Tutorial 6: Scaffolding and Motif Grafting

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Rosetta Workshop
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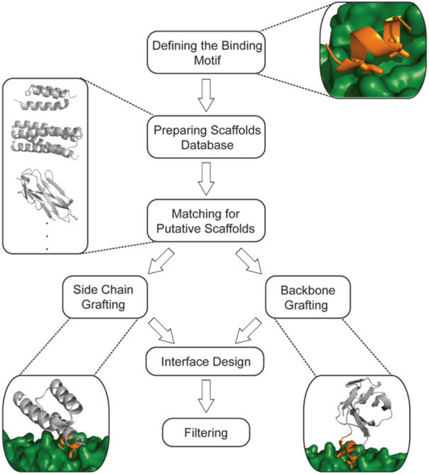
Various Grafting Methods in Rosetta

ROSETTA METHOD	Name	Function
ROSETTA XML SCRIPTS	Side Chain Grafting	Scaffold Design
	Backbone Grafting	Scaffold Design
ROSETTA APPLICATIONS	Rosetta Remodel	Motif/Loop Design
	Fold From Loops	Scaffold/Loop Design

Side chain and backbone grafting – an overview



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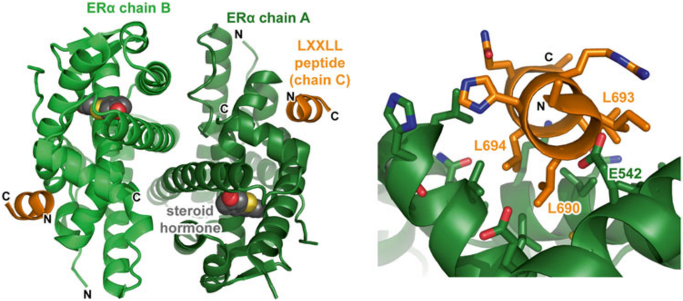
[1] Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

The Functional Binding Motif



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[1] 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)
- 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 Å)
2. Transplant side chains from functional motif onto scaffold
3. Design surrounding residues on the scaffold surface

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

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”/>
```

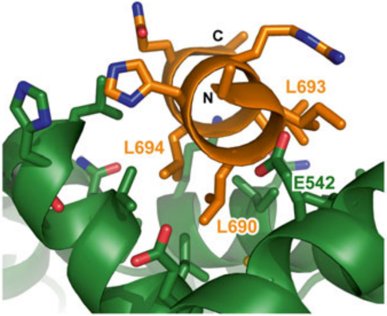

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Figure: Peptide structure contains LXXLL motif that is necessary for interaction.

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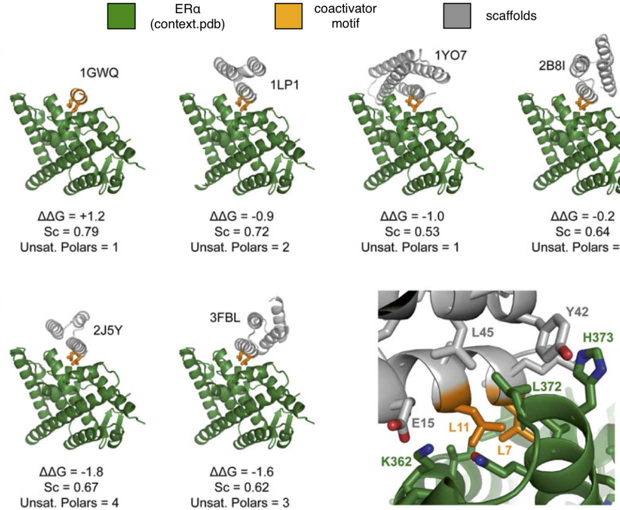
[1] Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304

Selecting accurate models



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[1] 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

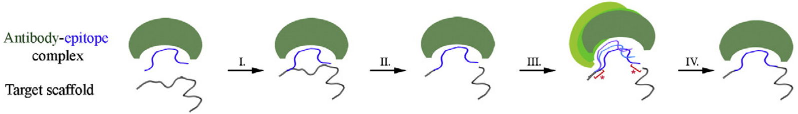

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Figure:

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

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[1] 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

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

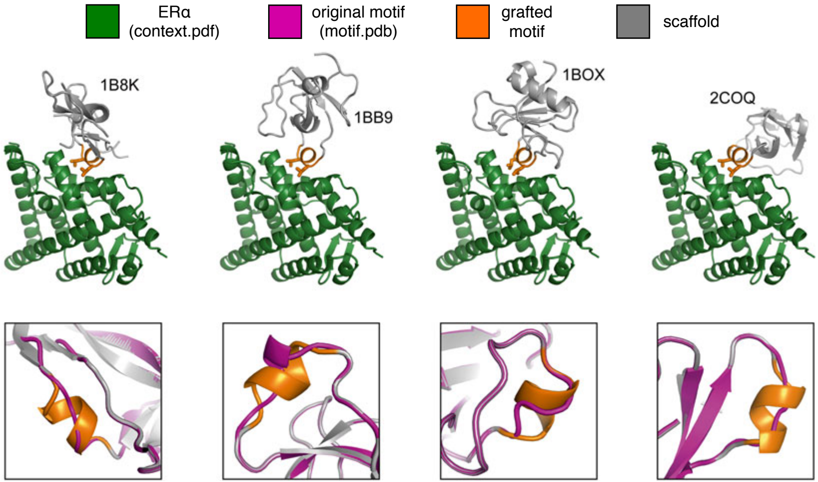
Backbone Grafting XML

```
<MotifGraft name=”motif_grafting” context_structure=”context.pdb”
motif_structure=”motif.pdb” RMSD_tolerance=”1.0”
NC_points_RMSD_tolerance=”1.0” clash_score_cutoff=”5”
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”/>
```

```
<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



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

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

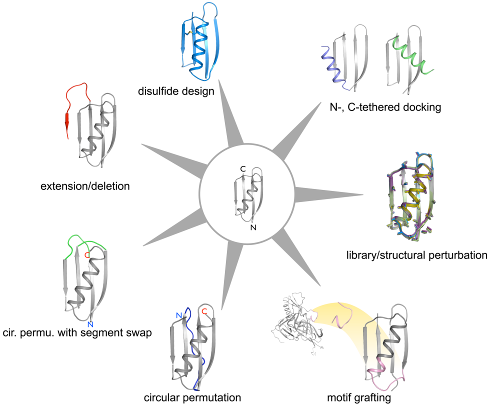
Results to Cull

- Buried charged residues
- Under-packed interfaces dominated by Ala residues

Selection of Designs and Optimization *Continued*

- 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

Rosetta Remodel



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[1] 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.

An example blueprint file for motif insertion

```
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 .
```

Motif grafting v. motif design

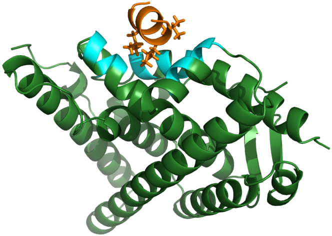

Figure: Today's Rosetta Remodel tutorial: Optimization of the ER binding site to the LxxLL motif

Motif Grafting	Motif Design
Requires two structures, the motif and scaffold	Requires one structure
May require docking to graft motif	Often requires presence of ligand or small molecule

Fold From Loops (FFL)

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.

Available now!

FFL2.0 is currently available in Rosetta 3.9

Questions?

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

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