

Motif grafting and scaffolding using Rosetta

Clara T. Schoeder

Meiler Lab

Virtual Rosetta Workshop August 2021



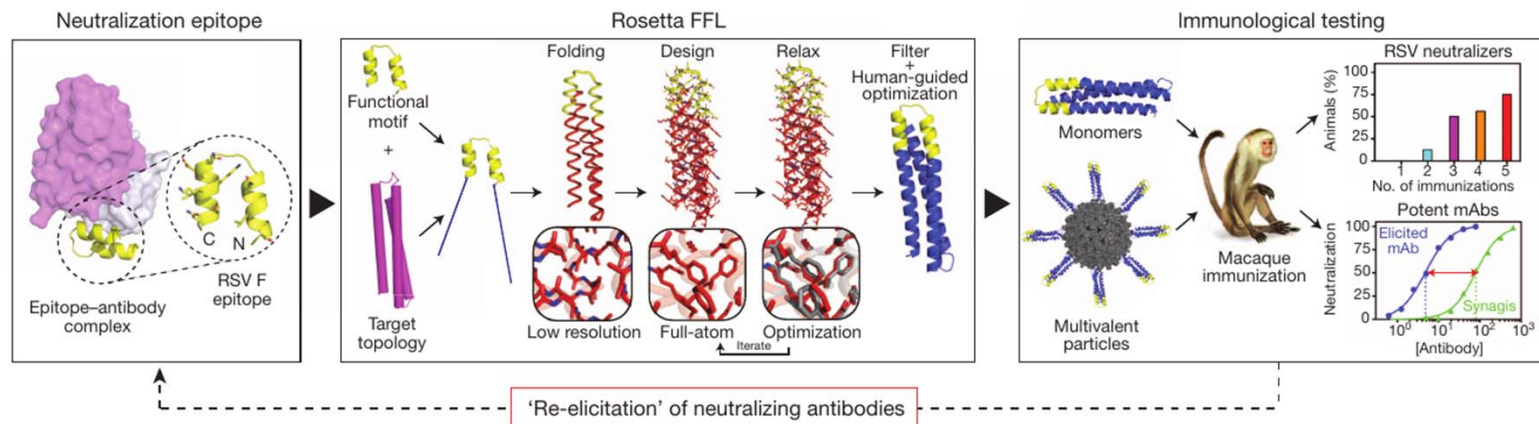
Content

- Why do we do grafting and scaffold design?
- Grafting protocols
 - Side chain grafting
 - Backbone grafting
- Scaffold design protocols
 - FunFoldes
 - TopoBuilder
 - RosettaRemodel



What can motif grafting and scaffolding be used for?

- 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



Correia, B. E.; Bates, J. T.; Loomis, R. J.; Baneyx, G.; Carrico, C.; Jardine, J. G.; Rupert, P.; Correnti, C.; Kalyuzhniy, 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., Proof of principle for epitope-focused vaccine design. *Nature* **2014**, 507 (7491), 201-6.

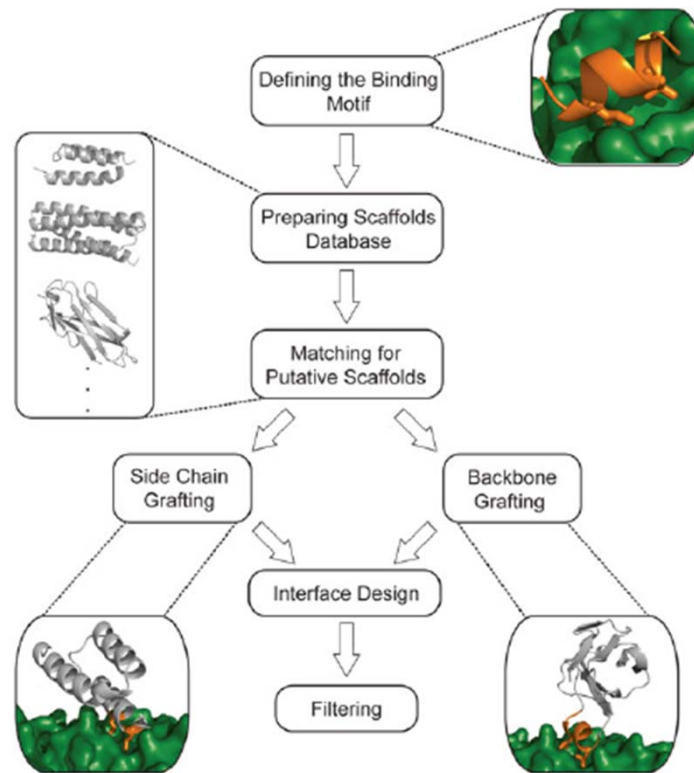


Rosetta supported protocols for motif grafting

Rosetta method	Name	Function	Mover
RosettaScripts	Side chain grafting	Scaffold design	MotifGraft
RosettaScripts	Backbone grafting	Scaffold design	MotifGraft
RosettaScripts	FunFoldDes (Fold from loops)	Loop/Scaffold design	NubInitio
Rosetta application	Rosetta remodel	Scaffold design / backbone design	Remodel



Side chain and backbone grafting



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



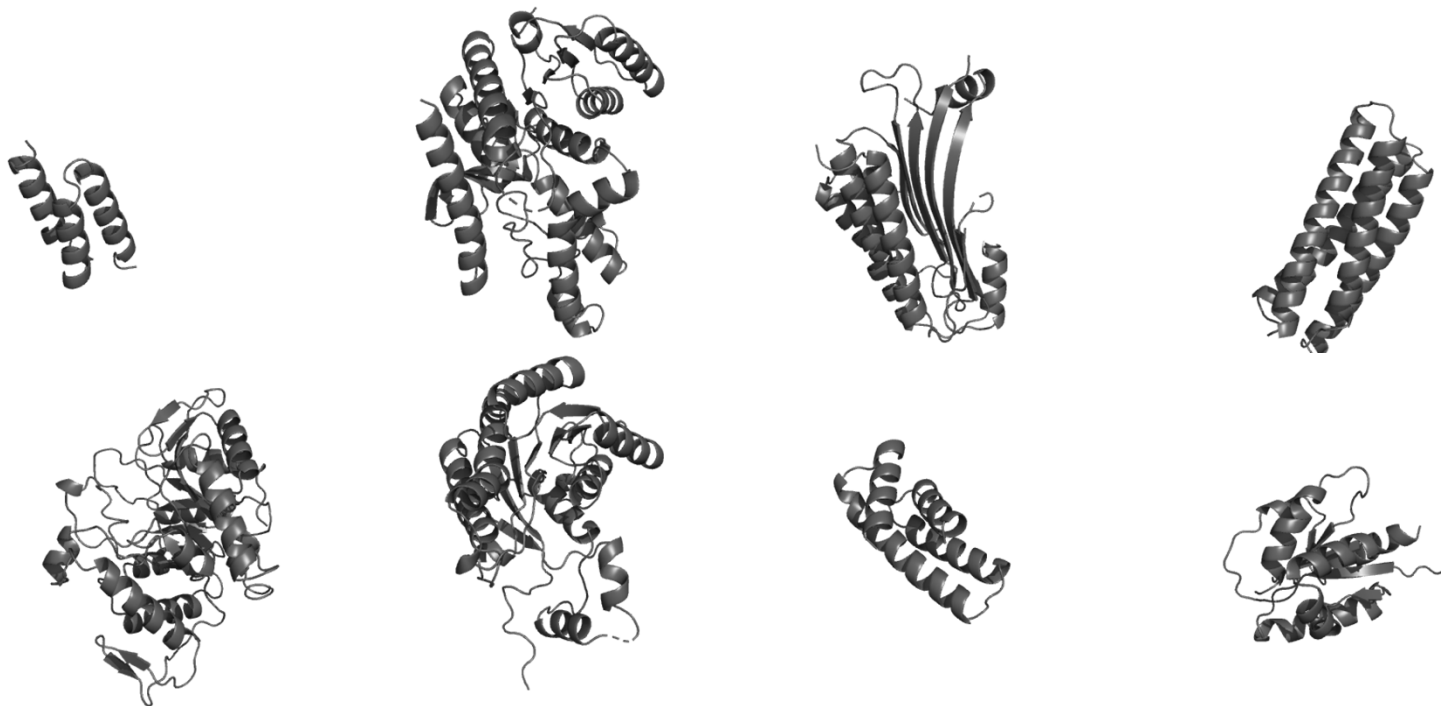
Requirements for the scaffold library

- 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

→ don't forget to relax your scaffold database



Scaffold library



Scaffold database in the tutorial



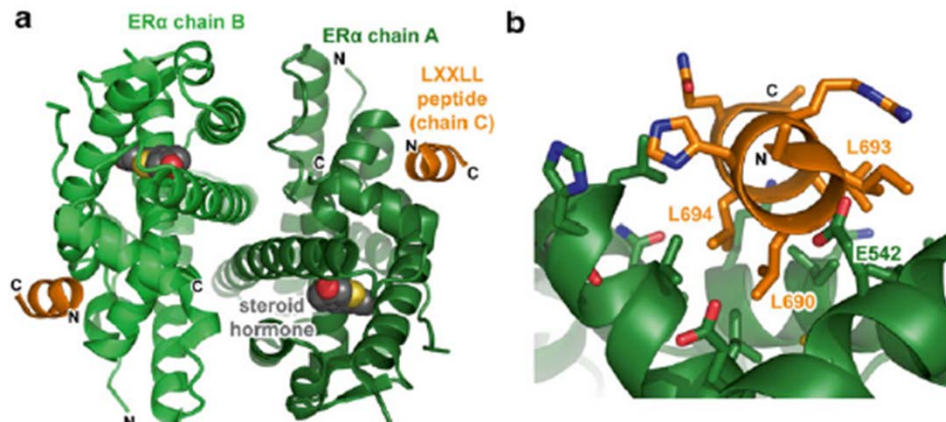
Side chain grafting – RosettaScripts protocol

```
<ROSETTASCRIPTS>
  <TASKOPERATIONS>
    <ProteinInterfaceDesign name="pido" repack_chain1="1" repack_chain2="1" design_chain1="0"
      design_chain2="1" interface_distance_cutoff="8.0"/>
    <OperateOnCertainResidues name="hotspot_repack">
      <ResiduePDBInfoHasLabel property="HOTSPOT"/>
      <RestrictToRepackingRLT/>
    </OperateOnCertainResidues>
  </TASKOPERATIONS>
  <SCOREFXNS>
  </SCOREFXNS>
  <FILTERS>
    <Ddg name="ddg" confidence="0"/>
    <BuriedUnsathbonds name="unsat" confidence="0"/>
    <ShapeComplementarity name="Sc" confidence="0"/>
  </FILTERS>
  <MOVERS>
    <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_replacement="1"
      revert_graft_to_native_sequence="1"/>
    <build_Ala_pose name="ala_pose" partner1="0" partner2="1" interface_cutoff_distance="8.0" task_operations="hotspot_repack"/>
    <Prepack name="ppk" jump_number="0"/>
    <PackRotamersMover name="design" task_operations="hotspot_repack,pido"/>
    <MinMover name="rb_min" bb="0" chi="1" jump="1"/>
  </MOVERS>
  <PROTOCOLS>
    <Add mover_name="motif_grafting"/>
    <Add mover_name="ala_pose"/>
    <Add mover_name="ppk"/>
    <Add mover_name="design"/>
    <Add mover_name="rb_min"/>
    <Add mover_name="design"/>
    <Add filter_name="unsat"/>
    <Add filter_name="ddg"/>
    <Add filter_name="Sc"/>
  </PROTOCOLS>
</ROSETTASCRIPTS>
```



Side chain grafting – RosettaScripts protocol

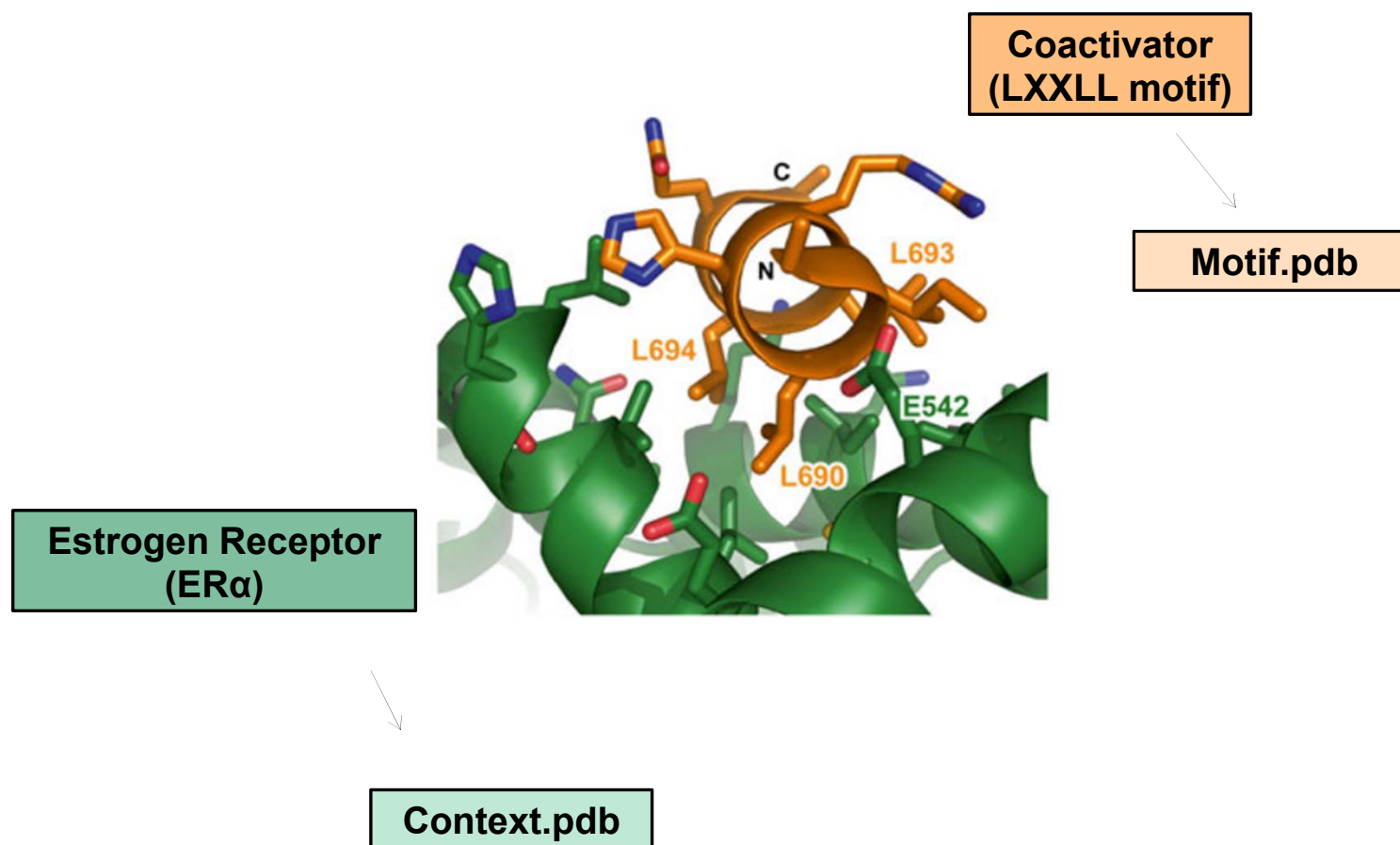
```
<MotifGraft name="motif_grafting" context_structure="context.pdb"  
motif_structure="motif.pdb" RMSD_tolerance="0.3"  
NC_points_RMSD_tolerance="0.5"  
clash_score_cutoff="5" clash_test_residue="GLY" hotspots="3:7"  
combinatory_fragment_size_delta="2:2" full_motif_bb_alignment="1"  
graft_only_hotspots_by_replacement="1"  
revert_graft_to_native_sequence="1"/>
```



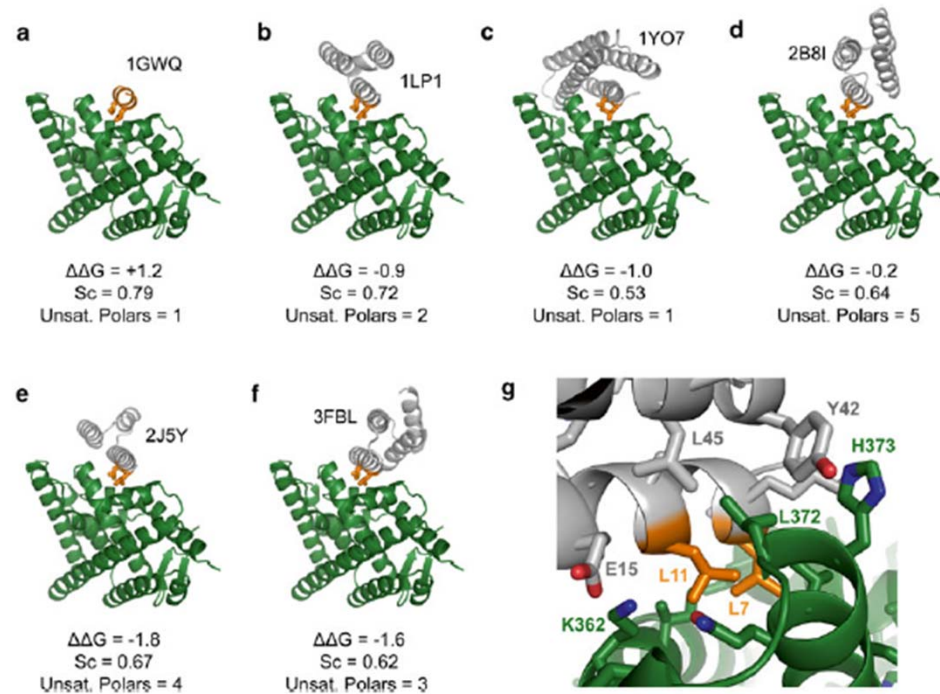
Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304



Design task in the tutorial – defining motif and context



Side chain grafting – RosettaScripts protocol

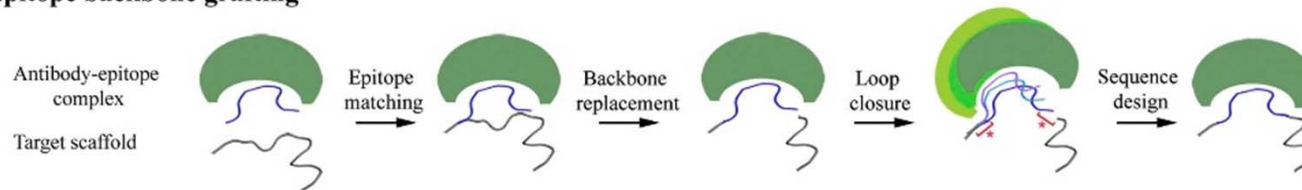


Silva, D., Correia, B.E., and Procko, E. (2016) Motif-driven Design of Protein-Protein Interactions. *Methods Mol. Biol.* 1414:285-304



Backbone grafting – principle

Epitope backbone grafting

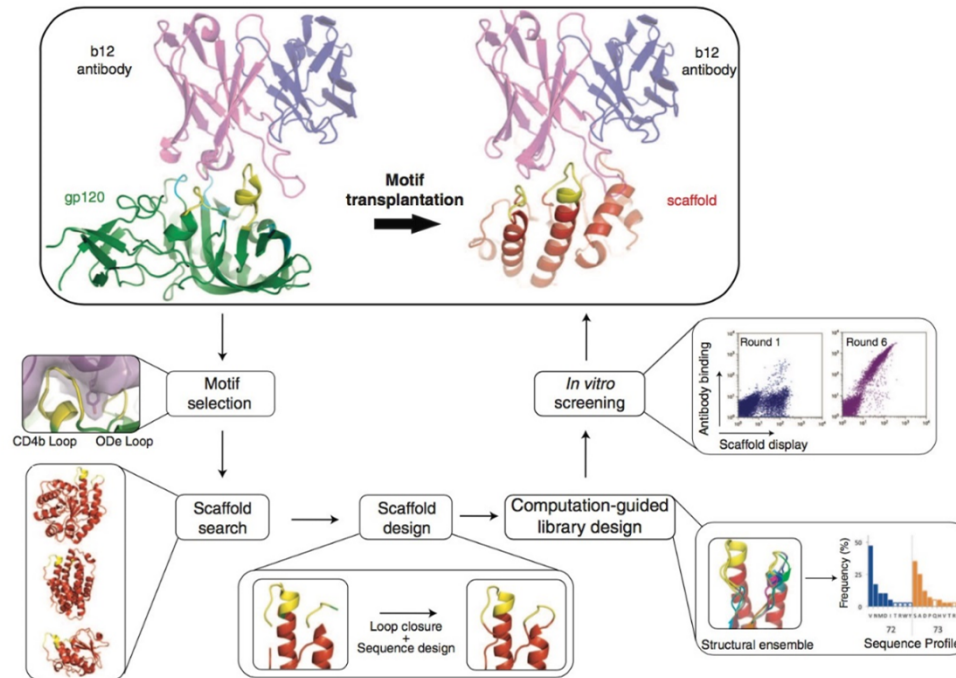


1. Align to target scaffold
2. Remove native scaffold backbone
3. Model new epitope between termini
4. Rigid-body orientation of new epitope and antibody relative to scaffold

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 – use cases



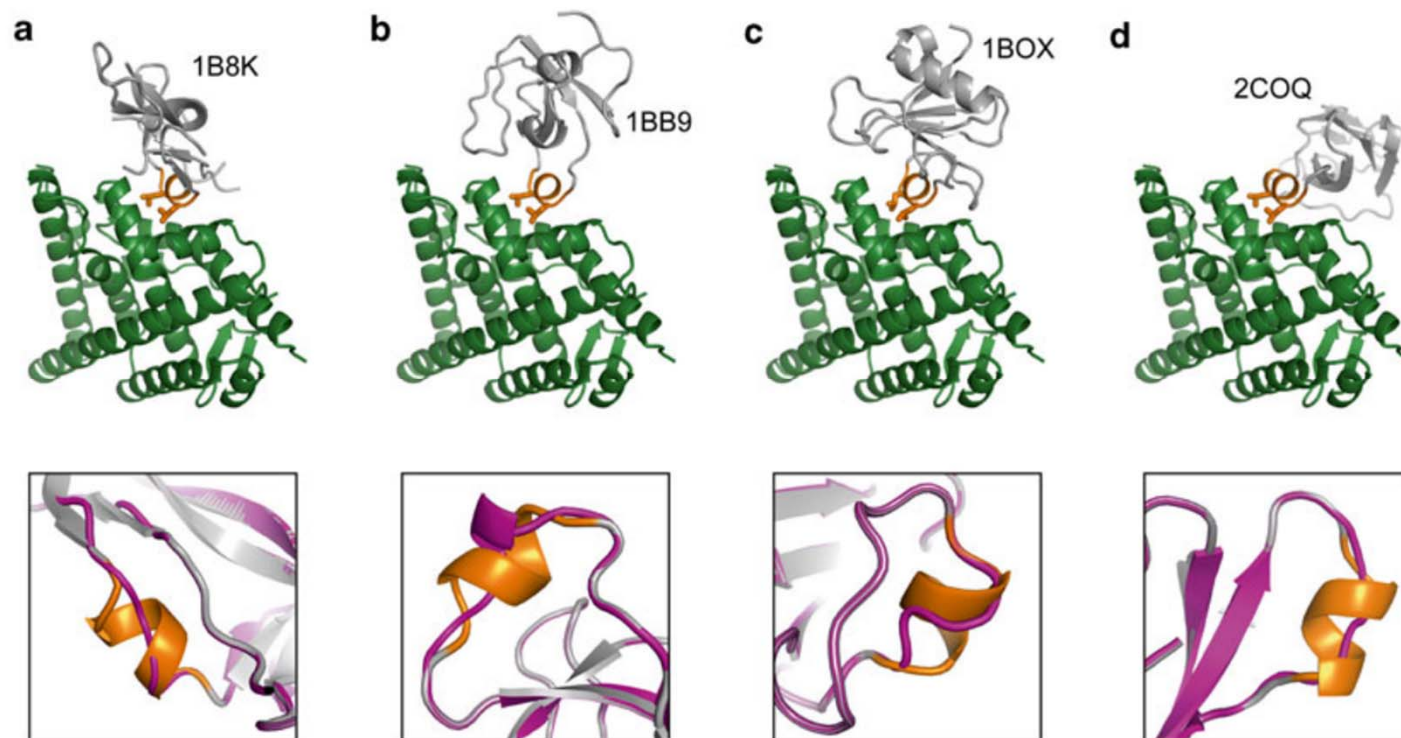
Azoitei, M.L., Correia, B.E., Ban, Y.E., Carrico, C., Kalyuzhiy, O., Chen, L., Schroeter, A., Huang, P.S., McLellan, J.S., Kwong, P.D., Baker, D., Strong, R.K., Schief, W.R. (2011) Computation-guided backbone grafting of a discontinuous motif onto a protein scaffold. *Science*. 334(6054):373-6. doi: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



Backbone grafting – RosettaScripts protocol

```
<ROSETTASCRIPTS>
<TASKOPERATIONS>
  <ProteinInterfaceDesign name="pido_far" interface_distance_cutoff="15.0"/>
  <ProteinInterfaceDesign name="pido_med" interface_distance_cutoff="12.0"/>
  <ProteinInterfaceDesign name="pido_near" interface_distance_cutoff="8.0"/>
  <OperateOnCertainResidues name="hotspot_repack">
    <ResiduePDBInfoHasLabel property="HOTSPOT"/>
    <RestrictToRepackingRLT/>
  </OperateOnCertainResidues>
  <SelectBySASA name="core" mode="sc" state="bound" probe_radius="2.2" core_asa="0"
    surface_asa="30" core="1" boundary="0" surface="0"/>
  <SelectBySASA name="core_and_boundary" mode="sc" state="bound" probe_radius="2.2"
    core_asa="0" surface_asa="30" core="1" boundary="1" surface="0"/>
</TASKOPERATIONS>
<FILTERS>
  <Ddg name="ddg" confidence="0"/>
  <BuriedUnsathbonds name="unsat" confidence="0"/>
  <ShapeComplementarity name="Sc" confidence="0"/>
</FILTERS>
<MOVERS>
  <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" combinatorial_fragment_size_delta="2:2" max_fragment_replacement_size_delta="-8:8"
    full_motif_bb_alignment="0" graft_only_hotspots_by_replacement="0"/>
  <build_Ala_pose name="ala_pose" partner1="0" partner2="1" interface_cutoff_distance="8.0" task_operations="hotspot_repack"/>
  <Prepack name="ppk" jump_number="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"/>
  <PackRotamersMover name="design_interface" task_operations="hotspot_repack,pido_near"/>
  <MinMover name="sc_min" bb="0" chi="1" jump="1"/>
</MOVERS>
<PROTOCOLS>
  <Add mover_name="motif_grafting"/>
  <Add mover_name="ala_pose"/>
  <Add mover_name="ppk"/>
  <Add mover_name="design_core"/>
  <Add mover_name="design_boundary"/>
  <Add mover_name="design_interface"/>
  <Add mover_name="sc_min"/>
  <Add filter_name="unsat"/>
  <Add filter_name="ddg"/>
  <Add filter_name="Sc"/>
</PROTOCOLS>
</ROSETTASCRIPTS>
```

Backbone grafting – RosettaScripts protocol



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



Scoring, filtering and selection of designs is an important step in your protocol

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

Side chain versus Backbone grafting – a comparison

	Side chain grafting	Backbone grafting
When?	• Backbone RMSD <0.5 • Defined secondary structure • Many scaffolds available	• Backbone RMSD for C- and N-terminus of motif align • Discontinuous epitopes • “challenging” backbones • Not many scaffolds for Side chain grafting available
Pro’s	• Less changes to the backbone → confidence in prediction	• Less dependent on scaffold availability than Side chain grafting
Con’s	• Availability of scaffolds	• Availability of scaffolds • More sampling, analyzing and testing necessary for successful design



Side chain and Backbone grafting - summary

- Define: motif, context, hotspots
- A scaffold library is needed
- Side chain grafting maintains the backbone coordinates
- Backbone grafting will tolerate more scaffolds
- Use the same protocol with different options
- Filtering steps need to be employed to select foldable designs



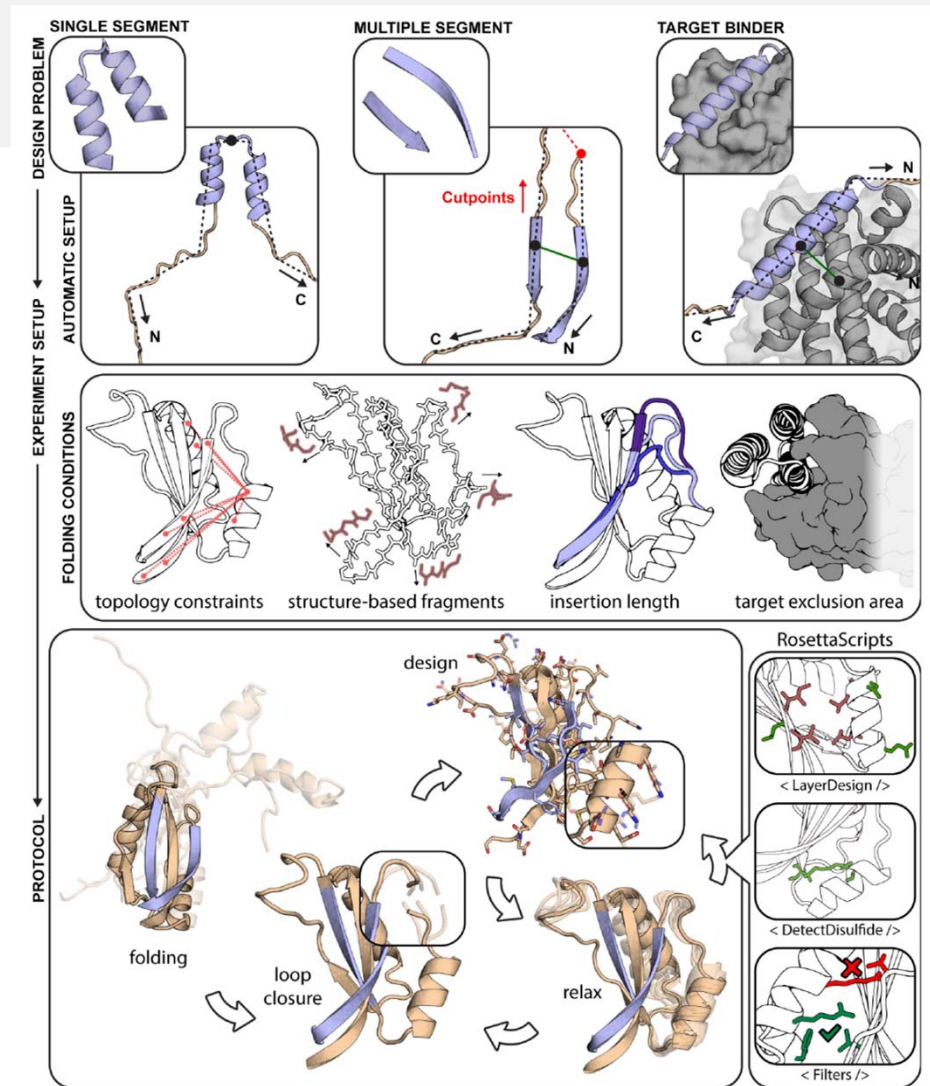
Protocols with scaffold redesign

- FunFoldDes (Functional Folding and Design)
 - Supersedes the FoldFromLoops protocol
- TopoBuilder
- RosettaRemodel



FunFoldDes

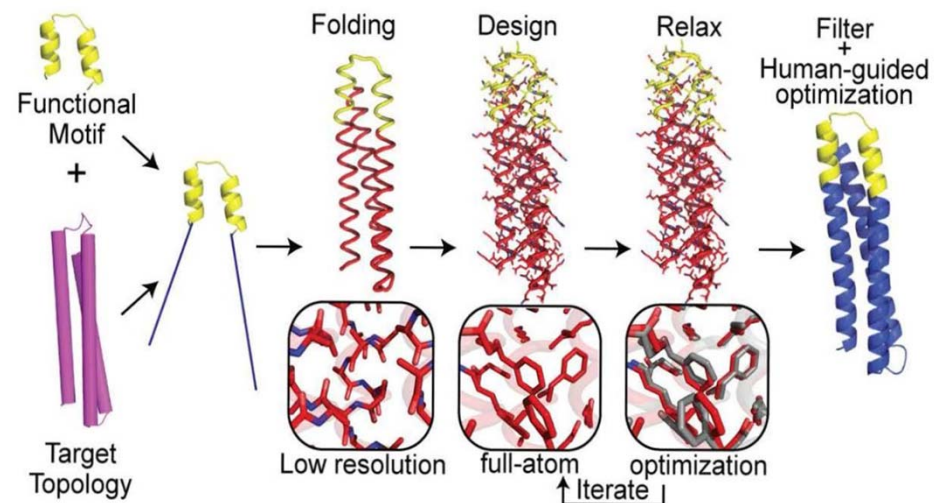
Bonet, J.; Wehrle, S.; Schriever, K.; Yang, C.; Billet, A.; Sesterhenn, F.; Scheck, A.; Sverrisson, F.; Veselkova, B.; Vollers, S.; Lourman, R.; Villard, M.; Rosset, S.; Krey, T.; Correia, B. E., Rosetta FunFoldDes - A general framework for the computational design of functional proteins. *PLoS Comput Biol* **2018**, 14 (11), e1006623.



FunFoldDes – Functional Folding and Design

- Improvements in design and folding capability
 - Design motif scaffolds in the presence of a binder/ligand and the motif.
 - Multi-segment or discontinuous motif can be grafted 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., 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.



ResidueSelectors tightly control grafting and design

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

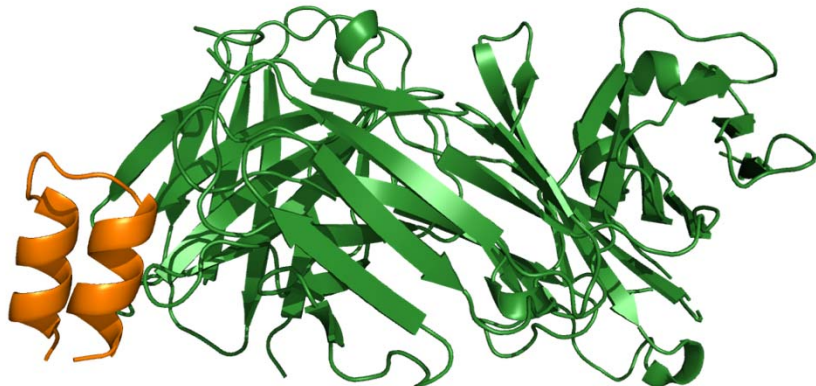
```
<RESIDUE_SELECTORS>  
  <Chain name="chA" chains="A"/>  
  <Index name="res1to10" resnums="1-10"/>  
</RESIDUE_SELECTORS>
```

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



FunFoldDes – Tutorial

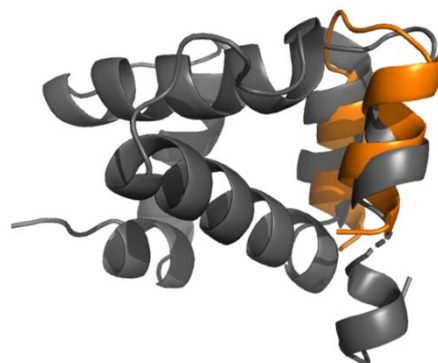
Respiratory Syncytial
Virus protein F (RSVF)
site II epitope



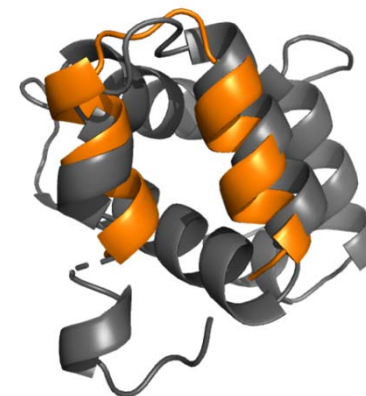
$K_D = 20 \text{ nM}$

Antibody
motavizumab
(mota)

Side View



Front View



90°

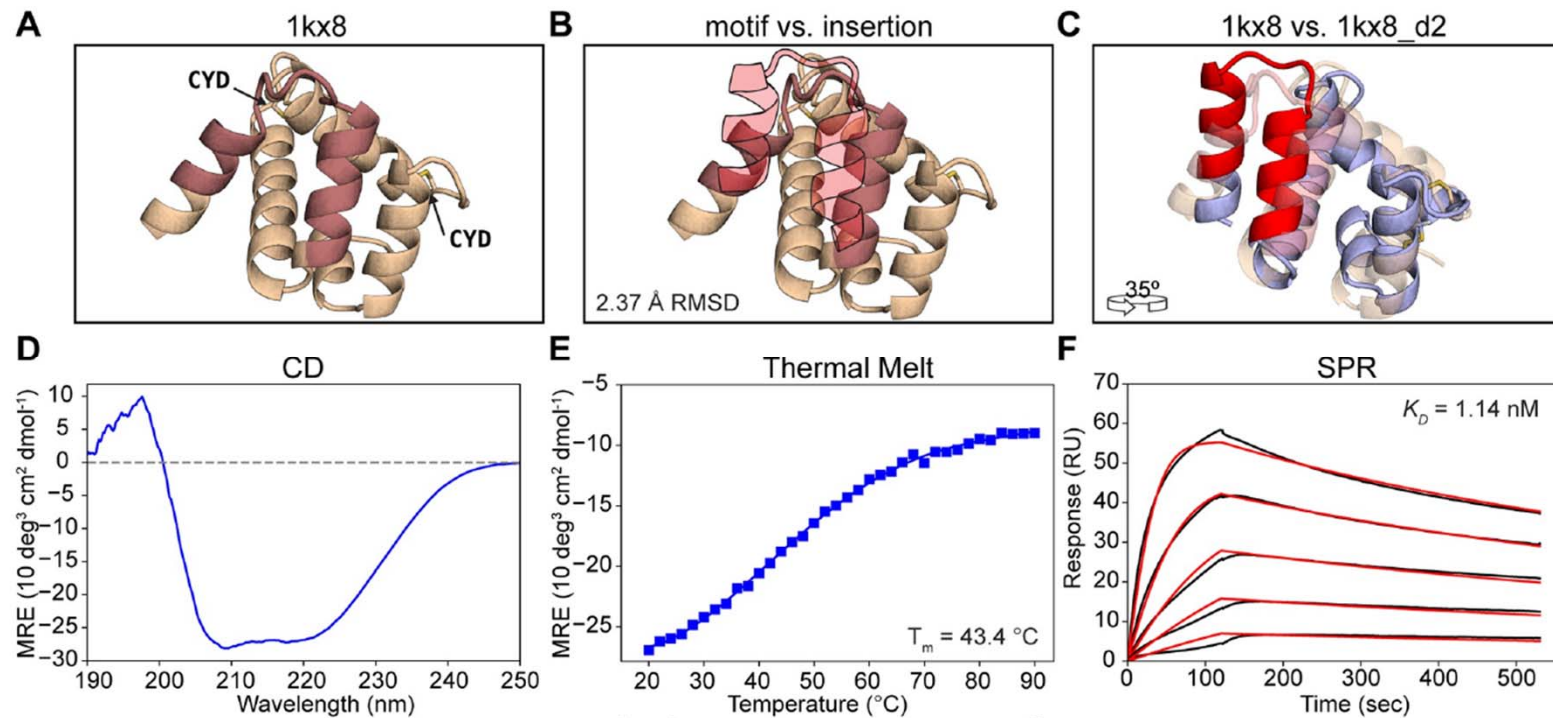


A6 protein - Antennal
Chemosensory
system (*Mamestra
brassicae*)

$RMSD = 2.37 \text{ \AA}$



FunFoldDes – grafting of the motavizumab epitope



Bonet, J.; Wehrle, S.; Schriever, K.; Yang, C.; Billet, A.; Sesterhenn, F.; Scheck, A.; Sverrisson, F.; Veselkova, B.; Vollers, S.; Lourman, R.; Villard, M.; Rosset, S.; Krey, T.; Correia, B. E., Rosetta FunFoldDes - A general framework for the computational design of functional proteins. *PLoS Comput Biol* **2018**, 14 (11), e1006623.

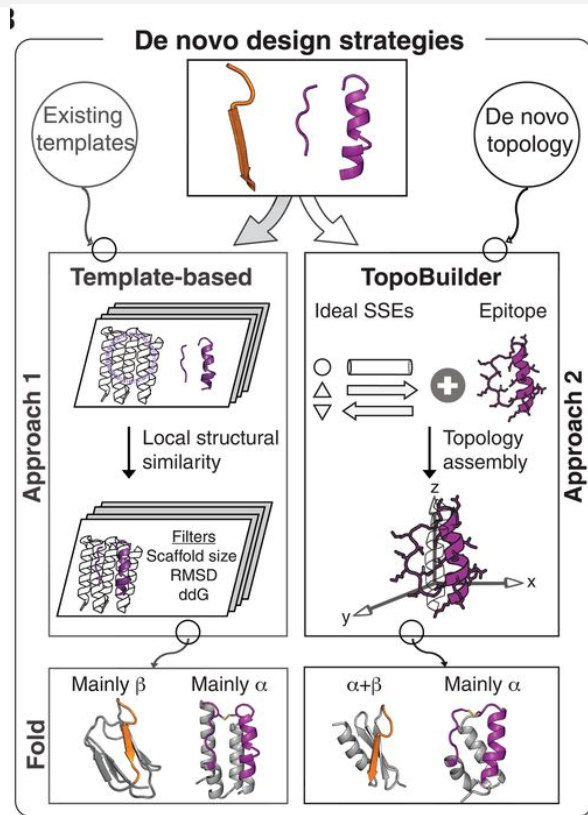


FunFoldes in comparison to Side chain and Backbone grafting

	Side chain grafting	Backbone grafting	FunFoldes
When?	• Backbone RMSD <0.5 • Defined secondary structure • Many scaffolds available	• Backbone RMSD for C- and N-terminus of motif align • Discontinuous epitopes • “challenging” backbones • Not many scaffolds for Side chain grafting available	• Discontinuous epitopes • Loops, flexible backbones • Scaffolds are sparse for Side chain and Backbone grafting
Pro’s	• Less changes to the backbone → confidence in prediction	• Less dependent on scaffold availability than Side chain grafting	• Less dependent on scaffold availability than Side chain and Backbone grafting
Con’s	• Availability of scaffolds	• Availability of scaffolds • More sampling, analyzing and testing necessary for successful design	• More sampling, analyzing and testing necessary for successful design

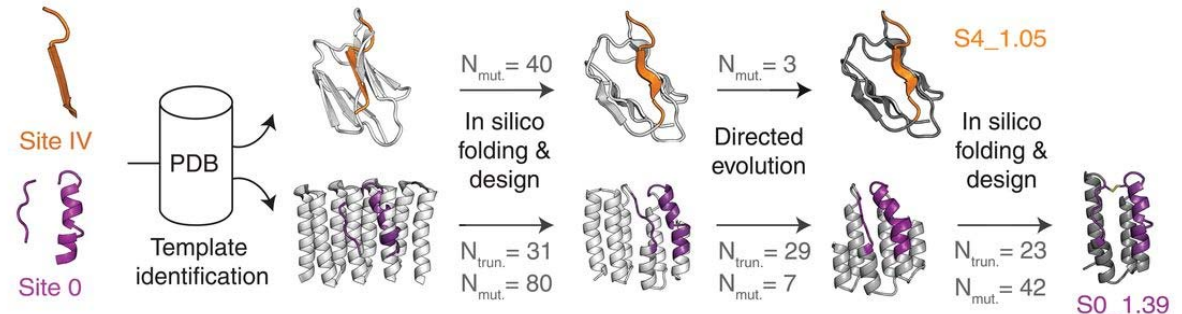


TopoBuilder

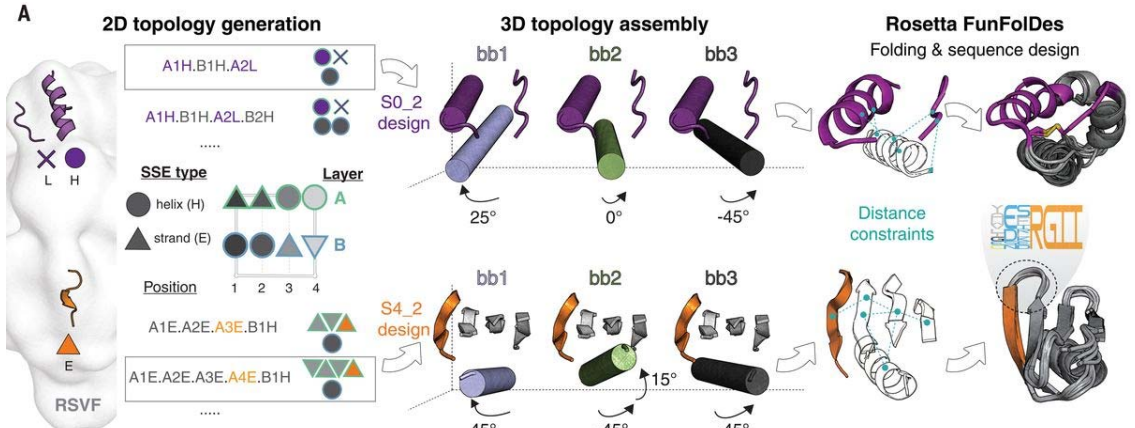


A

Template-based approach:

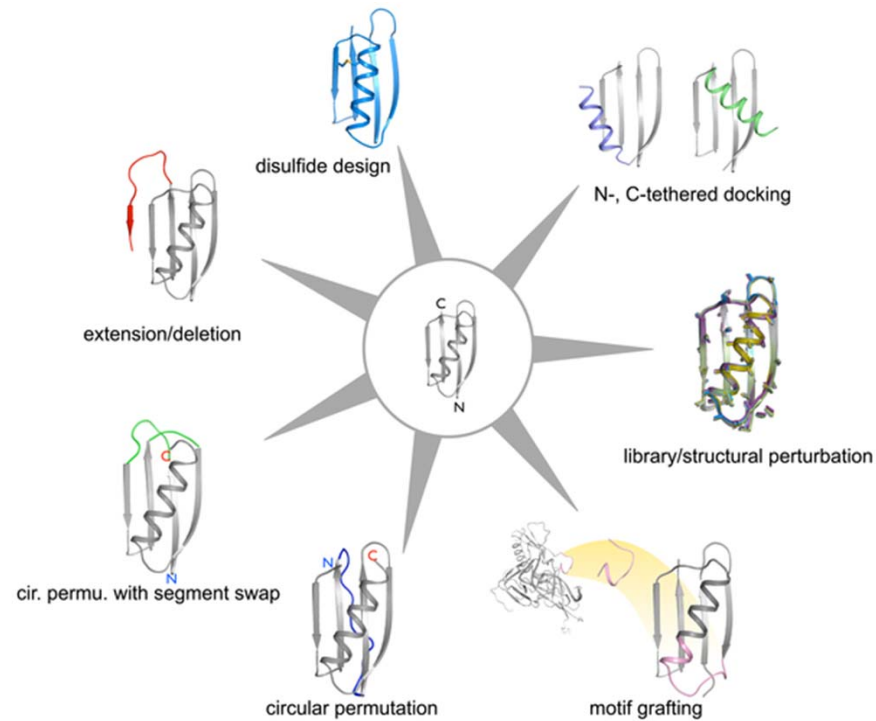


Template-free approach:



Sesterhenn, F.; Yang, C.; Bonet, J.; Cramer, J. T.; Wen, X.; Wang, Y.; Chiang, C. I.; Abriata, L. A.; Kucharska, I.; Castoro, G.; Vollers, S. S.; Galloux, M.; Dheilly, E.; Rosset, S.; Corthésy, P.; Georgeon, S.; Villard, M.; Richard, C. A.; Descamps, D.; Delgado, T.; Oricchio, E.; Rameix-Welti, M. A.; Más, V.; Ervin, S.; Eléouët, J. F.; Riffault, S.; Bates, J. T.; Julien, J. P.; Li, Y.; Jardetzky, T.; Krey, T.; Correia, B. E., De novo protein design enables the precise induction of RSV-neutralizing antibodies. *Science* **2020**, 368 (6492).

RosettaRemodel



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



RosettaRemodel

- 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



Scaffolding protocols – summary

- Redesign of scaffold proteins or *de novo* design of scaffold proteins overcomes the lack of scaffold proteins
- FunFoldes uses the topology of a scaffold protein, but drastically redesigns the scaffold protein sequence
- TopoBuilder is a new method for scaffold protein *de novo* design
- RosettaRemodel provides various methods to modify the scaffold protein



Thank you for your attention!

For questions please contact
clara.schoeder@medizin.uni-leipzig.de

