# TUTORIAL 6: SCAFFOLDING AND MOTIF GRAFTING

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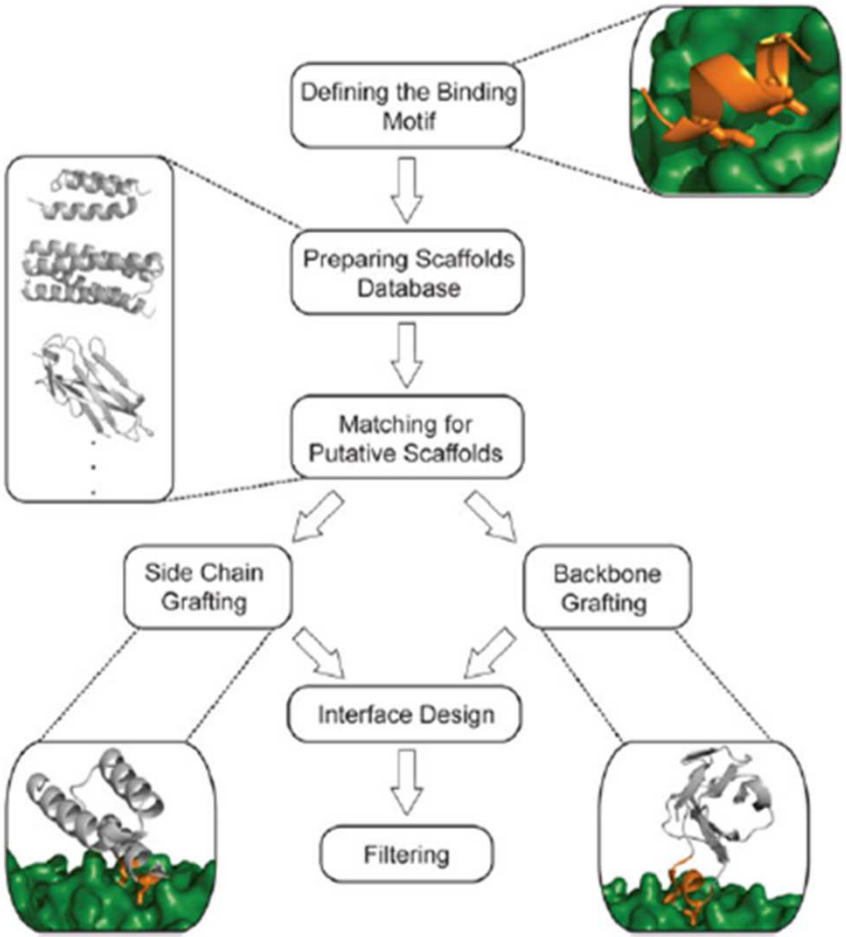
# INTRODUCTION
# WHY MOTIF GRAFTING AND SCAFFOLD DESIGN?

- 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 Å (better < 2.0 Å))
- 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 Å)
- 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

```
<MotifGraft name="motif_grafting" context_structure="context.pdb"
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NC_points_RMSD_tolerance="0.5"
clash_score_cutoff="5" clash_test_residue="GLY" hotspots="3:7"
combinatory_fragment_size_delta="2:2" full_motif_bb_alignment="1"
graft_only_hotspots_by_replacement="1"
revert_graft_to_native_sequence="1"/>
```

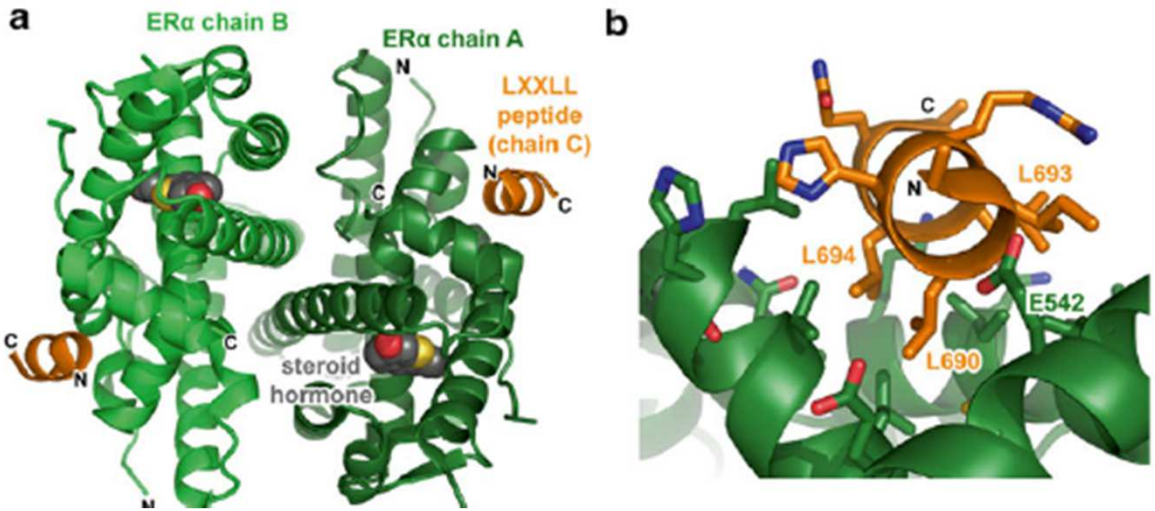


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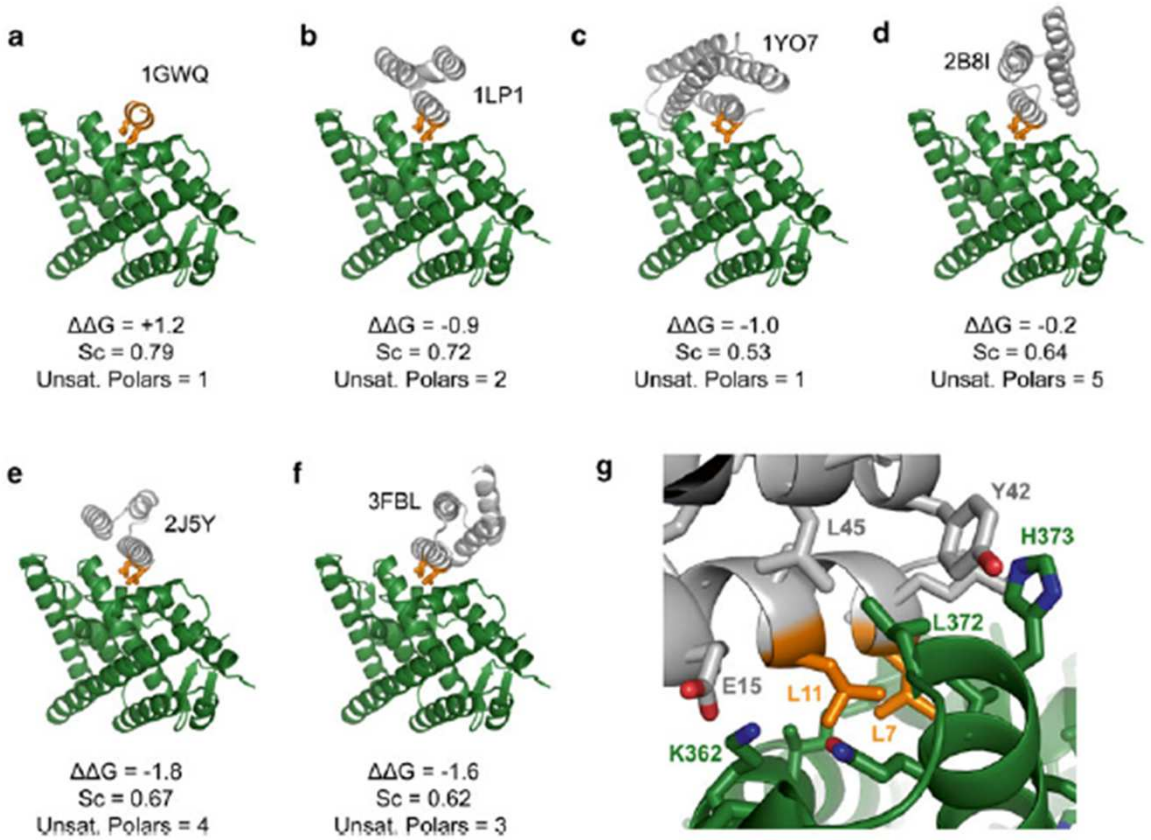
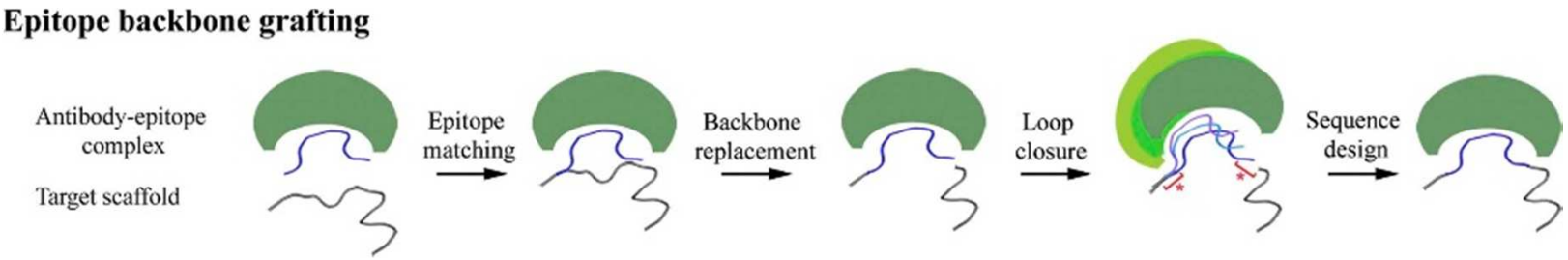
# MATCHING PUTATIVE SCAFFOLDS WITH SIDE CHAIN GRAFTING




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




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

```
<MotifGraft name=”motif_grafting” context_structure=”context.pdb”
motif_structure=”motif.pdb” RMSD_tolerance=”1.0”
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clash_test_residue=”GLY” hotspots=”3:7”
combinatory_fragment_size_delta=”2:2”
max_fragment_replacement_size_delta=”-8:8” full_motif_bb_alignment=”0”
graft_only_hotspots_by_replacement=”0”/>
```

# BACKBONE GRAFTING

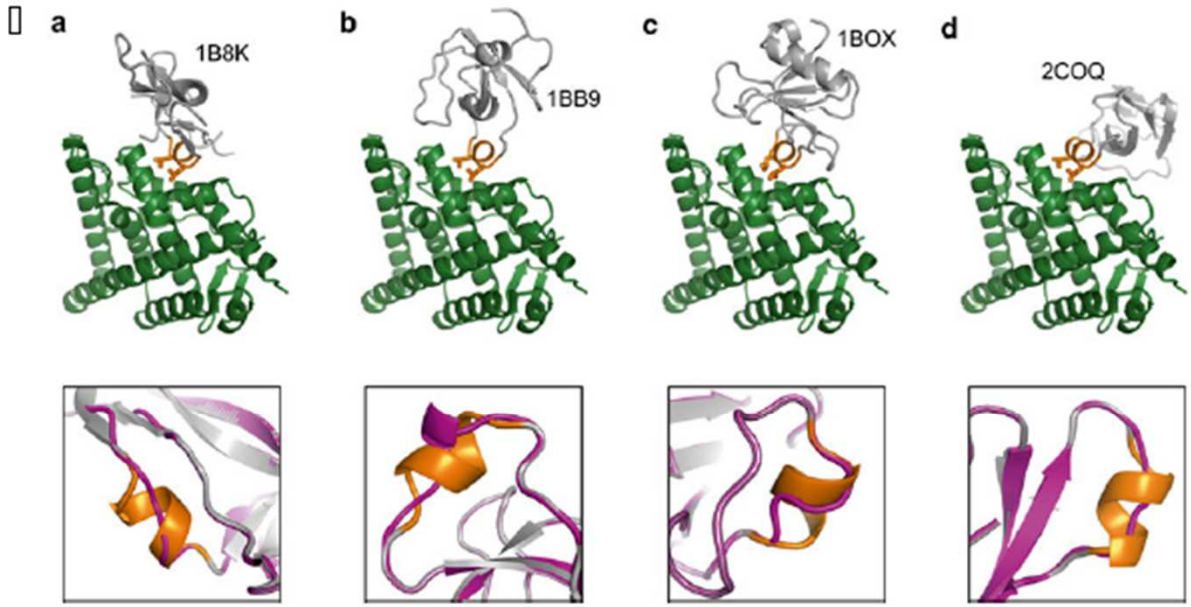
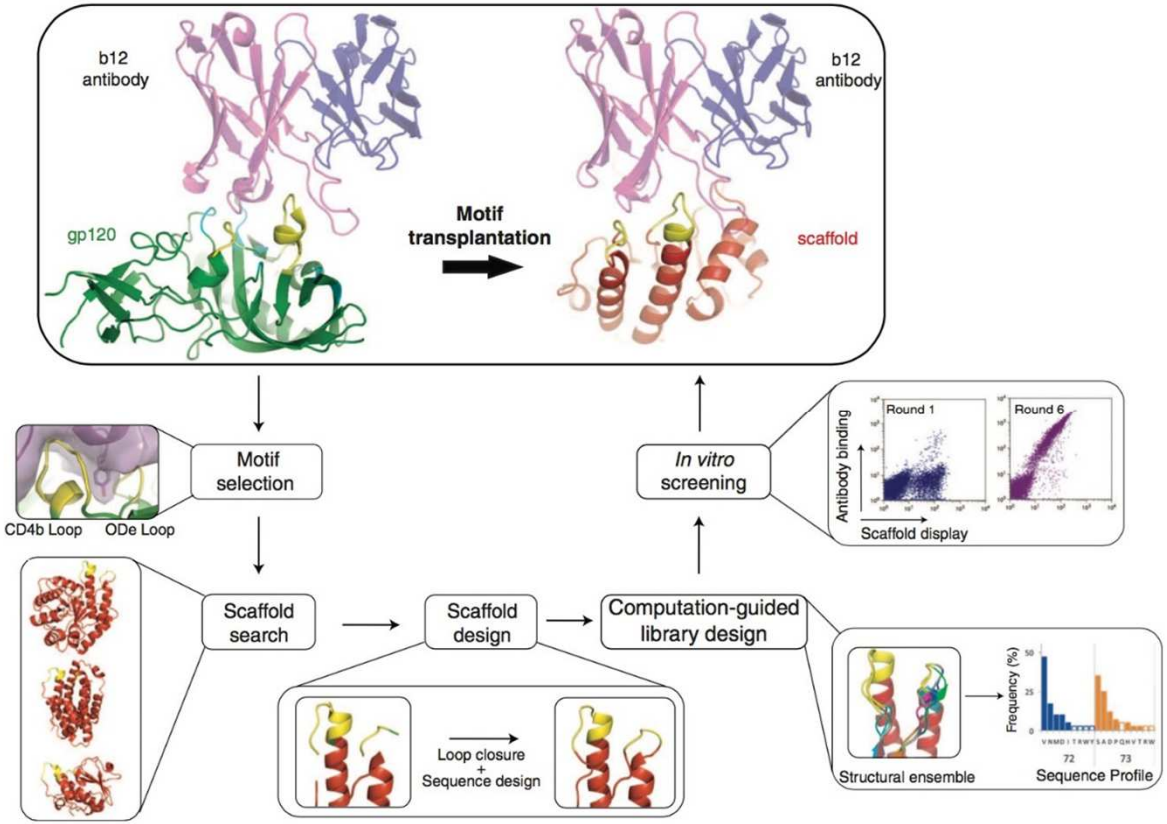



**Fig. 4** Examples of designs generated by backbone grafting. (**a–d**) In the *upper* images, the target ERα is shown in *green*, the scaffold in *grey*, and the grafted motif in *orange*. The scaffold PDB is labeled. In the *lower* images, the designed proteins (scaffold and motif regions are in *grey* 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

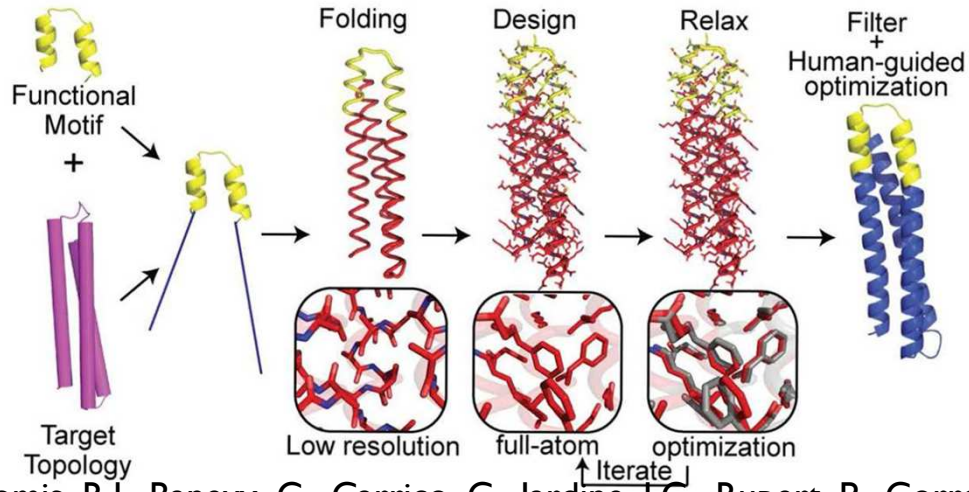
- Results to keep:
  - Favorable binding energy ($\Delta\Delta G$)
  - High shape complementarity
  - Low number of buried unsatisfied H-bonding atoms
- Results to cull:
  - Buried charged residues
  - Under-packed interfaces dominated by Ala residues

# 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

# FUNFOLDES (FOLDFROMLOOPS)

- Improvements in design capability
  - Design motif scaffolds in the presence of a binder/ligand.
  - Multi-segment or discontinous 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, Kalyuzhnly, O., Vittal, V., Connell, M.J., Stevens, E., Schroeter, A., Chen, M., Macpherson, S., Serra, A.M, Adachi, Y., Holmes, M.A. Li, Y., Klevit, 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 IS CONTROLLED BY RESIDUE SELECTORS

- Residue selectors in Rosetta allow to perform an action on only a subset of residues of your pdb

```
<RESIDUE_SELECTORS>
   <Chain name="chA" chains="A"/>
   <Index name="res1to10" resnums="1-10"/>
</RESIDUE_SELECTORS>
```

- www.rosettacommons.org/docs/latest/scripting_documentation/RosettaScripts/ResidueSelectors/ResidueSelectors

# FUNFOLDES KEY PART IS THE NUBINITIOMOVER

```
<NubInitioMover name="FFD" fragments_id="frags"
    template_motif_selector="acceptor" rmsd_threshold="5"
    fullatom_scorefxn="fullatom" >
  <Nub reference_name="motif_pose" residue_selector="donor"
binder_selector="target" >
    <Segment order="1" n_term_flex="3" c_term_flex="3"
editable="3,6,10,16,20" />
  </Nub>
</NubInitioMover>
```

- NubInitioMover is an abinitio protocol!!! (with restraints)

# MODEL EVALUATION

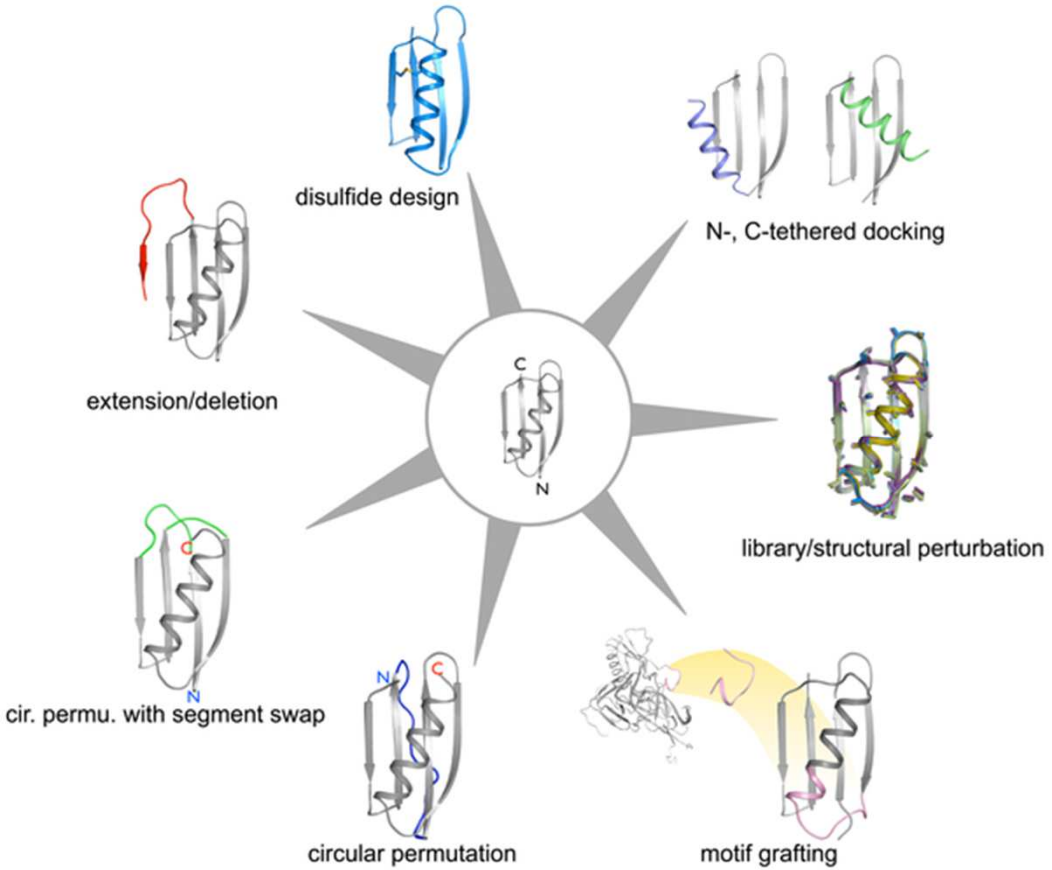
- Filtering is important!
  - RMSD
  - Total score
  - dG_separated
  - Holes scores/core packing
  - Buried unsatisfied hydrogen bond donors and acceptors

  - … what ever is important for your project!

# 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 |

# 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

# SCAFFOLD DESIGN

- What to do when there is no scaffold?
- Rosetta Remodel can also be used to generate new scaffold based on known folds.

- Useful sources are Koga et al. Nature, 2012 and Rocklin 2017, Science

# QUESTIONS?


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



## Acknowledgement

- Jaume Bonet