

Tutorial 6: Scaffolding and Epitope Grafting

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 - Preparing input files
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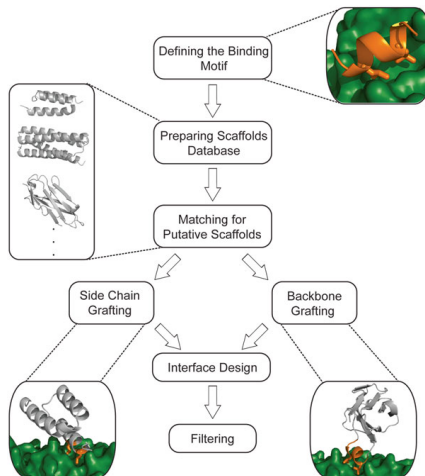
Why use scaffolding?

- Transplantation of functional motifs onto protein target
- Reduce entropic penalty of binding by embedding flexible peptide within a stable backbone
- Increase favorable interactions with protein target

Various Scaffolding Methods in Rosetta

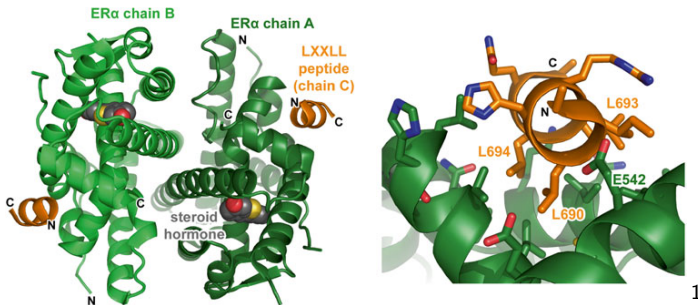
ROSETTA XML SCRIPTS	Side Chain Grafting Backbone Grafting
ROSETTA APPLICATIONS	Fold From Loops Rosetta Remodel

Side chain and backbone grafting – an overview



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The Functional Binding Motif



¹Silva, 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 ($< 2.5 \text{ \AA}$)
- 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

For future reference

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

Matching putative scaffolds with Side Chain Grafting

- ① Choose motif and scaffold backbones that superimpose with very low RMSD ($< 0.5 \text{ \AA}$)
- ② Transplant side chains from functional motif onto scaffold
- ③ Design surrounding residues on the scaffold surface

Possible Considerations

Pros

Minimal number of changes to the scaffold

Increase chances of correctly folded designs

Cons

Often motif and scaffold structures are too dissimilar, limiting availability of scaffolds

Side Chain Grafting XML

```
<MotifGraft name="motif_grafting"
context_structure="context.pdb"
motif_structure="motif.pdb"
RMSD_tolerance="0.3"
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_replace-
ment="1"
revert_graft_to_native_sequence="1"/>
```

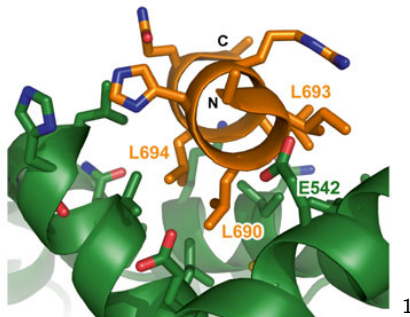
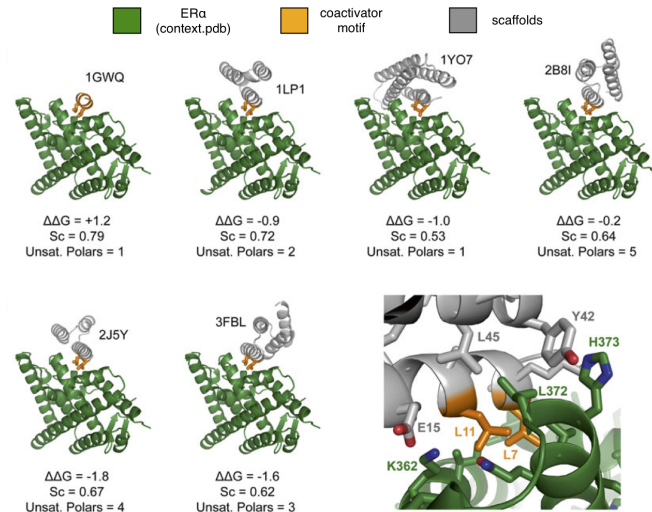


Figure: Peptide structure contains LXXLL motif that is necessary for interaction.

Selecting accurate models



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Backbone Grafting Overview

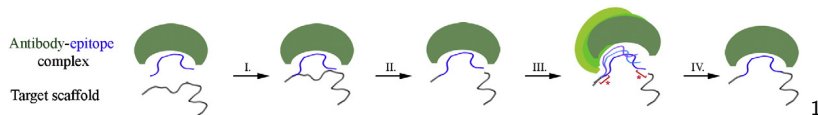


Figure:

- 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

¹Azoitei, M.L., Ban, Y.A., Julien, J., Bryson, S., Schroeter, A., Kalyuzhnyi, 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

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

Possible Considerations

Pros

Extremely versatile – a loop in the scaffold can be replaced by a different secondary structure or even with a different amino acid length

Cons

Can disrupt the overall fold in the scaffold

Redesign of the hydrophobic core and interface introduces unfavorable mutations to the scaffold

Careful filtering of designs

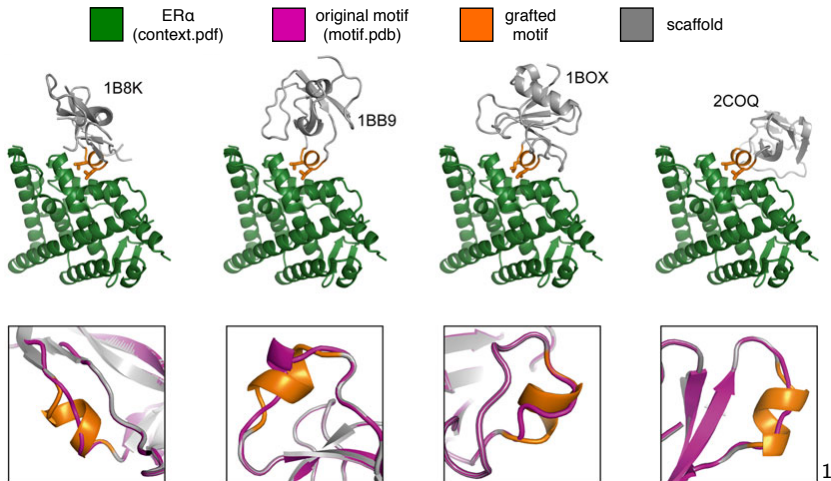
Backbone Grafting XML

```
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NC_points_RMSD_tolerance="1.0" clash_score_cutoff="5"  
clash_test_residue="GLY" hotspots="3:7" combinatorial_fragment_size_delta="2:2"  
max_fragment_replacement_size_delta="-8:8" full_motif_bb_alignment="0"  
graft_only_hotspots_by_replacement="0"/>
```

```
<PackRotamersMover name="design_core"  
task_operations="hotspot_repack, pido_far, core"/>
```

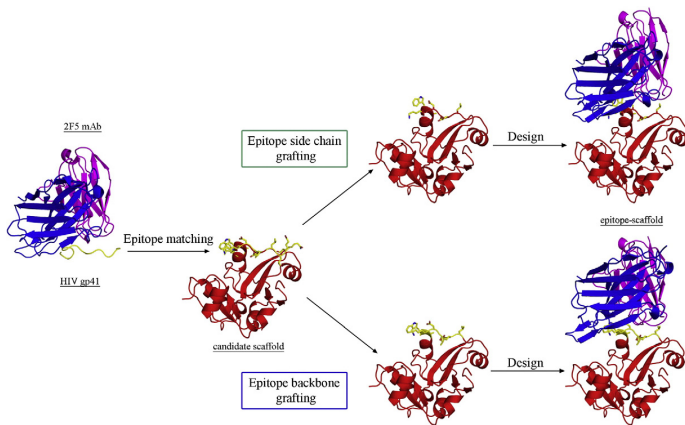
```
<PackRotamersMover name="design_boundary"  
task_operations="hotspot_repack, pido_med, core_and_boundary"/>
```

Backbone Grafting Examples



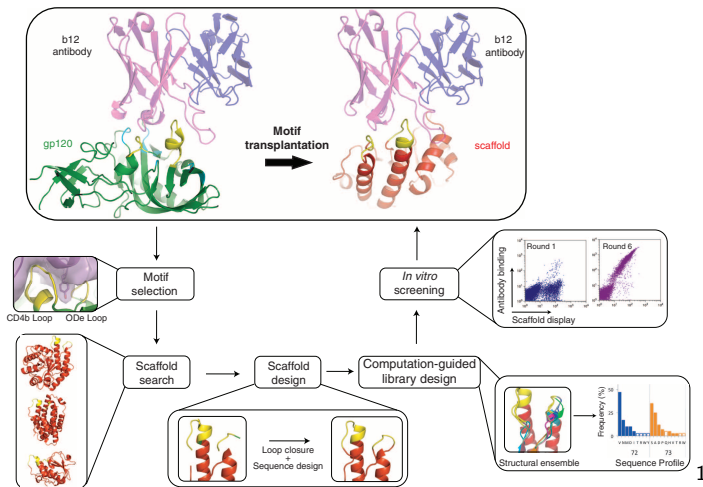
¹Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

Side Chain vs. Backbone Grafting



¹Azoitei, M.L., Ban, Y.A., Julien, J., Bryson, S., Schroeter, A., Kalyuzhnyi, 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

Grafting a discontinuous motif



¹Azoitei, M.L., Correia, B.E., Ban, Y.A., Carrico, C., Kalyuzhnyi, O., Chen, L., Schroeter, A., Huang, P., 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* 334:373-376

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 to the RMSD from the native model
- You may have to manually adjust designs

Fold From Loops (FFL)

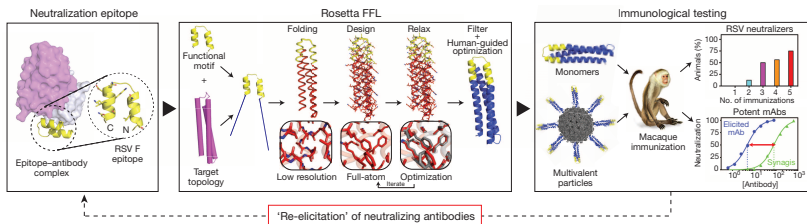
Design Considerations

- 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.

Caveats

FFL is not available in the Rosetta master branch or in RosettaScripts

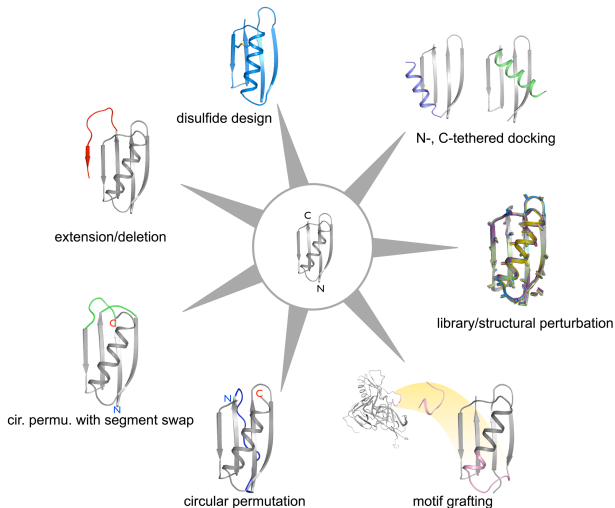
Proof of principle using FFL for an RSV Vaccine



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¹Correia, B.E., Bates, J.T., Loomis, R.J., Baneyx, G., Carrico, C., Jardine, J.G., Rupert, P., Correnti, C., Kalyuzhnyi, 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., Johnson, P.R., Schief, W.R. (2014) Proof of principle for epitope-focused vaccine design. *Nature* 507:201-206

Rosetta Remodel



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¹Huang, 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

Questions?

All material for this tutorial can be found in
~/rosetta_workshop/tutorials/scaffolding/

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