



TUTORIAL 6: SCAFFOLDING AND MOTIF GRAFTING

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INTRODUCTION

WHY MOTIF GRAFTING AND SCAFFOLD DESIGN?

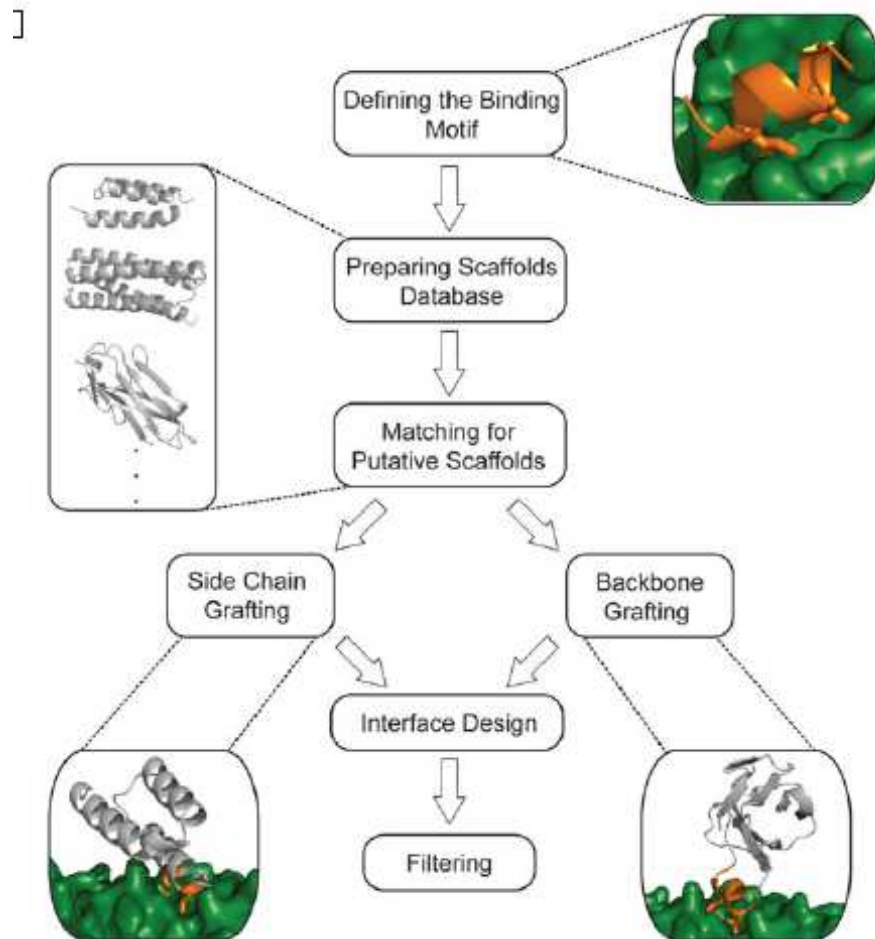
- Transplantation of the functional sites onto another protein
 - Immunological applications (e.g. immunogen and vaccine design)
 - Useful when targeting a secondary epitope
- Flexible peptide can be embedded into a stable protein that will reduce the entropic penalty of binding
- Can create new favorable interactions with the target

INTRODUCTION

GRAFTING METHODS IN ROSETTA

Rosetta method	Name	Function
Rosetta_scripts	Side chain grafting	Scaffold design
Rosetta_scripts	Backbone grafting	Scaffold design
Rosetta_scripts	FunFoldDes (Fold from loops)	Loop/Scaffold design
Rosetta application	Rosetta remodel	Scaffold design / backbone design

SIDE CHAIN AND BACKBONE GRAFTING – AN OVERVIEW



Silva, D., Correia, B.E., and Procko, E.
(2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

REQUIREMENTS FOR PREPARING A SCAFFOLD DATABASE

- High-resolution diffraction data ($< 2.5 \text{ \AA}$ (better $< 2.0 \text{ \AA}$))
- Protein has been reported to be expressed in *E. coli*
- Single protein chain as an asymmetric unit
- No bound ligand or modified residues
- Scaffold proteins must be energy minimized using Rosetta

→ A motif-focused library may be more useful, e.g. only including helical scaffolds for a helical motif.

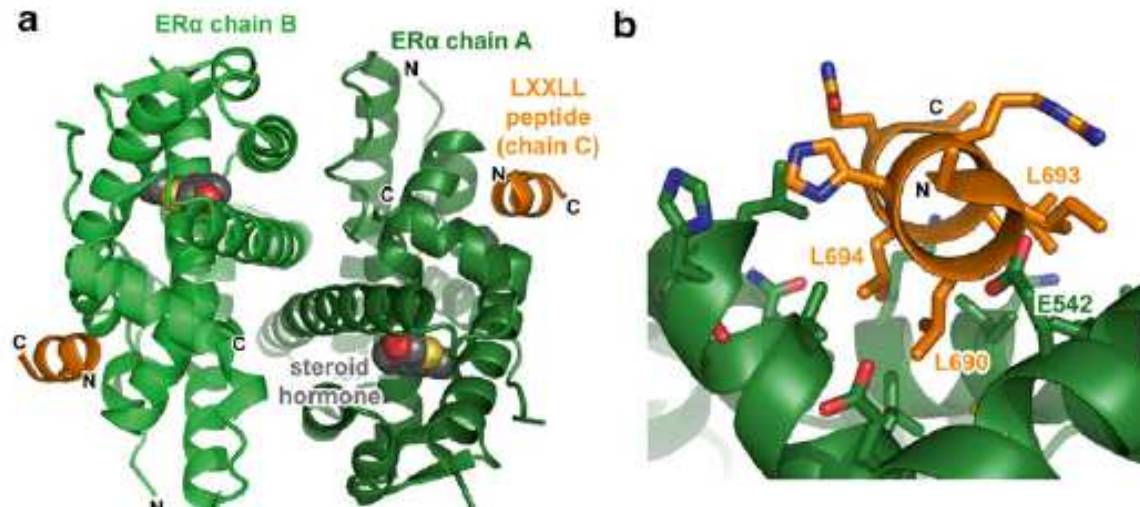
MATCHING PUTATIVE SCAFFOLDS WITH SIDE CHAIN GRAFTING

- Choose motif and scaffold backbones that superimpose with very low RMSD ($<0.5 \text{ \AA}$)
- Transplant side chains from functional motif onto scaffold
- Design surrounding residues on the scaffold surface

Pros	Cons
Minimal number of changes to the scaffold → Increase chances of correctly folded designs	Often motif and scaffold to the scaffold structures are too dissimilar, limiting availability of scaffolds

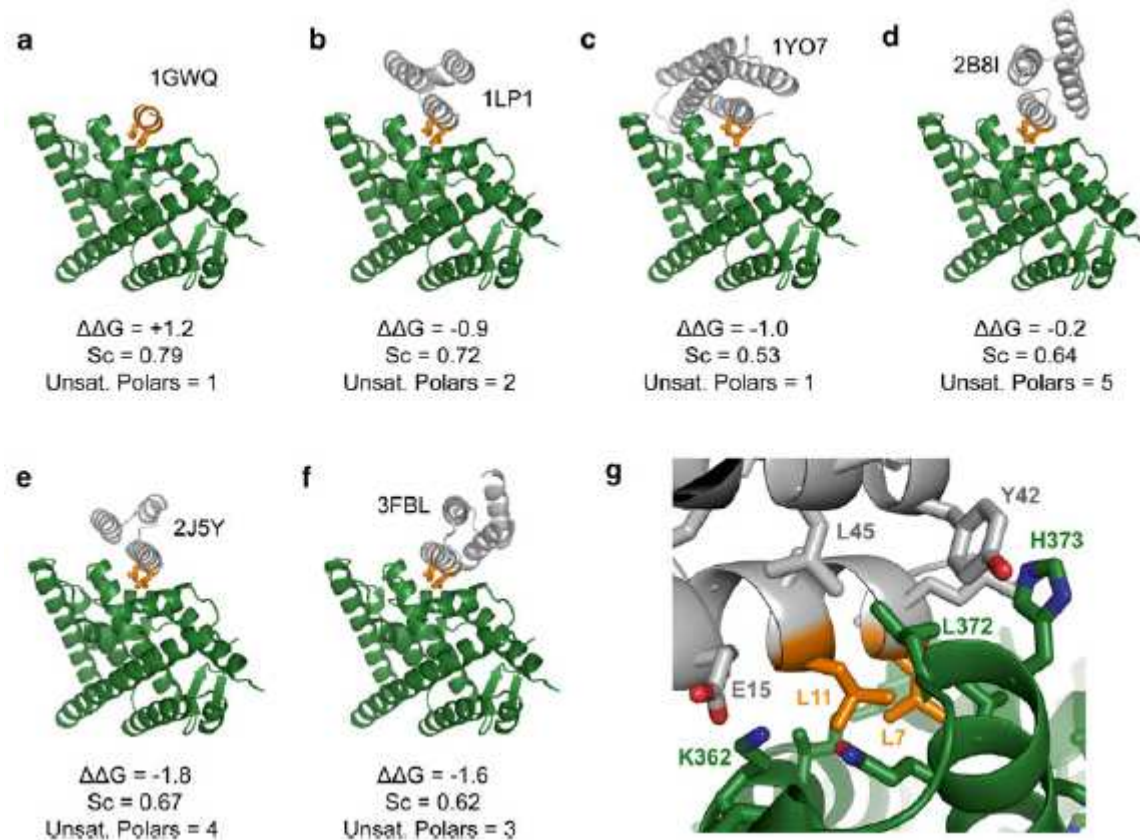
MATCHING PUTATIVE SCAFFOLDS WITH SIDE CHAIN GRAFTING

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combinatory_fragment_size_delta="2:2" full_motif_bb_alignment="1"  
graft_only_hotspots_by_replacement="1"  
revert_graft_to_native_sequence="1"/>
```



Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

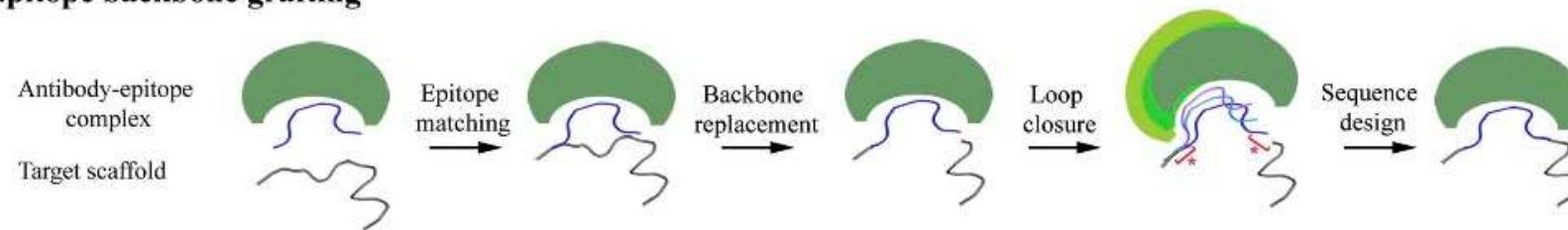
MATCHING PUTATIVE SCAFFOLDS WITH SIDE CHAIN GRAFTING



Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

BACKBONE GRAFTING - OVERVIEW

Epitope backbone grafting



1. Align to target scaffold
2. Remove native scaffold backbone
3. Model new epitope between termini
4. Rigid-body orientation of new epitope and antibody relative to scaffold

Azoitei, M.L., Ban, Y.A., Julien, J., Bryson, S., Schroeter, A., Kalyuzhniy, O., Porter, J.R., Adachi, Y., Baker, D., Pai, E.F., and Schief, W.R. (2012) Computational Design of High-Affinity Epitope Scaffolds by Backbone Grafting of a Linear Epitope. *J. Mol. Biol.* 415:175-192

BACKBONE GRAFTING

- Search for segments of scaffolds that align closely to the termini of the motif (both N- and C- terminal sides)
- The scaffold segment between these alignment points is replaced by the motif

Pros	Cons
Extremely versatile – a loop in the scaffold can be replaced by a different secondary structure or even with a different amino acid length	Can disrupt the overall fold in the scaffold
Can be used for discontinuous epitopes	Redesign of the hydrophobic core and interface introduces unfavorable mutations to the scaffold
	Careful filtering of designs

BACKBONE GRAFTING XML

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max_fragment_replacement_size_delta="-8:8" full_motif_bb_alignment="0"
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task_operations="hotspot_repack, pido_far, core"/>
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<PackRotamersMover name="design_boundary"
task_operations="hotspot_repack, pido_med, core_and_boundary"/>
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BACKBONE GRAFTING

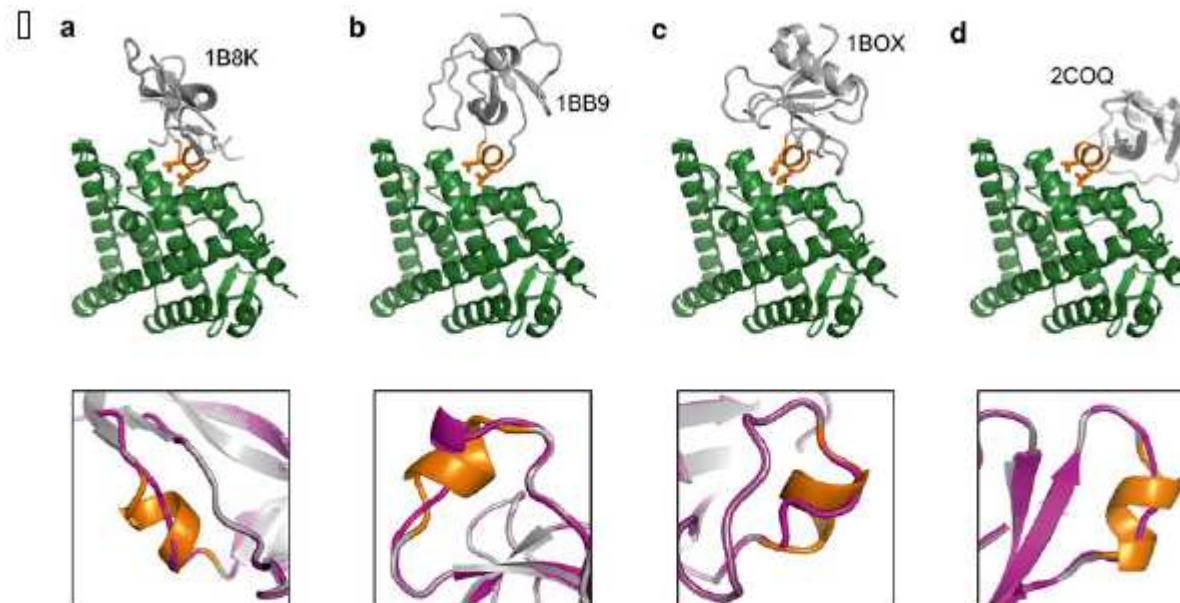
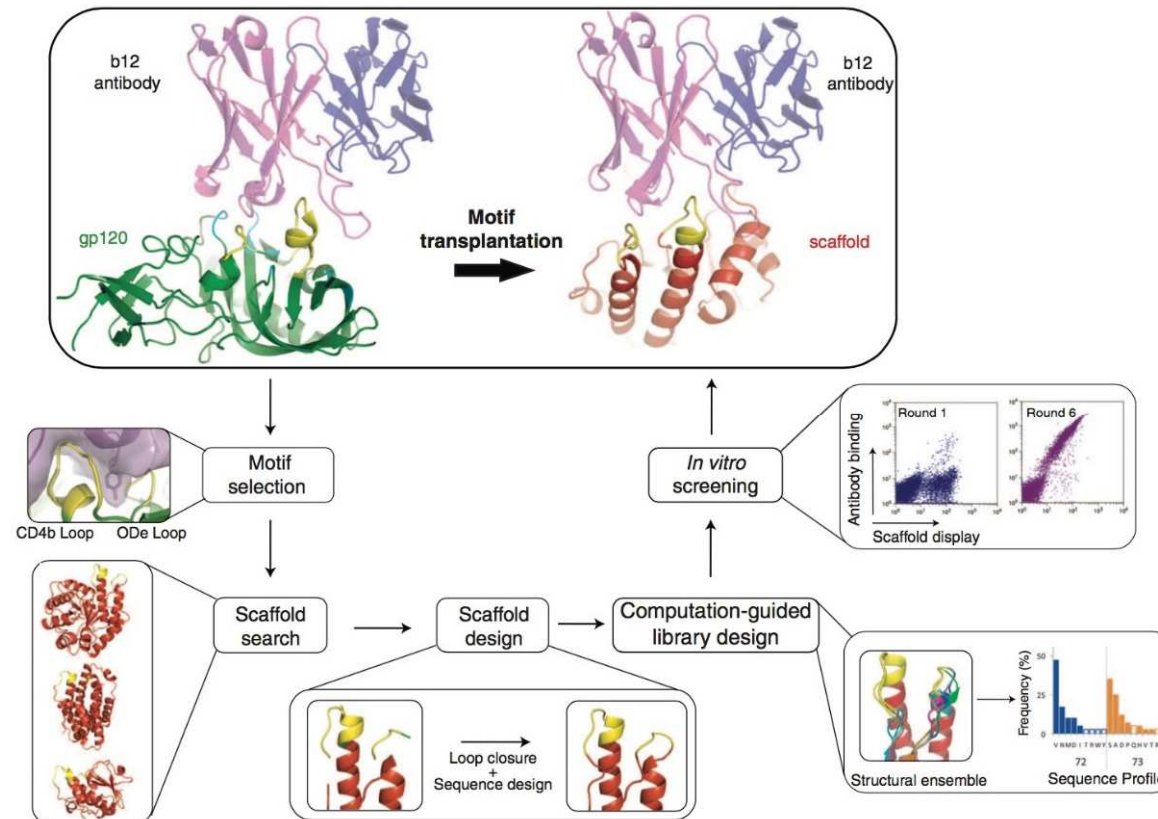


Fig. 4 Examples of designs generated by backbone grafting. (a–d) In the *upper* images, the target ERα is shown in *green*, the scaffold in *grey*, and the grafted motif in *orange*. The scaffold PDB is labeled. In the *lower* images, the designed proteins (scaffold and motif regions are in *grey* and *orange*, respectively) are superimposed with the original scaffold PDBs in *magenta*. Notice that scaffold loops of very different lengths and conformations were replaced with the helical motif

Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

BACKBONE GRAFTING



Azoitei, M.L., Correia, B.E., Ban, Y.E., Carrico, C., Kalyuzhiy, O., Chen, L., Schroeter, A., Huang, P.S., McLellan, J.S., Kwong, P.D., Baker, D., Strong, R.K., Schief, W.R. (2011) Computation-guided backbone grafting of a discontinuous motif onto a protein scaffold. *Science*. 334(6054):373-6. doi: 10.1126/science.1209368

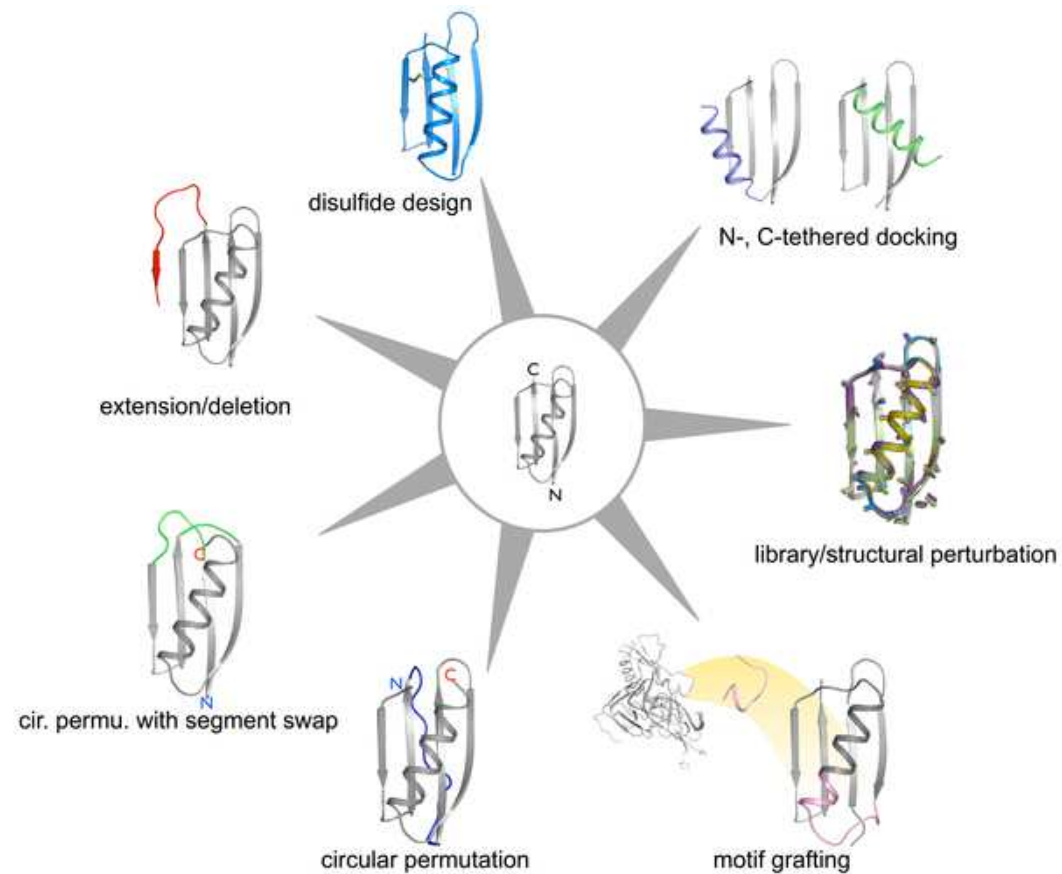
SELECTION OF DESIGNS AND OPTIMIZATION

- Results to keep:
 - Favorable binding energy ($\Delta\Delta G$)
 - High shape complementarity
 - Low number of buried unsatisfied H-bonding atoms
- Results to cull:
 - Buried charged residues
 - Under-packed interfaces dominated by Ala residues

SELECTION OF DESIGNS AND OPTIMIZATION

- Insert as few mutations as possible because probability of a designed sequence to properly fold is inversely correlated with the number of mutations imposed on the scaffold during the design process
- Check if the design is “stable” by comparing the score vs. RMSD to the native scaffold
- You may have to manually adjust designs

ROSETTA REMODEL



Huang, P.H., Ban, Y.A., Richter, F., Andre, I., Vernon, R., Schief, W.R., and Baker, D. (2011) RosettaRemodel: A generalized framework for flexible backbone protein design. *PLoS One* 6(8):e24109. doi:10.1371/journal.pone.0024109.g001

THE BLUEPRINT FILE

- Rosetta Remodel has 3 required inputs: the input PDB(s) – and also specify the chain to be remodeled, database location, and **blueprint file**.

```
1 V .  
2 L .  
3 E .  
4 I .  
5 L L PIKAA L  
0 x I NATAA  
0 x I NATAA  
0 x I NATAA  
0 x I NATAA  
6 N L PIKAA L  
7 G .
```

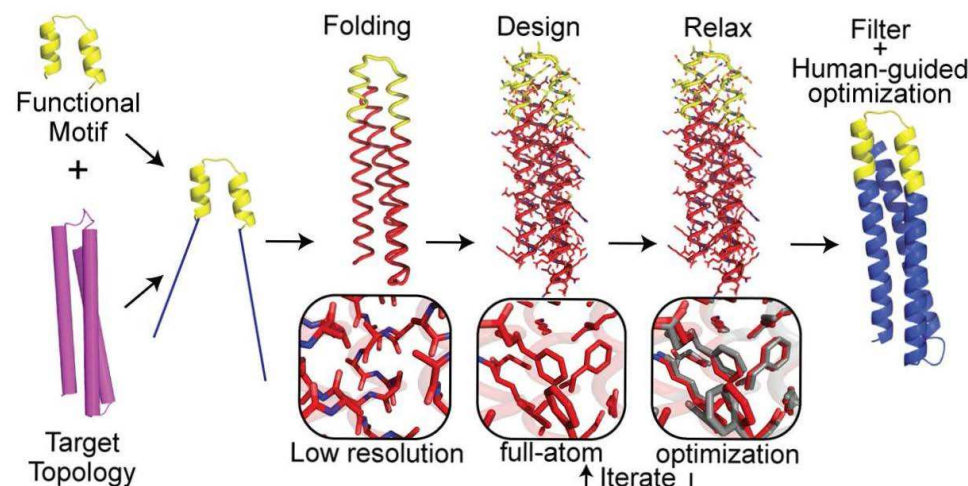
Example blueprint file for motif insertion

MOTIF GRAFTING V. MOTIF DESIGN

Motif grafting	Motif design
Requires two structures, the motif and scaffold	Requires one structure
Can be used for discontinuous epitopes	Redesign of the hydrophobic core and interface introduces unfavorable mutations to the scaffold
	Careful filtering of designs

FUNFOLDES (FOLDFROMLOOPS)

- Improvements in design capability
 - Design motif scaffolds in the presence of a binder/ligand.
 - Multi-segment or discontinuous motif can be put into a scaffold using a multi-loop file.
 - Motif does not have to be the same length as the segment being replaced.
 - Can also be used in presence of a binder



Correia, B.E., Bates, J.T., Loomis, R.J., Baneyx, G., Carrico, C., Jardine, J.G., Rupert, P., Correnti, C., Kalyuzhny, O., Vittal, V., Connell, M.J., Stevens, E., Schroeter, A., Chen, M., Macpherson, S., Serra, A.M., Adachi, Y., Holmes, M.A., Li, Y., Kleivit, R.E., Graham, B.S., Wyatt, R.T., Baker, D., Strong, R.K., Crowe, J.E. Jr., Johnson, P.R., Schief, W.R. (2014) Proof of principle for epitope-focused vaccine design *Nature*. Mar 13;507(7491):201-6. doi: 10.1038/nature12966.

FUNFOLDES (FOLDFROMLOOPS)

Pros	Cons
Extremely useful for loops	Can disrupt the overall fold in the scaffold
Can be used for discontinuous epitopes	Redesign of the hydrophobic core and interface introduces unfavorable mutations to the scaffold
	Careful filtering of designs



QUESTIONS?

- Contact: clara.t.schoeder@vanderbilt.edu