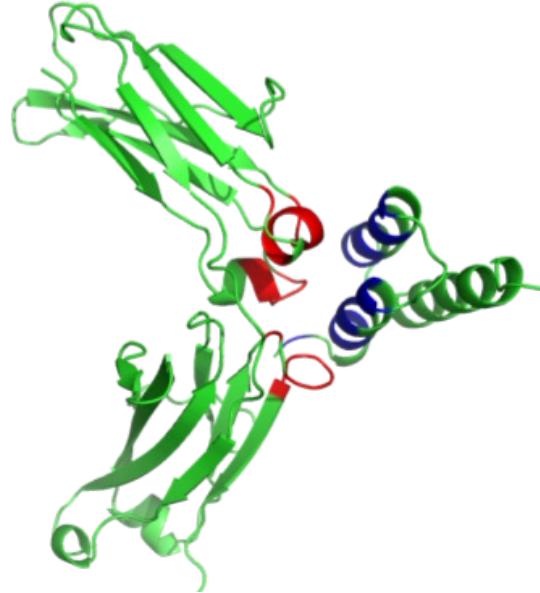
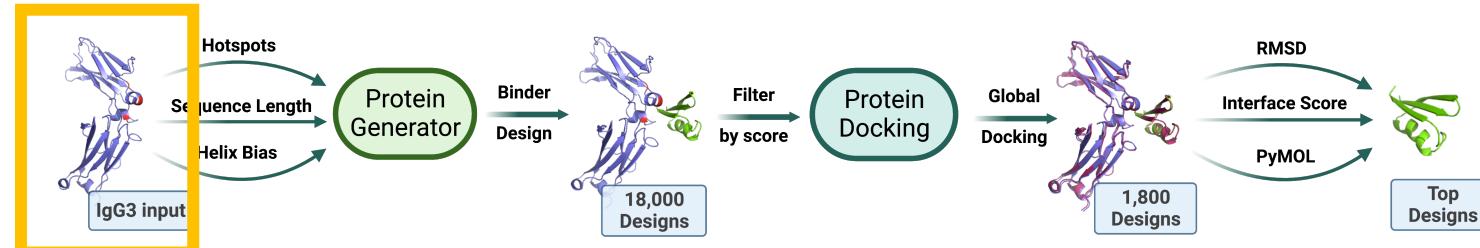


Fig 3. IgG3 Fc domain in complex with Protein A. IgG3 interacting residues in red.



Generate input file:

- Align IgG1 + Protein A PDB to IgG3 PDB
- Result: IgG3 + Protein A (then FastRelax)

Define binding mode:

- Run Rosetta *define_interface*
- Identifies interacting residues

ProteinGenerator Design

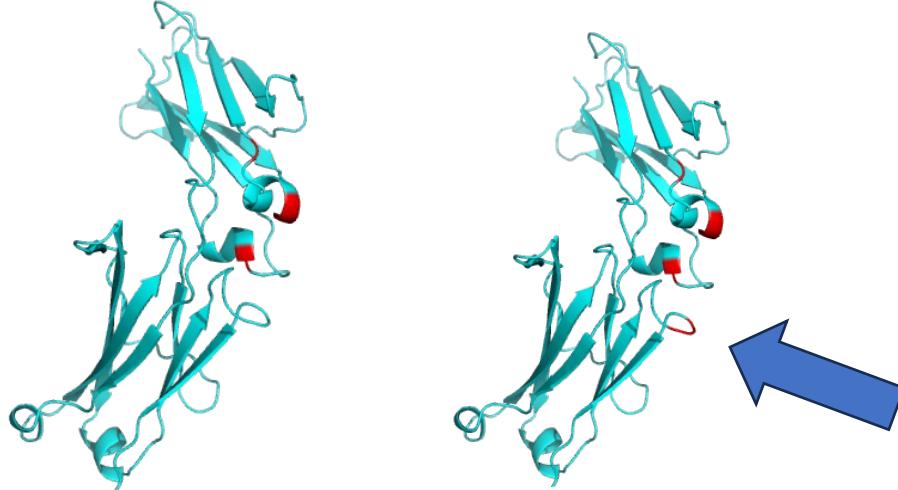
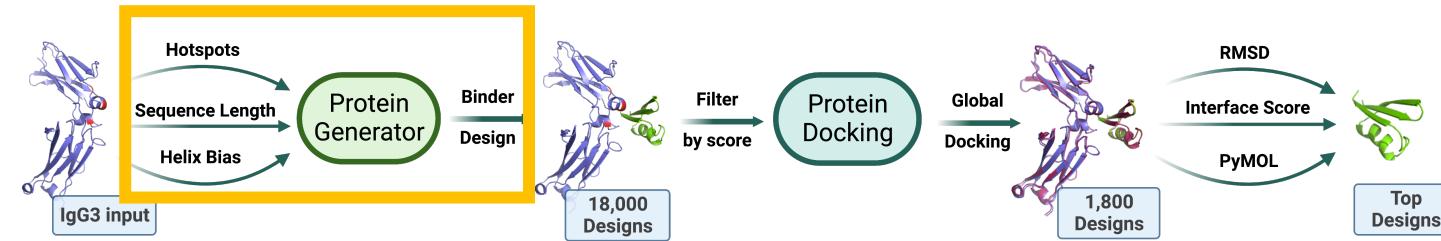
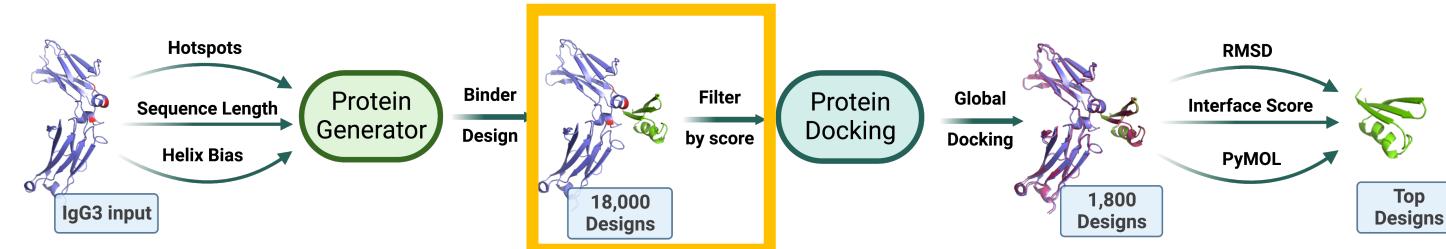


Fig 4. IgG3 Fc domain with hotspot residues in red. Additional residues in image on the right.

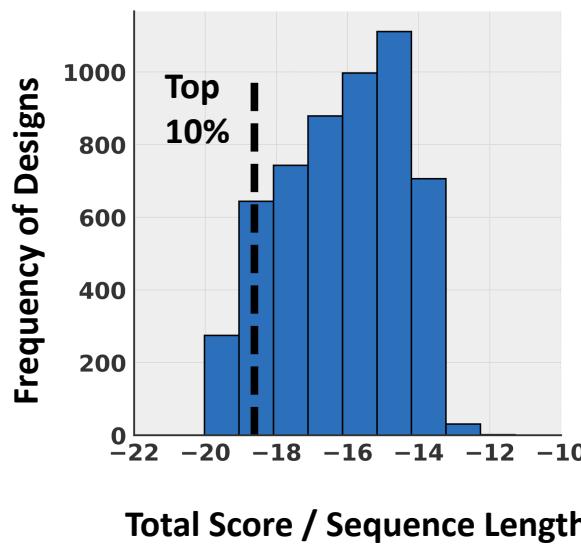
Output	Hotspots (residue #)	Sequence Length	Helix Bias
High Helix	15, 74, 75, 81	40 - 60	0.05
Many Hotspots	15, 74, 75, 81, 197, 198	40 - 75	0.03
Long Sequence	15, 74, 75, 81	40 - 75	0.01

In total: 17939 designs generated

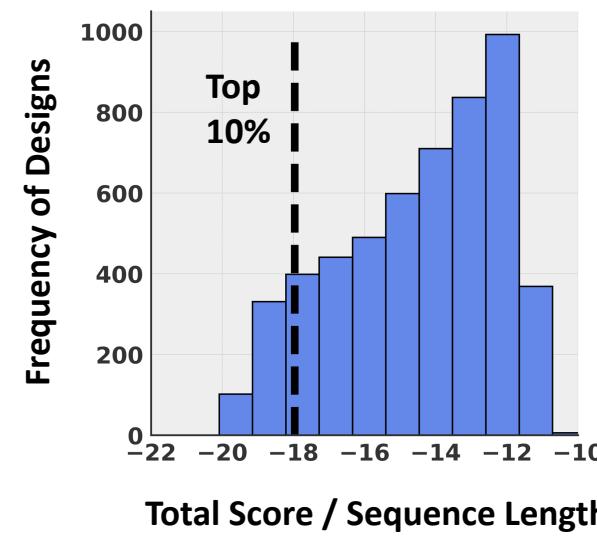
FastRelax Filter Step



Post Fast Relax Filter Step:
High Helix



Post Fast Relax Filter Step:
Many Hotspots



Post Fast Relax Filter Step:
Long Sequence

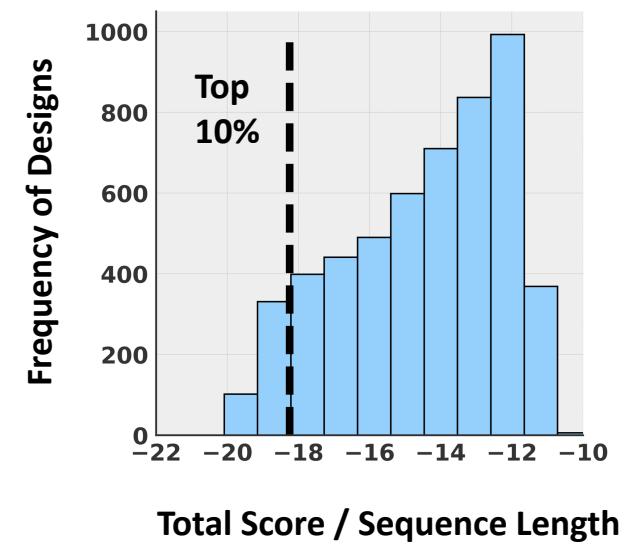
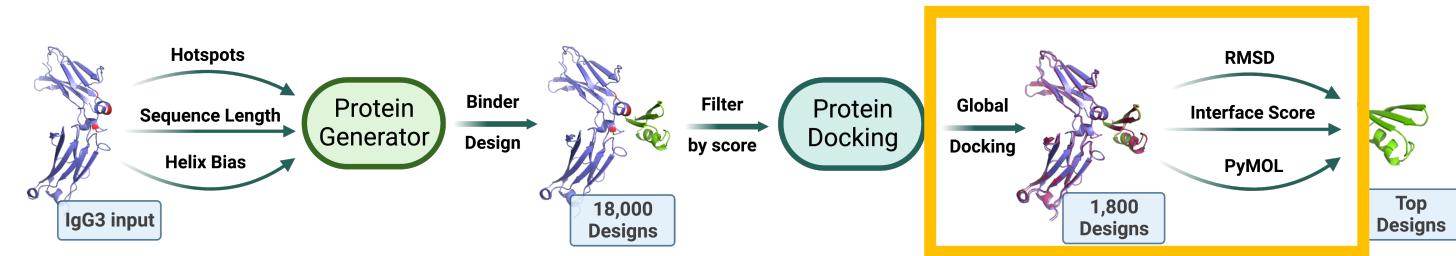
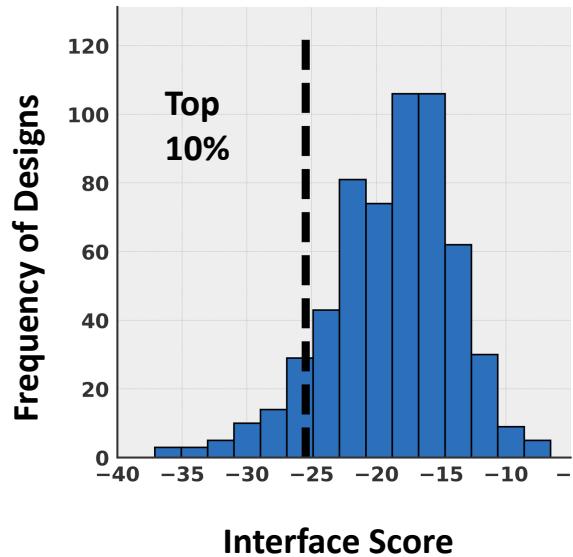


Fig 5. Visualization of top 10% scoring designs in first filter step after protein relaxation

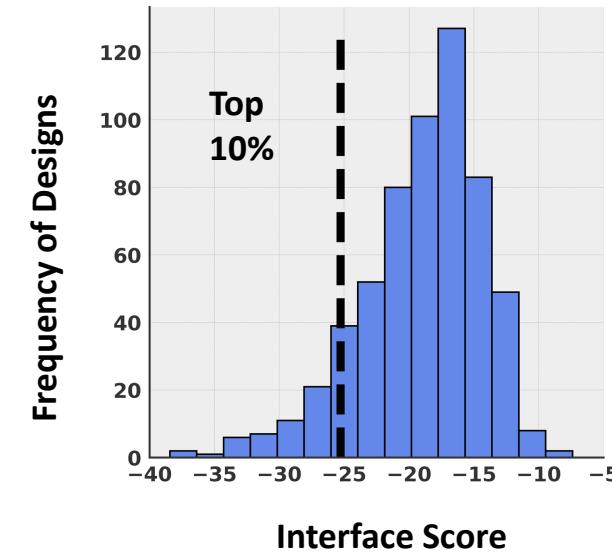
Protein Docking Filter Step



Post Docking Filter Step:
High Helix



Post Docking Filter Step:
Many Hotspots



Post Docking Filter Step:
Long Sequence

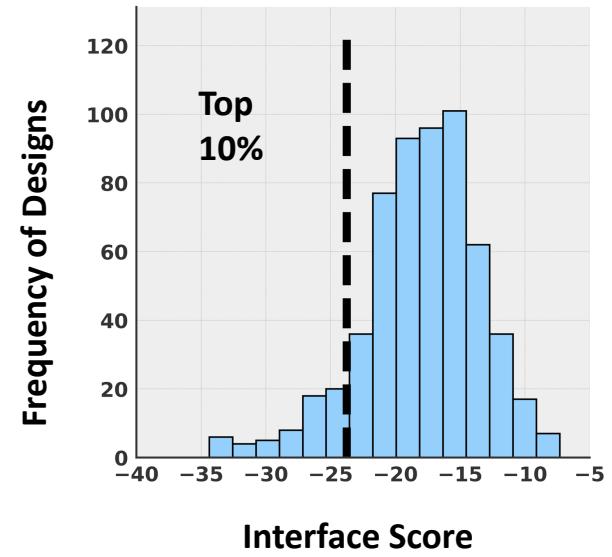
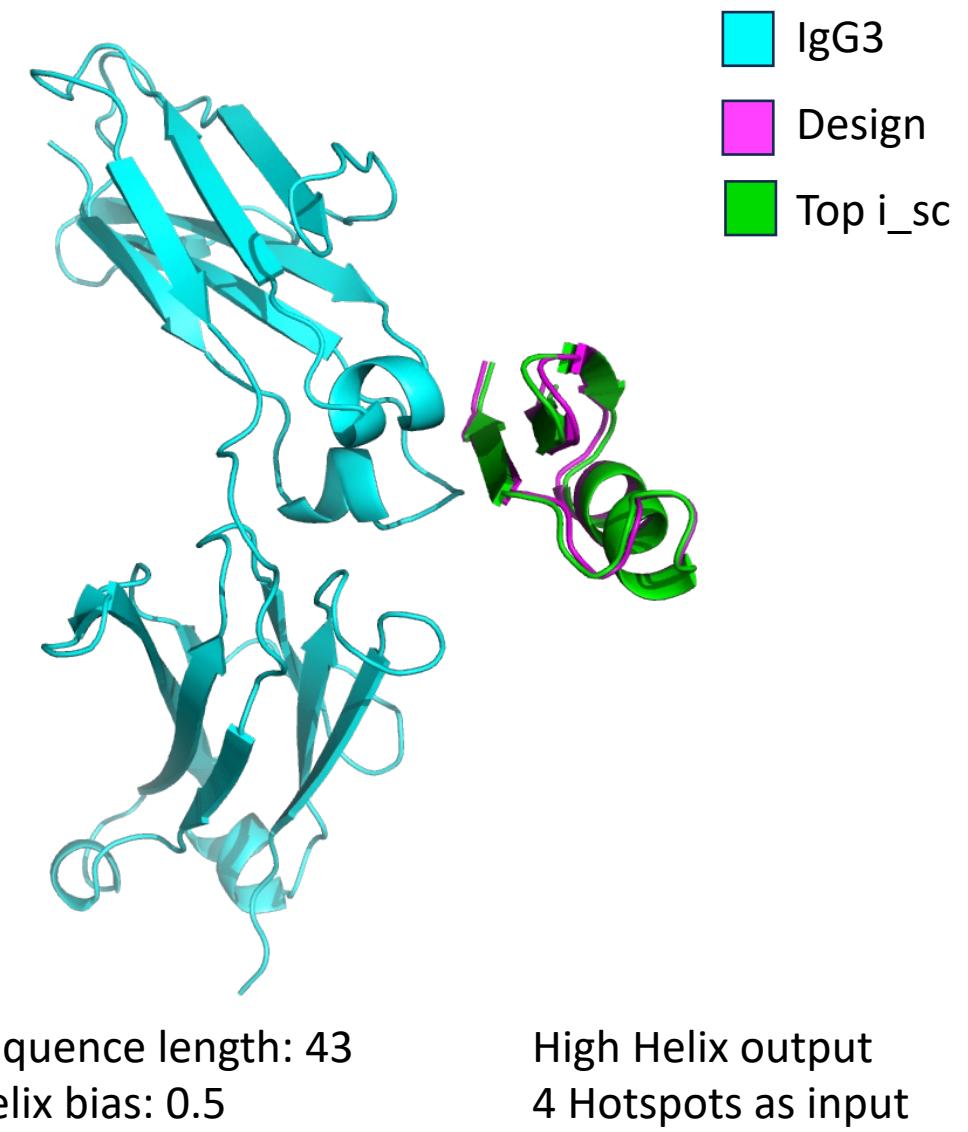
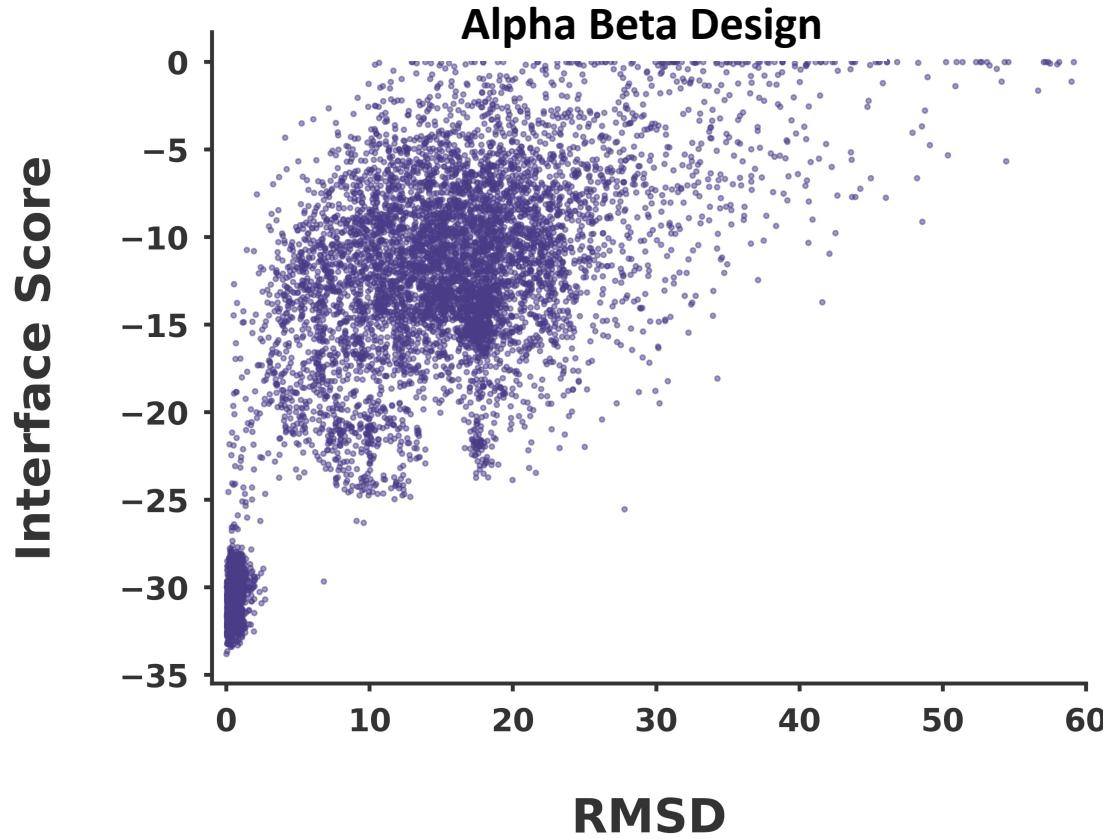


Fig 6. Visualization of top 10% scoring designs in last filter step post docking

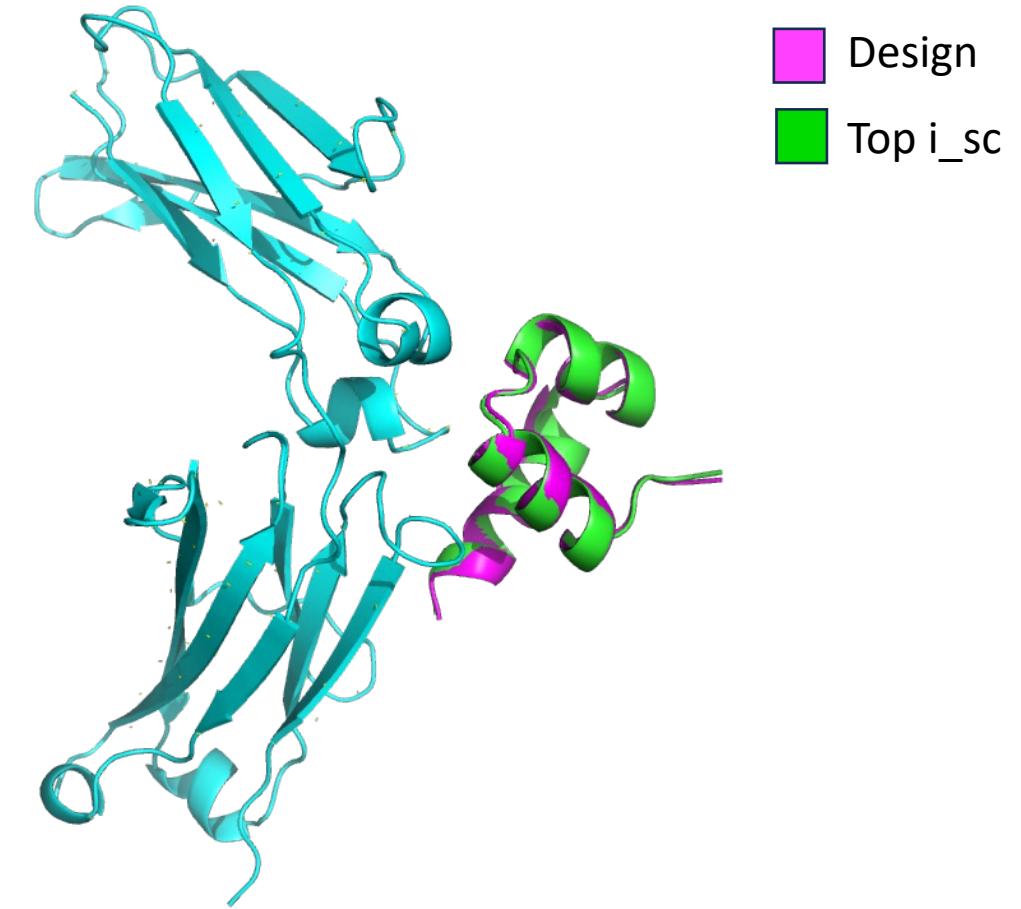
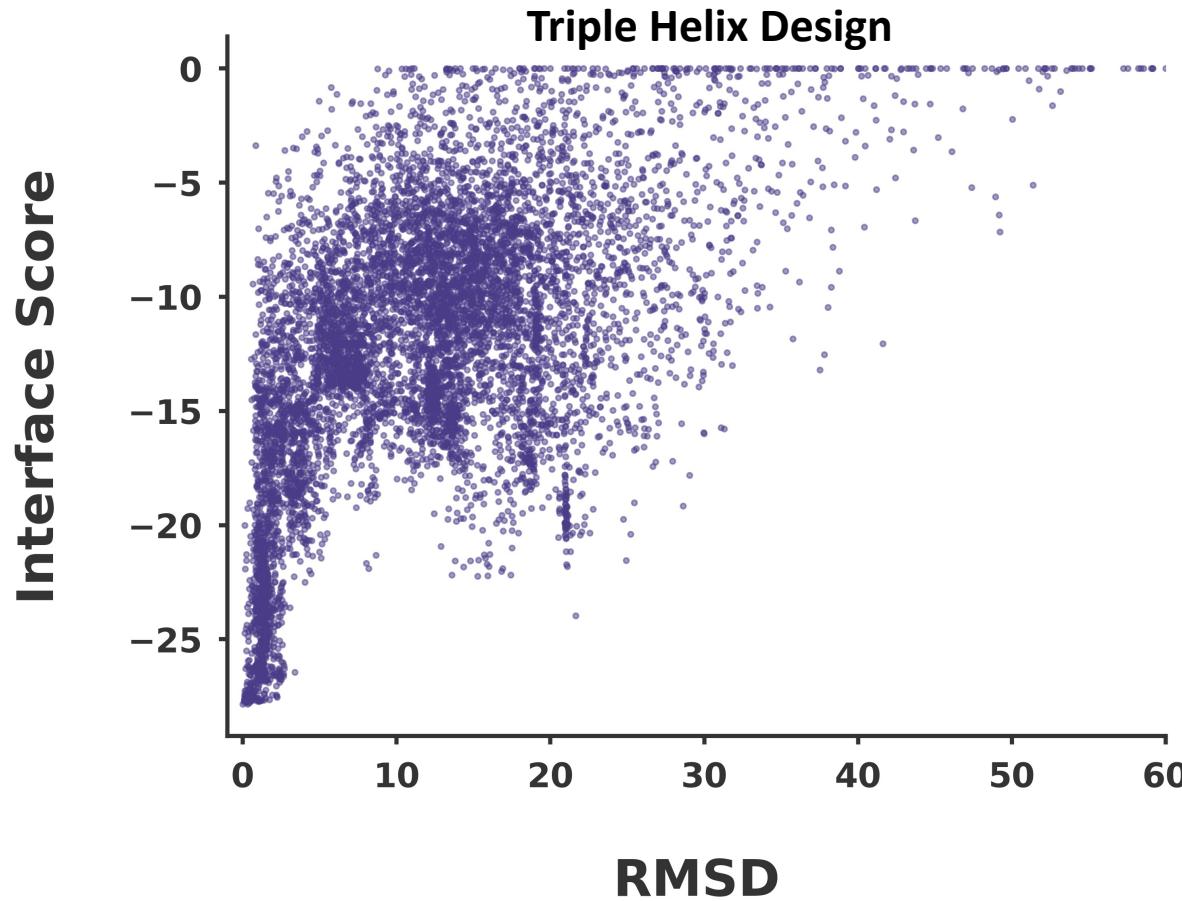
Results

Relationship Between I_sc and RMSD:



Results

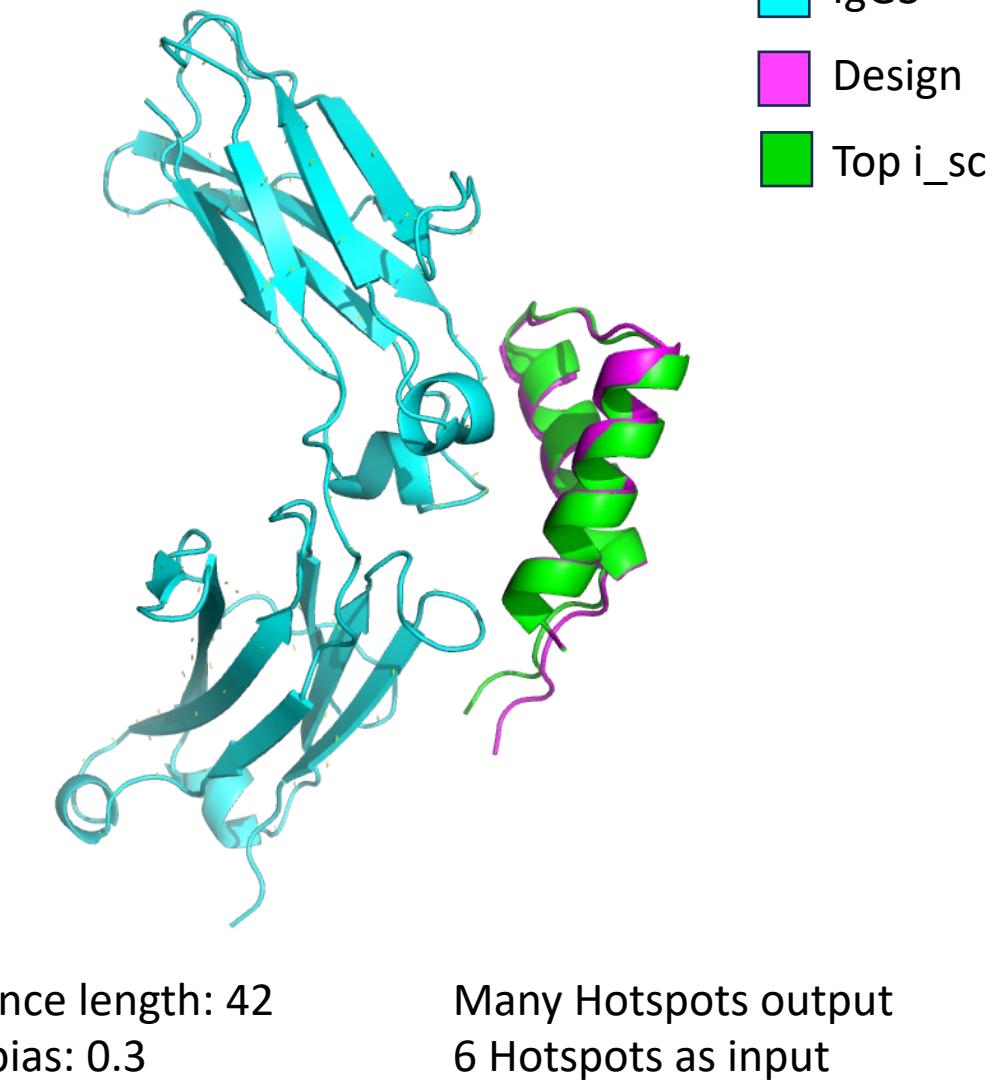
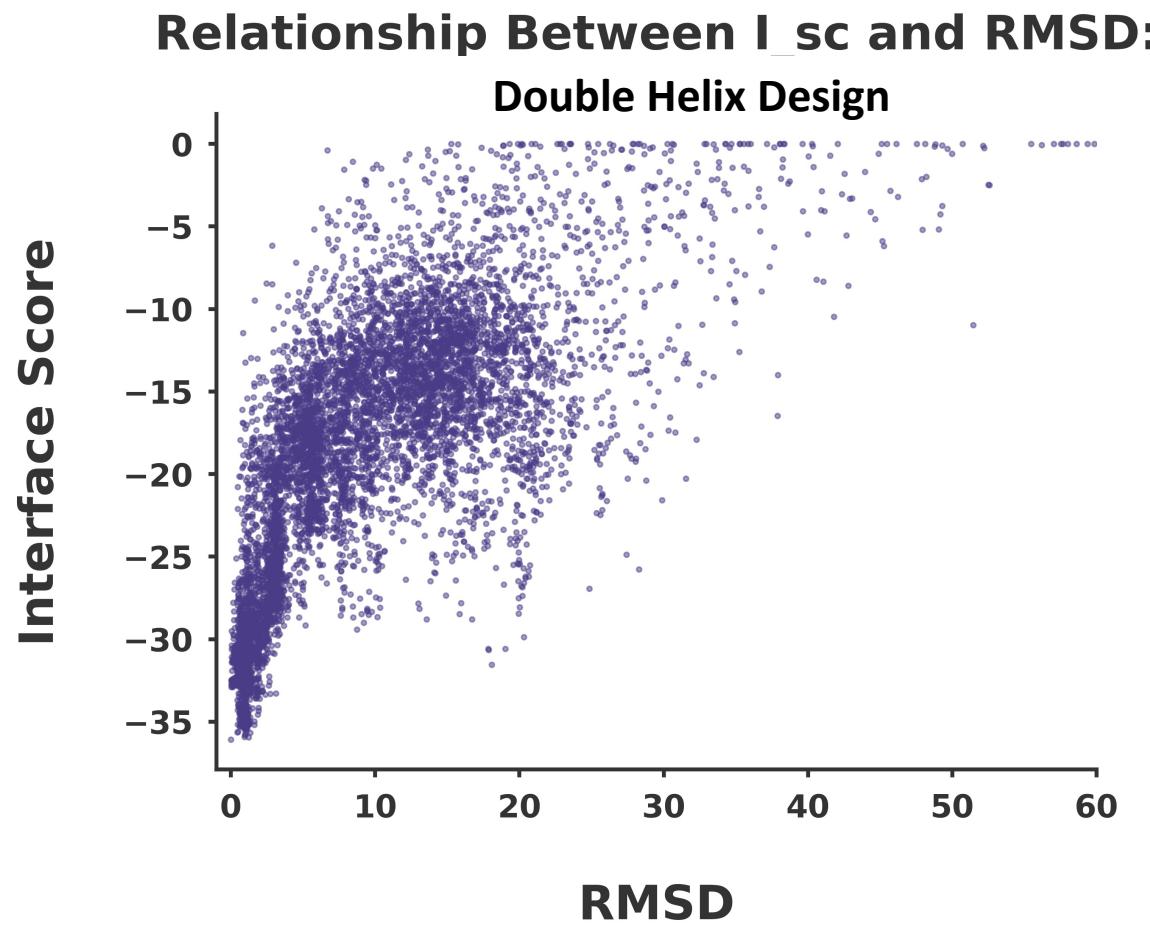
Relationship Between I_{sc} and RMSD:



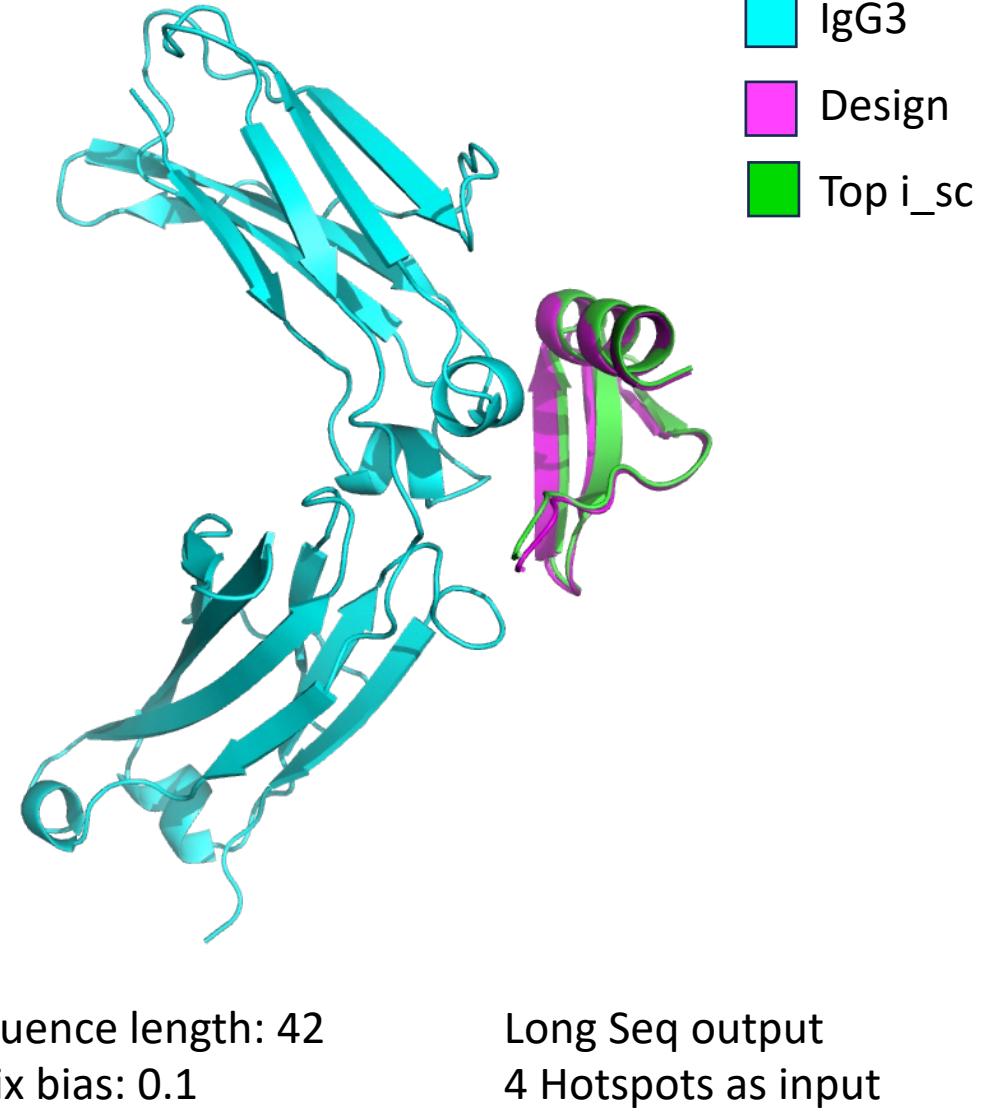
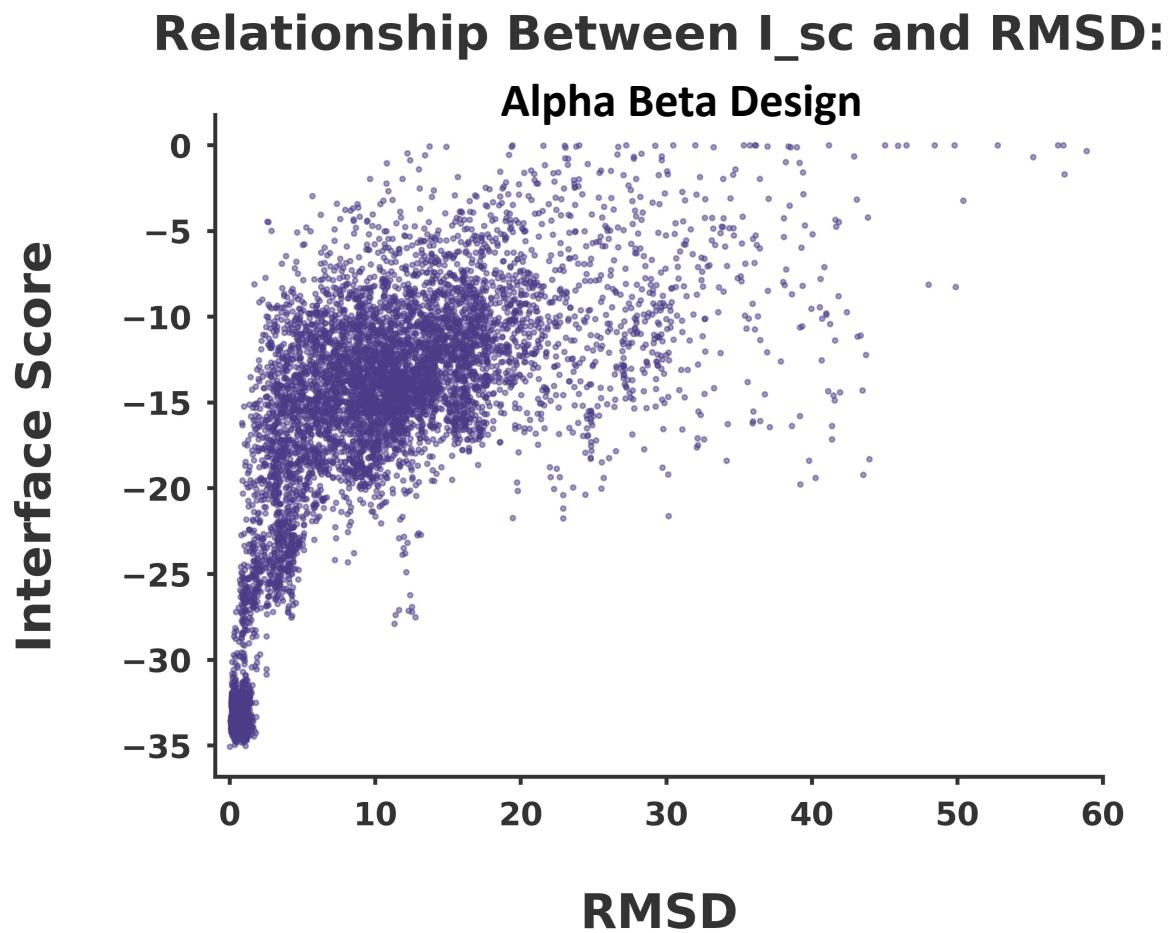
Sequence length: 40
Helix bias: 0.3

Many Hotspots output
6 Hotspots as input

Results

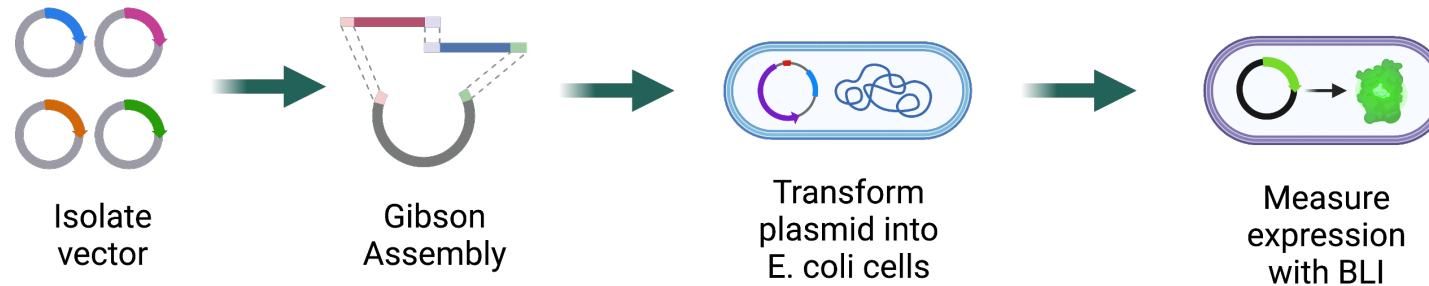


Results



This Week

Wet Lab Workflow



- The expression of 15 designs will be measured
- Conclusions will be drawn about ProteinGenerator's functionality after analysis of protein expression

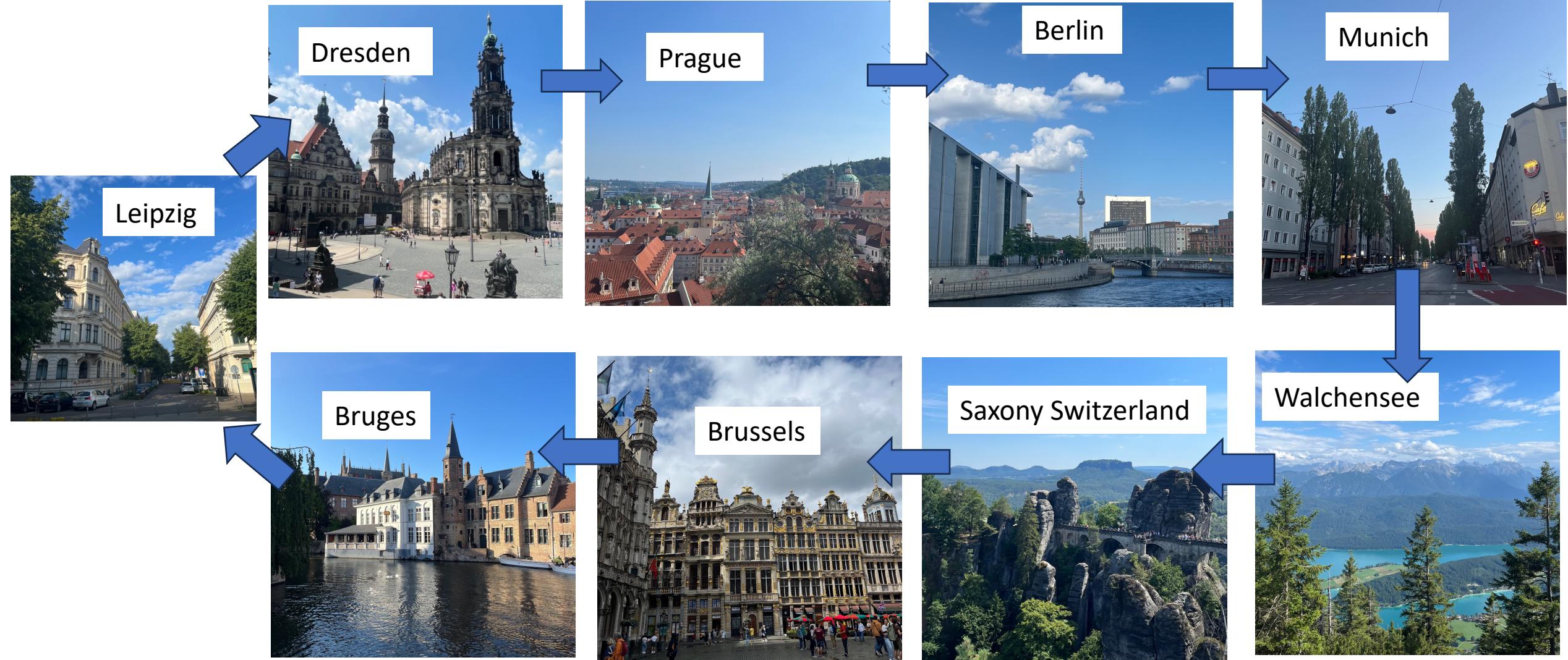
Summary

- ProteinGenerator was used to output 18000 IgG3 binder designs with different input parameters
- Designs were filtered by average score, interface score, and RMSD
- Protein expression will be measured for 15 top designs

Future Work

- The proteins that express will then be tested as IgG3 binders with Biolayer Interferometry

Travel Workflow



Thank you!

Erin Ahern

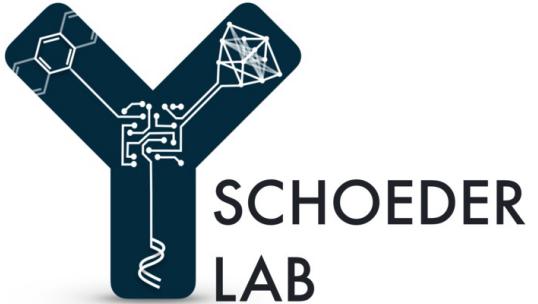
Rosetta Commons Intern

Special thanks to ...

Dr. Clara T. Schoeder

Johannes Klier

and everyone in the lab!



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