

Rosetta Scaffolding

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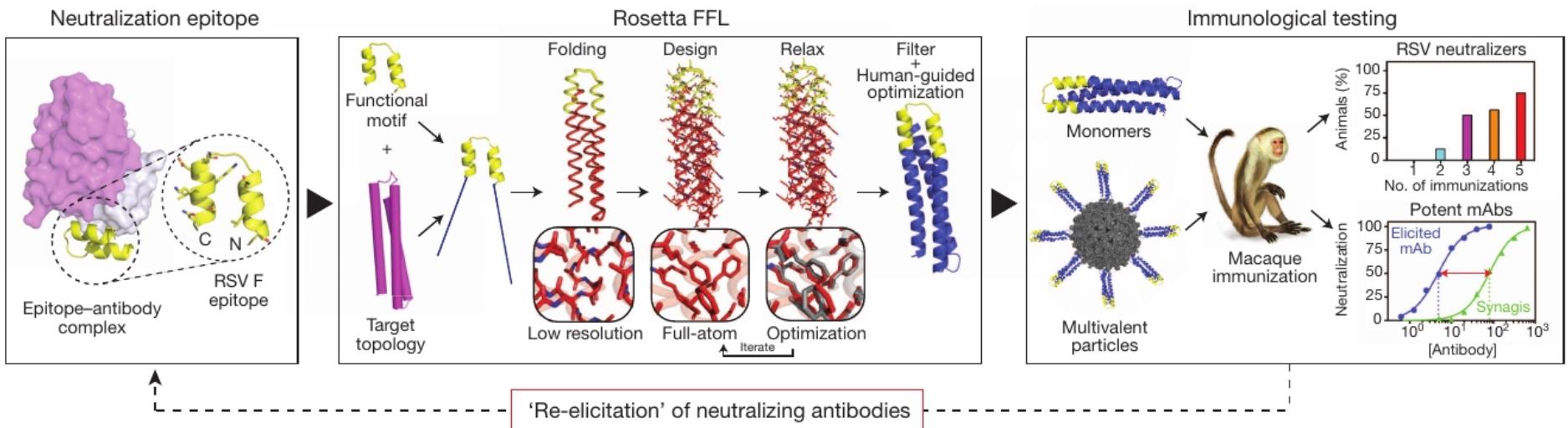
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**Why we need
motif grafting and
scaffold design?**

Why scaffolding?

Engineering therapeutics

- immunogen design
- vaccine design
- antibody design



Why scaffolding?

Engineering therapeutics

- immunogen design
- vaccine design
- antibody design

Stabilizing flexible domains

Adding functions/interaction

**Which options
does Rosetta
offer?**

Rosetta scaffolding:

Rosetta Motif Graft:

1- SideChain graft Rosetta_script (xml file) Scaffold design

2- Backbone graft Rosetta_script (xml file) Scaffold design

Rosetta Functional Folding and Design:

3- FunFoldes Rosetta_script (xml file) Scaffold/Loop design

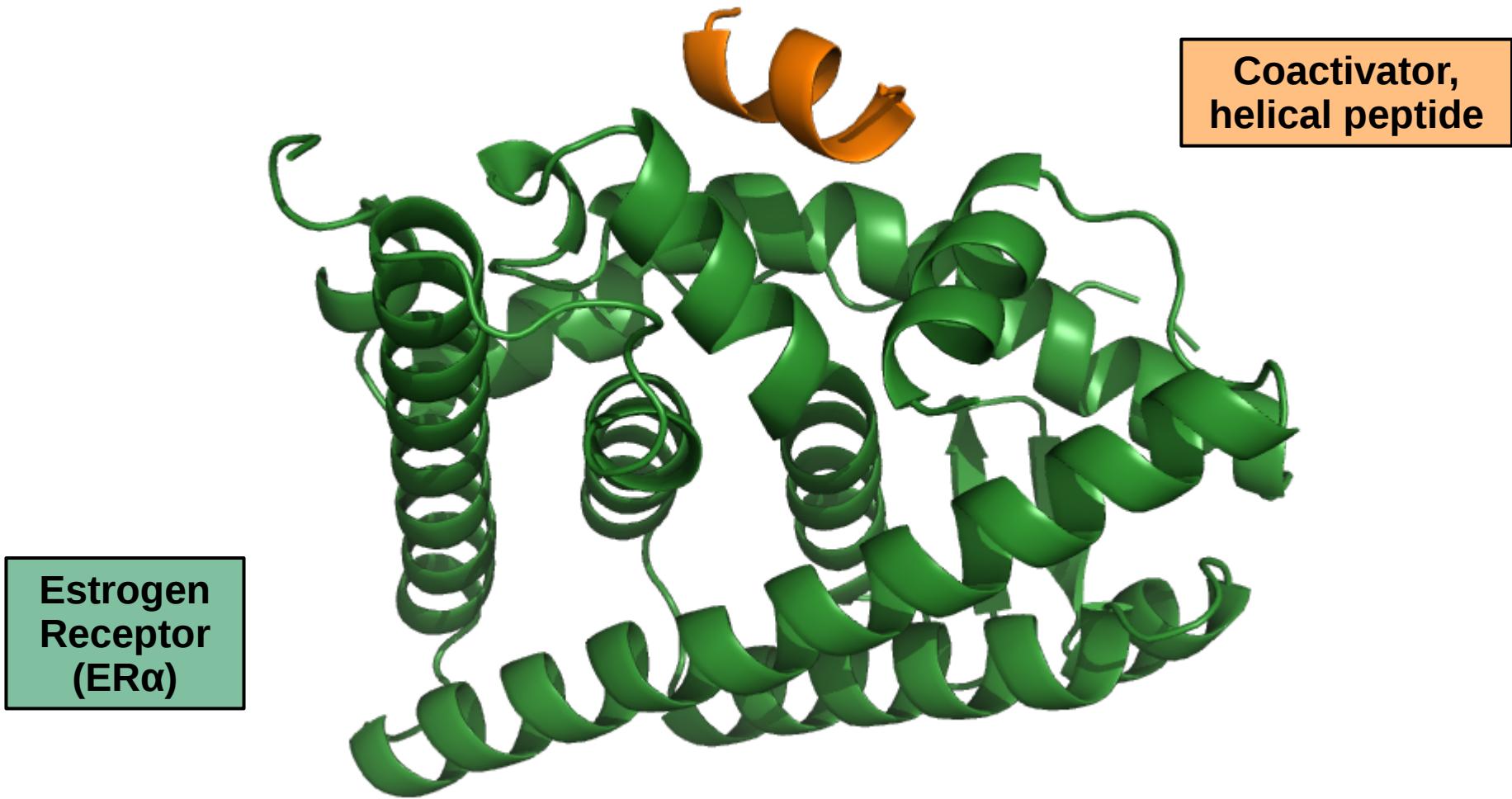
Rosetta Motif Design:

4- RosettaRemodel Rosetta application Scaffold/Backbone design

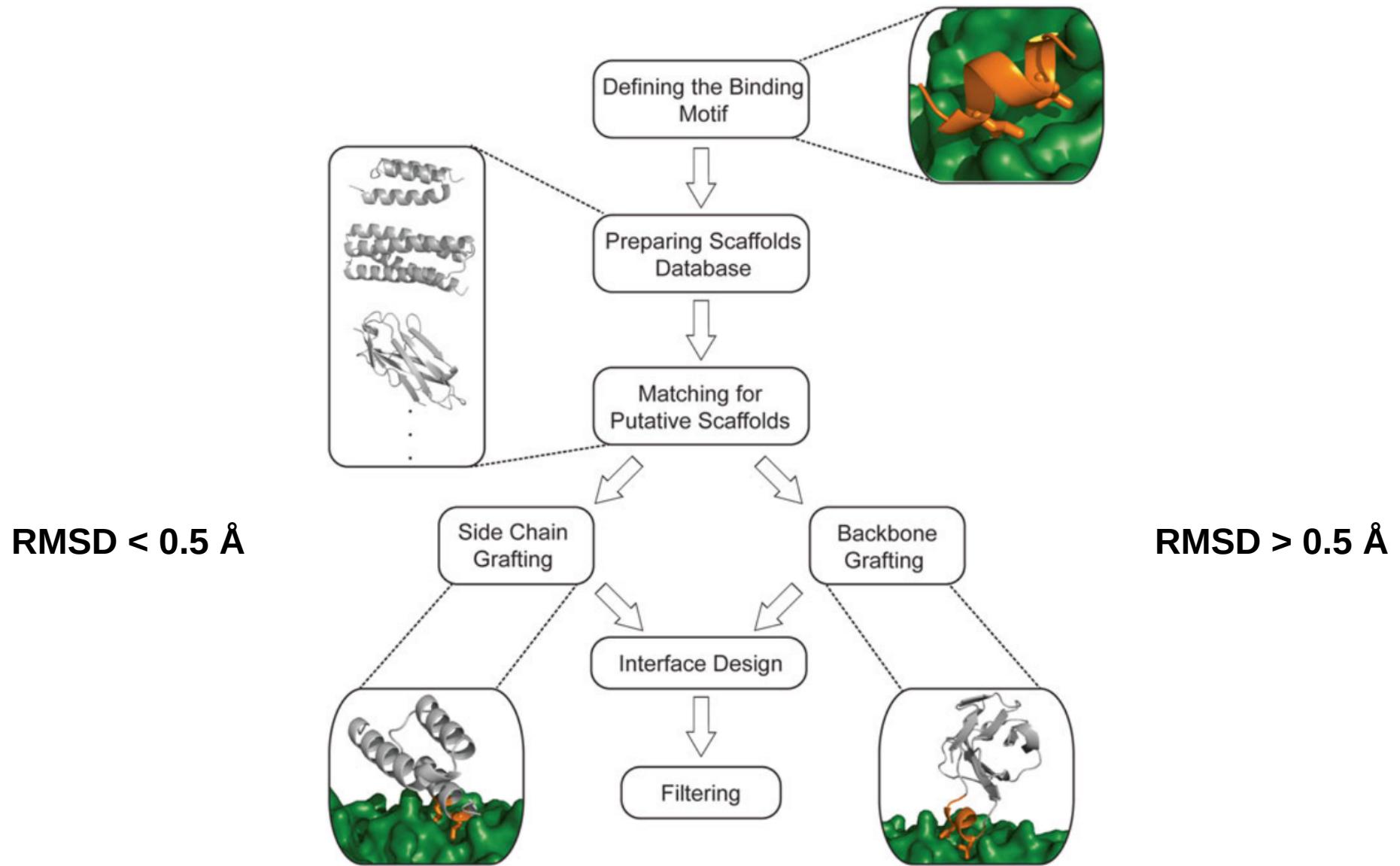
Rosetta Motif Graft

(tutorials 1 and 2)

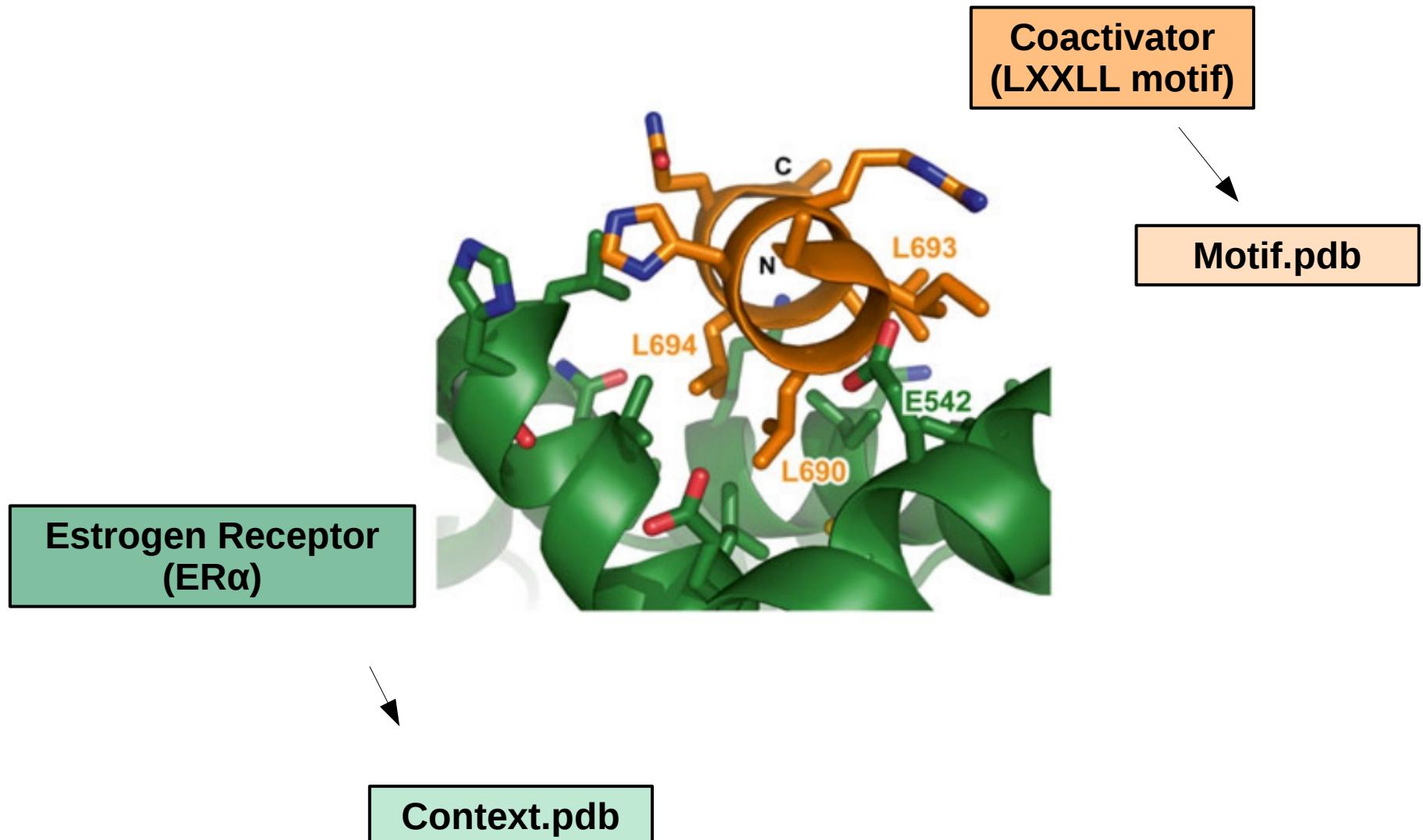
Scaffolding Tutorial:



1 & 2 - Rosetta Grafting:



Defining the Binding Motif:



Scaffold Database:

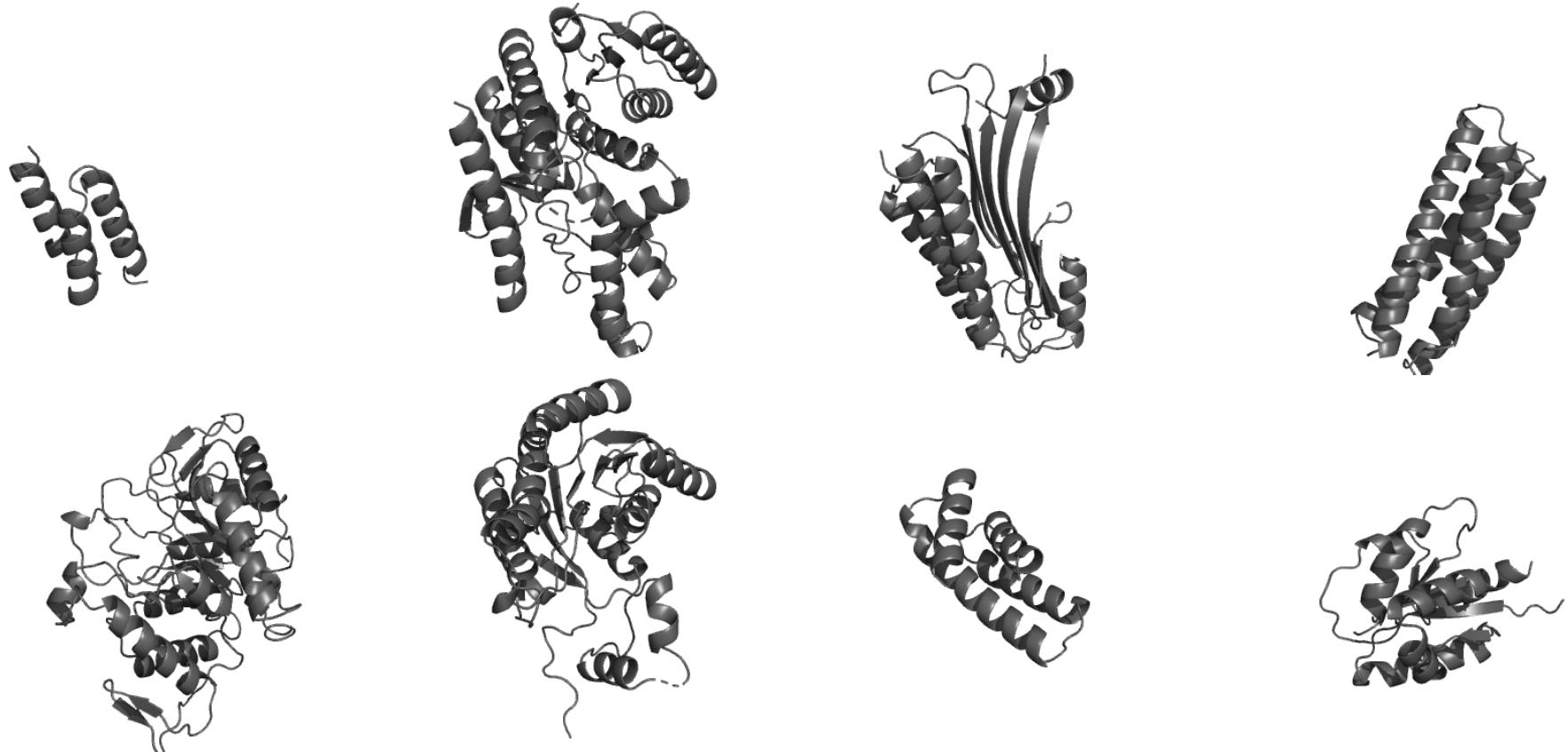
Advanced search from RCSB-PDB:

- 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 in the asymmetric unit
- No bound ligand or modified residues



Scaffold proteins must be energy minimized using Rosetta

Scaffold Database:



Scaffold(s).pdb

1- Side-Chain Grafting

1- Side-Chain Grafting:

Protocol (xml file):

1- Transplant Side-chains

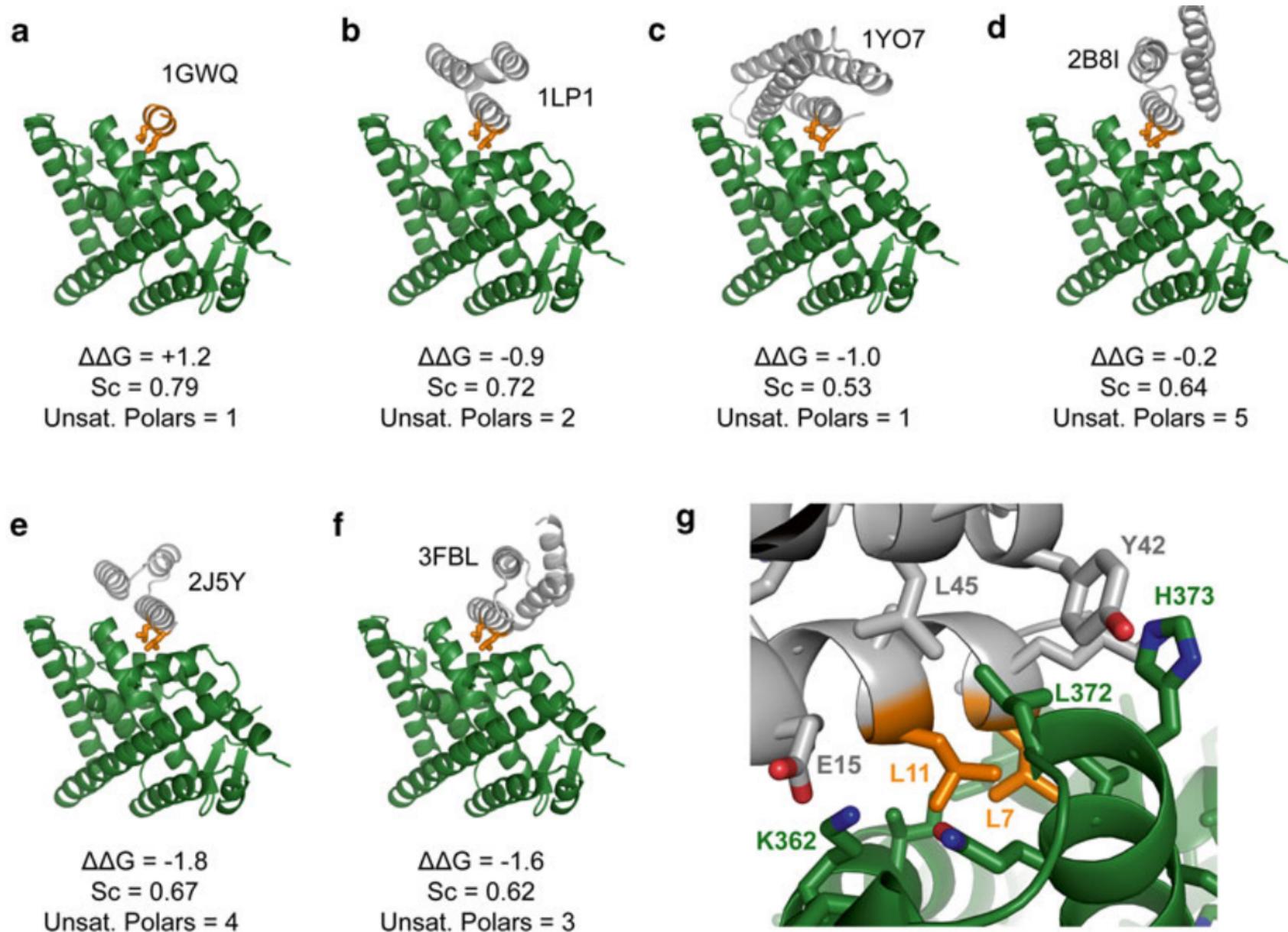
2- Re-design surroundings residues

Filtering (user):

Select best models

- binding energy ($\Delta\Delta G < 0$)
- shape complementarity ($Sc > 0.65$)
- buried unsatisfied H-bonds (low)

1- Side-Chain Grafting:



1- Side-Chain Grafting:

XML file:

```
<ROSETTASCRIPTS>
  <TASKOPERATIONS>
    <ProteinInterfaceDesign name="pid" 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,pid"/>
      <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>
```

2- Backbone Grafting

2- Backbone Grafting:

Protocol (xml file):

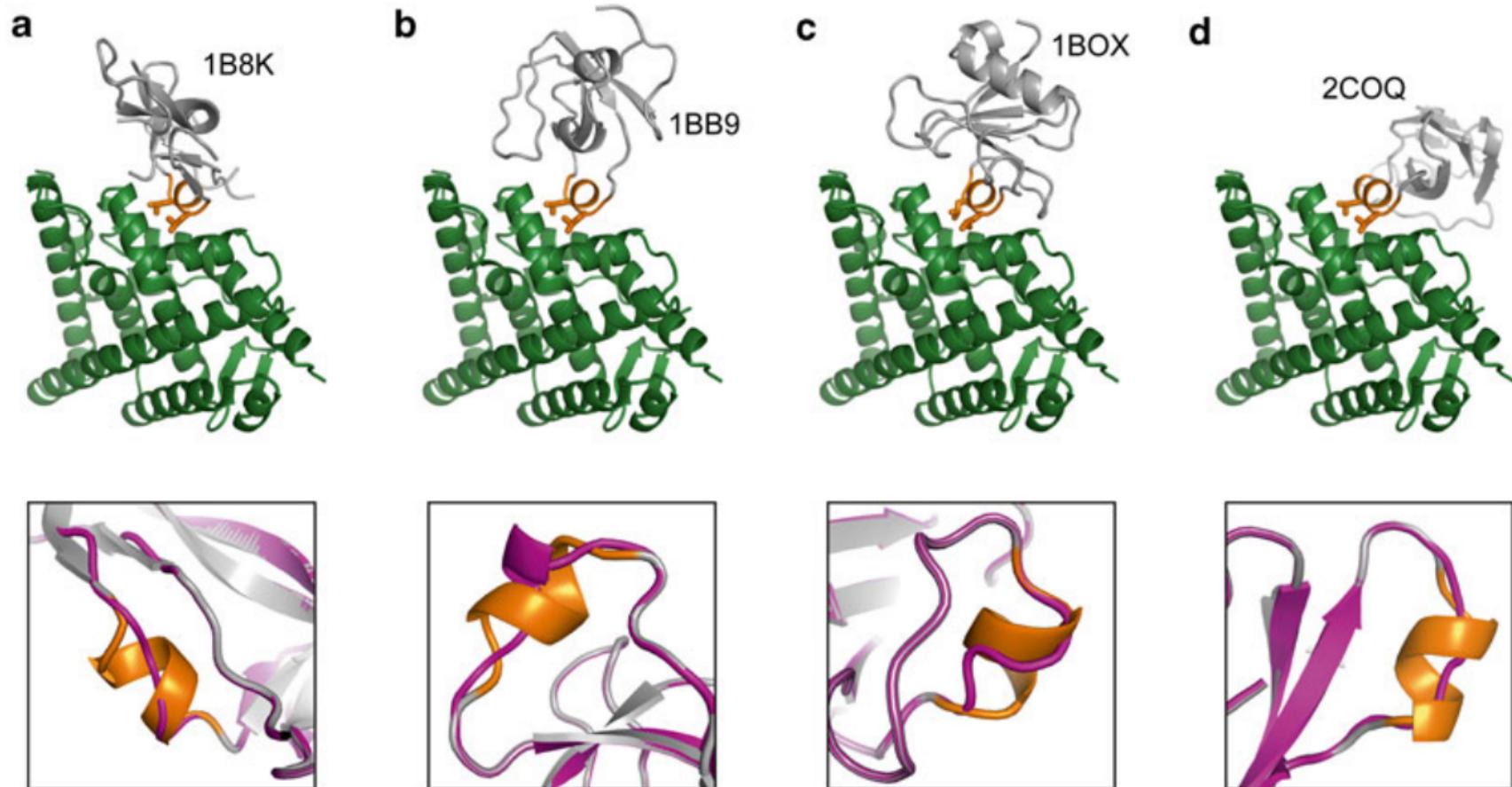
- 1- Remove native scaffold backbone**
- 2- Model new epitope between termini**
- 3- Re-design surrounding residues**

Filtering (user):

Select best models

- binding energy ($\Delta\Delta G < 0$)
- shape complementarity ($Sc > 0.65$)
- buried unsatisfied H-bonds (low)

2- Backbone Grafting:



2- Backbone Grafting:

XML file:

```
<ROSETTASCRIPTS>
<TASKOPERATIONS>
    <ProteinInterfaceDesign name="pid0_far" interface_distance_cutoff="15.0"/>
    <ProteinInterfaceDesign name="pid0_med" interface_distance_cutoff="12.0"/>
    <ProteinInterfaceDesign name="pid0_near" interface_distance_cutoff="8.0"/>
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        <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>
<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="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"/>
    <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>
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    <Add mover_name="sc_min"/>
    <Add filter_name="unsat"/>
    <Add filter_name="ddg"/>
    <Add filter_name="Sc"/>
</PROTOCOLS>
</ROSETTASCRIPTS>
```

Rosetta Motif Graft:

1- Side-Chain Grafting:

WHEN:

- RMSD < 0.5 Å

PRO:

- Minimal number of changes to the scaffold
- Higher chance of correctly folded designs

CONS:

- Limited availability of scaffolds (i.e. weird dihedral angles in the motif can not be fitted in the existing scaffolds)

2- Backbone Grafting:

WHEN:

- RMSD > 0.5 Å
- Similar distance for N- and C- terms

PRO:

- Extremely versatile (different SS and lengths)
- Discontinuous epitopes

CONS:

- Might disrupt overall fold
- Unfavorable mutation in scaffold core

Rosetta FunFoldes

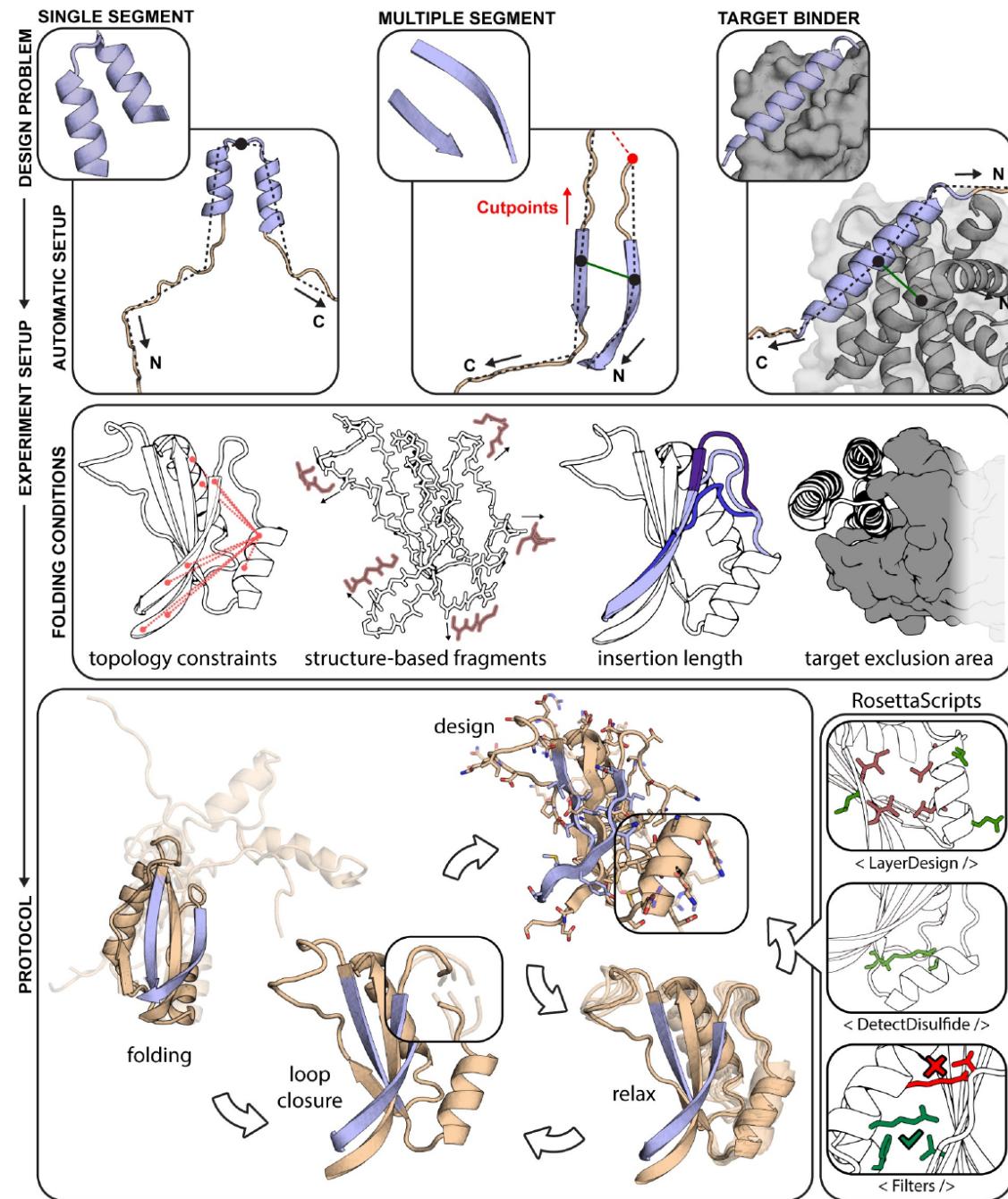
(Tutorial 3)

3- FunFolDes:

(derived from Fold From Loops (FFL))

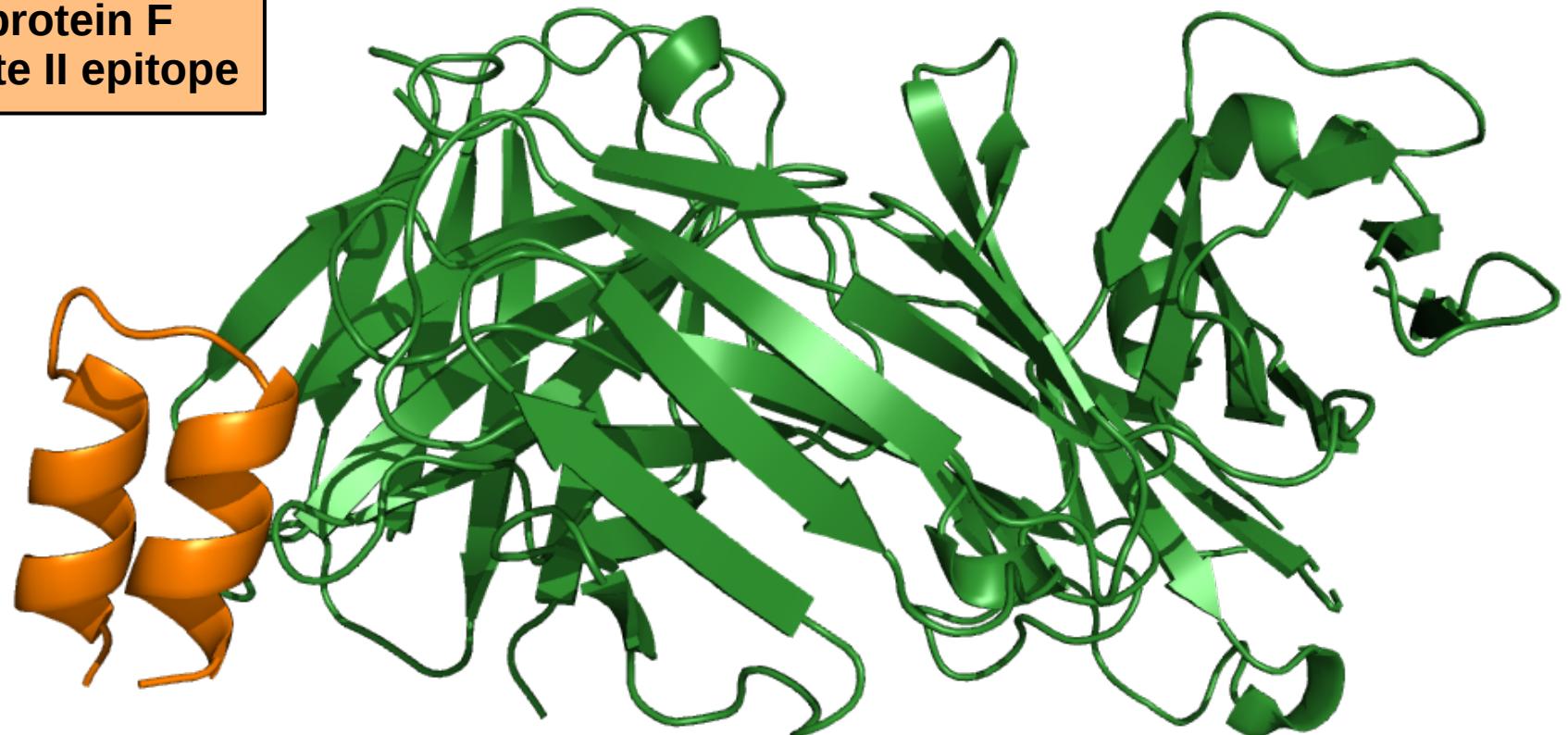
- intended for scaffold with low structural similarity
- accepts multiple discontinuous backbone segments
 - can swap them if necessary!
- accepts binding partners (including small ligand!)
- allows flexibility across the overall structure
- couple folding and design:
 - 1- Motif is inserted in the scaffold and kept fixed (static)
 - 2- Scaffold is folded through fragment picking
 - 3- Model is accepted if RMSD < threshold (defined by user)
 - 4- Sequence design and relax

3- FunFoldes:



The Motif:

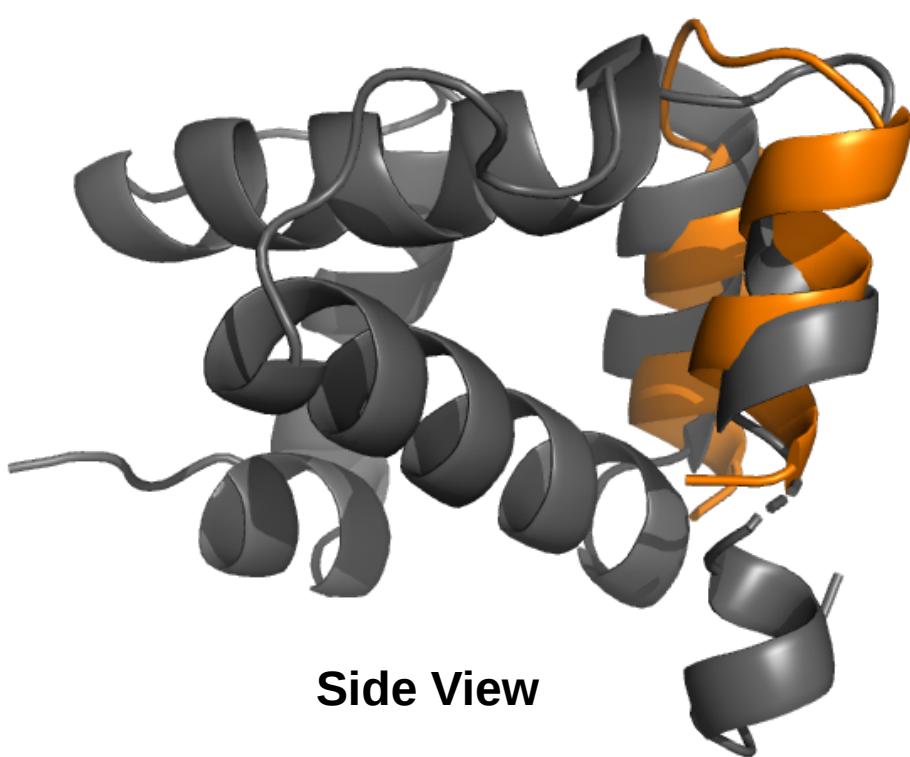
Respiratory Syncytial
Virus protein F
(RSVF) site II epitope



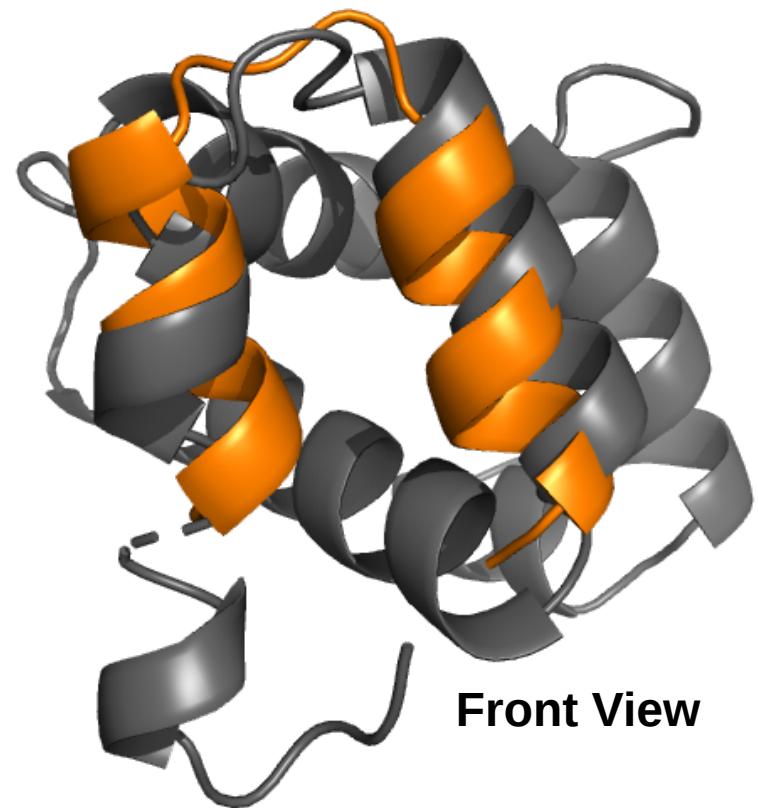
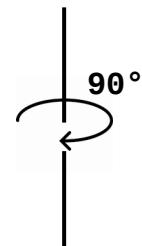
$K_D = 20 \text{ nM}$

Antibody
motavizumab
(mota)

The Scaffold:



Side View



Front View

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



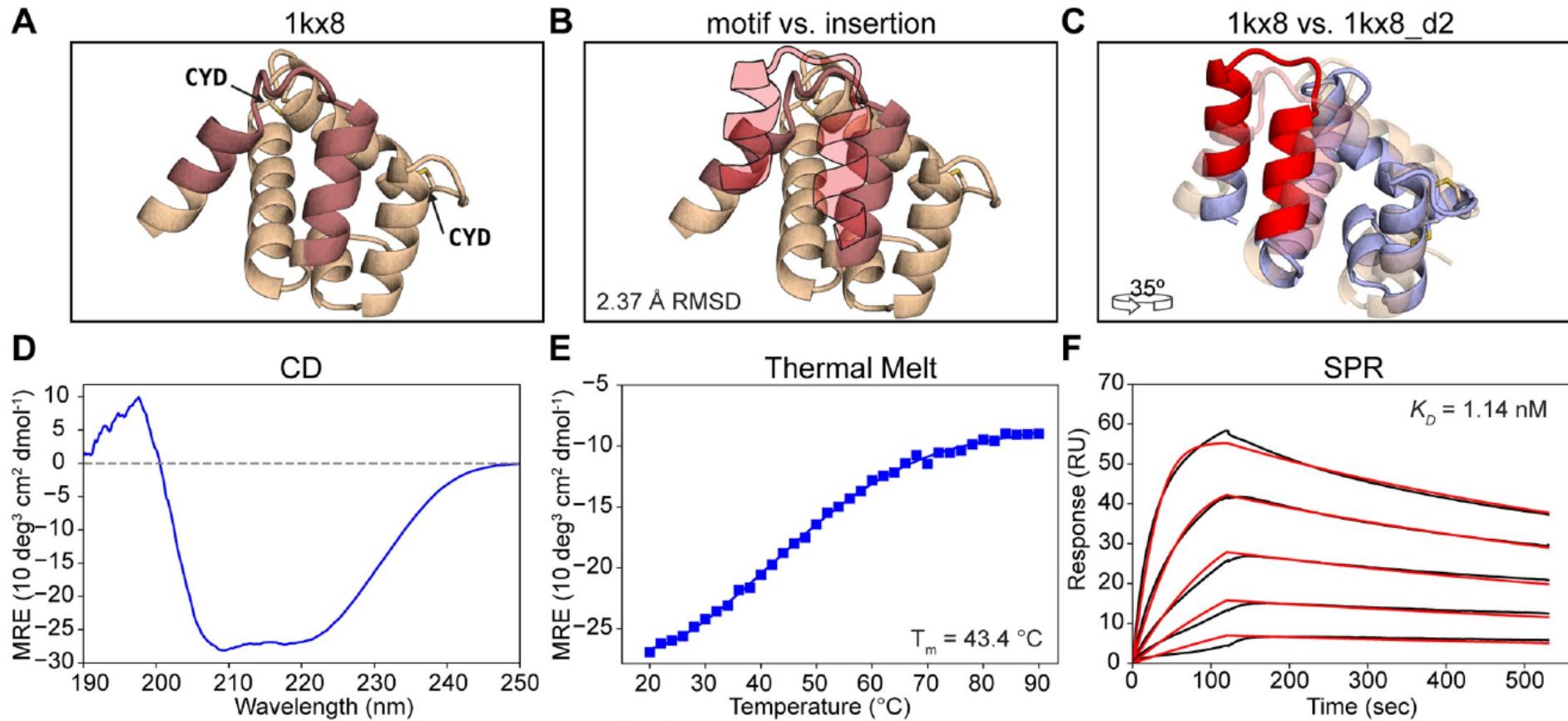
RMSD = 2.37 Å

3- FunFolDes:

Protocol (xml file):

- 1- Generate fragment database for scaffold**
- 2- Folding of the scaffold**
- 3- Re-design surrounding residues**
- 4- Select best models**
 - RMSD & Total score
 - Binding energy
 - buried unsatisfied H-bonds
 - Holes score / Core packing

3- FunFoldes:



3- FunFolDes:

1- Side-Chain Grafting:

WHEN:

- RMSD < 0.5 Å

PRO:

- Minimal number of changes to the scaffold
- Higher chance of correctly folded designs

CONS:

- Limited availability of scaffolds (i.e. weird dihedral angles in the motif can not be fitted in the existing scaffolds)

2- Backbone Grafting:

WHEN:

- RMSD > 0.5 Å
- Similar distance for N- and C- terms

PRO:

- Extremely versatile (different SS and lengths)
- Discontinuous epitopes

CONS:

- Might disrupt overall fold
- Unfavorable mutation in scaffold core

3- FunFolDes (ex FFL):

WHEN:

- High RMSD

PRO:

- Extremely versatile (different SS and lengths)
- Discontinuous epitopes
- Allows ligands/DNA/RNA
- Higher rate of folded proteins in experiments

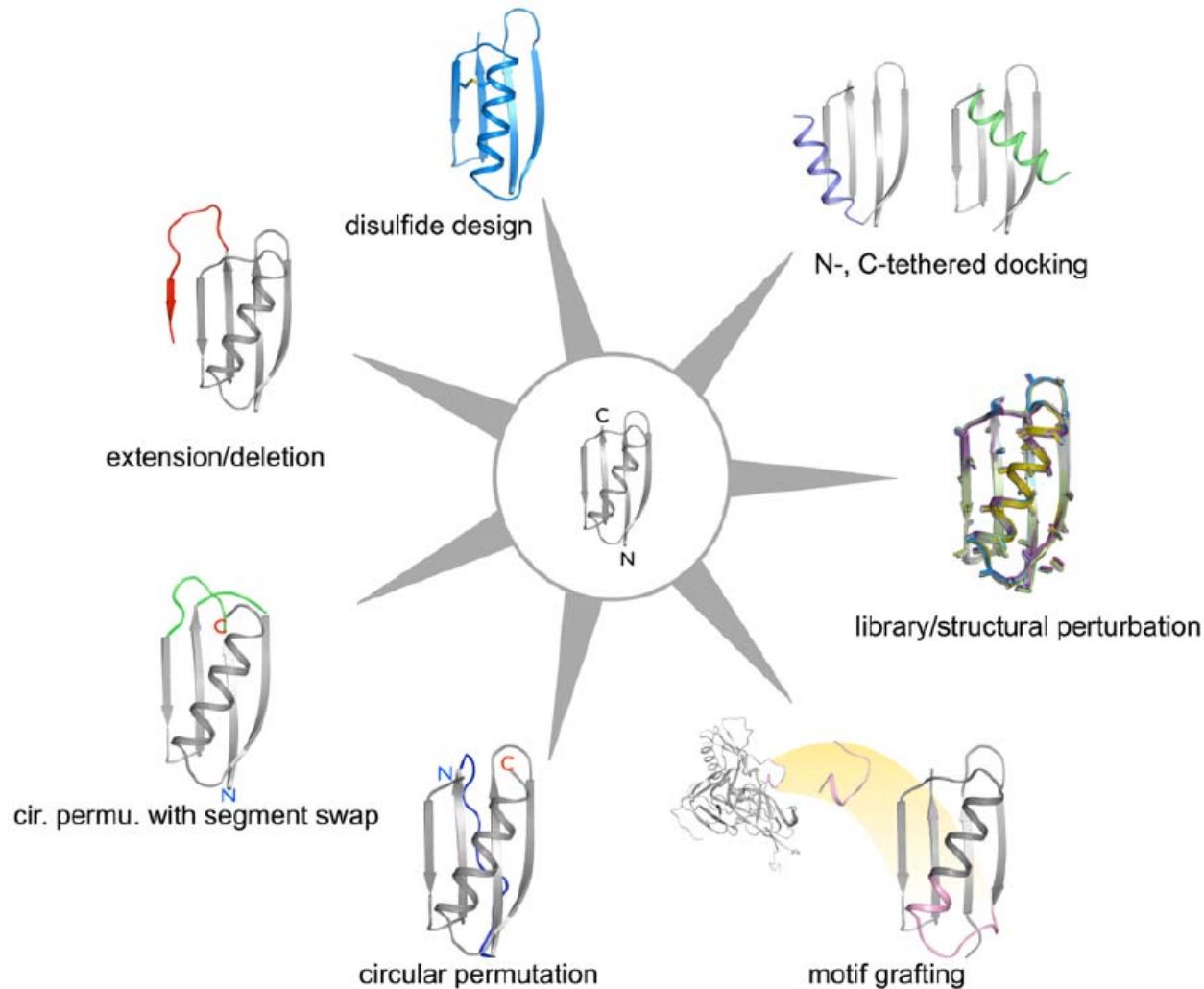
CONS:

- Computationally more expensive in time and CPU

RosettaRemodel

(optional)

3 - RosettaRemodel:



Blueprint file:

```
4 I . ALLAA
5 L H
0 X H
6 N H
7 G .
9 F . PIKAA R
```

References:

Rosetta Motif Graft:

- Silva, D. et al. (2016) **Motif-driven Design of Protein-Protein Interactions**. *Methods Mol. Biol.* DOI: [10.1007/978-1-4939-3569-7_17](https://doi.org/10.1007/978-1-4939-3569-7_17)
- Azoitei, M.L. et al. (2012) **Computational Design of High-Affinity Epitope Scaffolds by Backbone Grafting of a Linear Epitope**. *J. Mol. Biol.* DOI: [10.1016/j.jmb.2011.10.003](https://doi.org/10.1016/j.jmb.2011.10.003)
- Azoitei, M.L. et al. (2011) **Computation-guided backbone grafting of a discontinuous motif onto a protein scaffold**. *Science*. DOI: [10.1126/science.1209368](https://doi.org/10.1126/science.1209368)

Rosetta FunFolDes:

- Bonet, J. et al. (2018) **Rosetta FunFolDes - A general framework for the computational design of functional proteins**. *PLOS Computational Biology*. DOI: [10.1371/journal.pcbi.1006623](https://doi.org/10.1371/journal.pcbi.1006623)
- Correia, B.E. et al. (2014) **Proof of principle for epitope-focused vaccine design**. *Nature*. DOI: [10.1038/nature12966](https://doi.org/10.1038/nature12966)

RosettaRemodel:

- Huang, P.H. et al. (2011) **RosettaRemodel: A generalized framework for flexible backbone protein design**. *PLoS One*. DOI: [10.1371/journal.pone.0024109.g001](https://doi.org/10.1371/journal.pone.0024109.g001)