Scaffolding and Epitope Grafting

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Why design scaffolds?

- Transplantation of the functional sites onto another protein

Example: put an antibody specific epitope onto a new protein (epitope focused vaccine design) or design other functional proteins

- 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

Various Scaffolding Methods in Rosetta

1. Side Chain Grafting
2. Backbone Grafting

Rosetta XML scripts

3. Fold From Loops
4. Rosetta Remodel

Rosetta Applications

Side Chain and Backbone Grafting

Motif-Driven Design of Protein–Protein Interfaces

Daniel-Adriano Silva, Bruno E. Correia, and Erik Procko

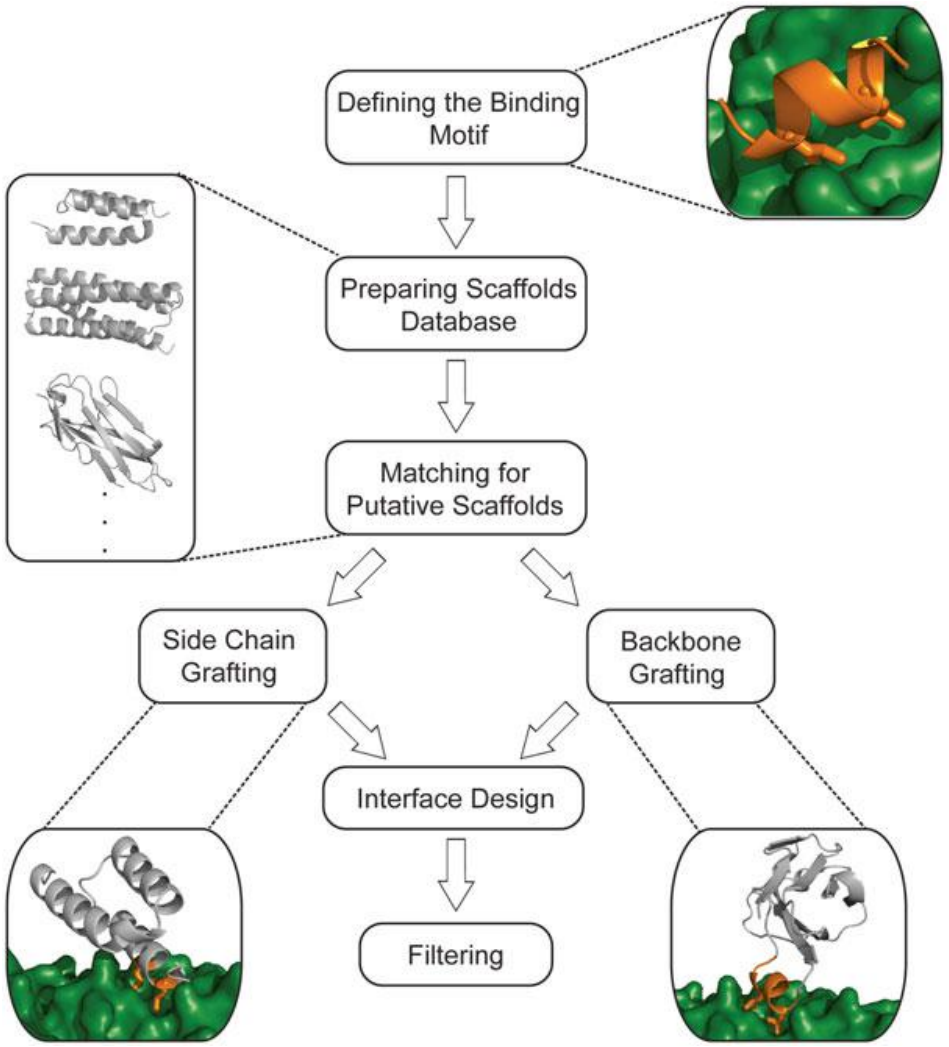
Abstract

Protein–protein interfaces regulate many critical processes for cellular function. The ability to accurately control and regulate these molecular interactions is of major interest for biomedical and synthetic biology applications, as well as to address fundamental biological questions. In recent years, computational protein design has emerged as a tool for designing novel protein–protein interactions with functional relevance. Although attractive, these computational tools carry a steep learning curve. In order to make some of these methods more accessible, we present detailed descriptions and examples of ROSETTA computational protocols for the design of functional protein binders using seeded protein interface design. In these protocols, a motif of known structure that interacts with the target site is grafted into a scaffold protein, followed by design of the surrounding interaction surface.

Methods Mol Biol. 2016;1414:285-304. doi: 10.1007/978-1-4939-3569-7_17.

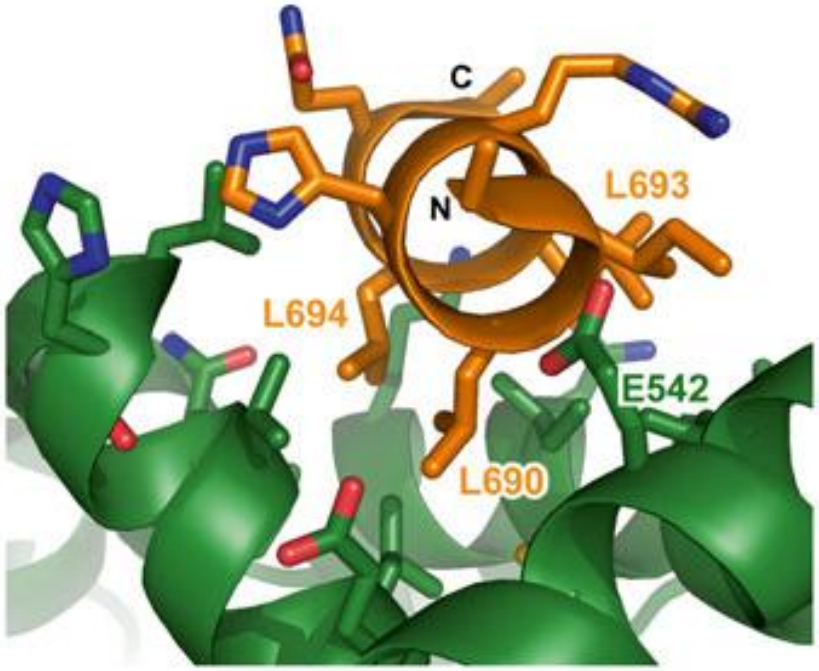
https://www.ncbi.nlm.nih.gov/pubmed/27094298

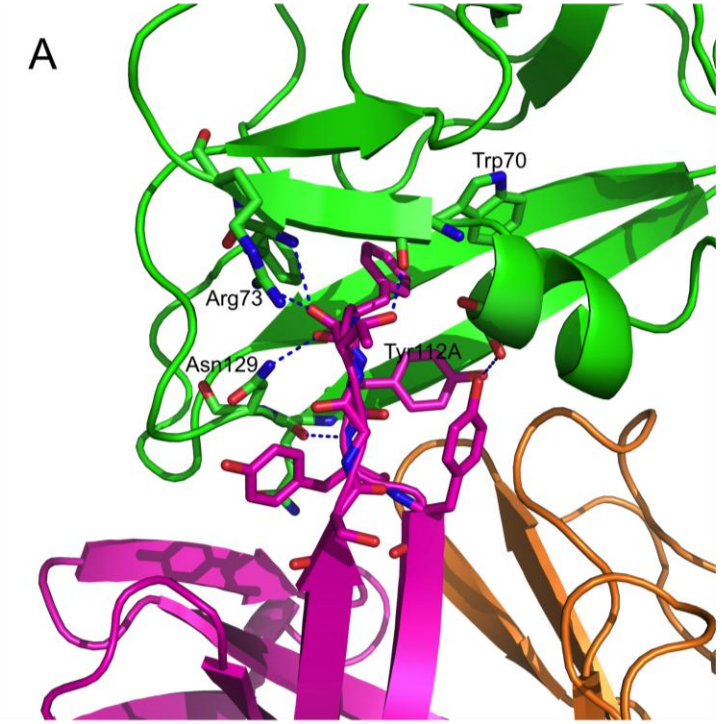
Side Chain & Backbone Grafting



Structure (2010), 18, 1116-1126.

The Functional/Binding Motif





Preparing Scaffold Database

- Crystal structures with high-resolution x-ray diffraction data (<2.5 Å)
- Protein has been reported to be expressed in *E. Coli*
- Single protein chain in the asymmetric unit (monomeric scaffolds as grafting targets)
- No bound ligand or modified residues

Scaffold PDBs are then energy minimized using Rosetta

Note: A motif focused library may be more useful.

Matching for Putative Scaffolds for Side Chain Grafting

- Choose PDBs where Motif and Scaffold backbones superimpose with very low RMSD (<0.5 Å)
- Transplantation of side chains from functional motif onto scaffold
- Design the surrounding residues on the scaffold surface

Pros	Cons
Minimal number of changes in the scaffold Increased chances of correctly folded designs	Often motif and scaffold structures are too dissimilar, limited availability of scaffolds.

Side Chain Grafting

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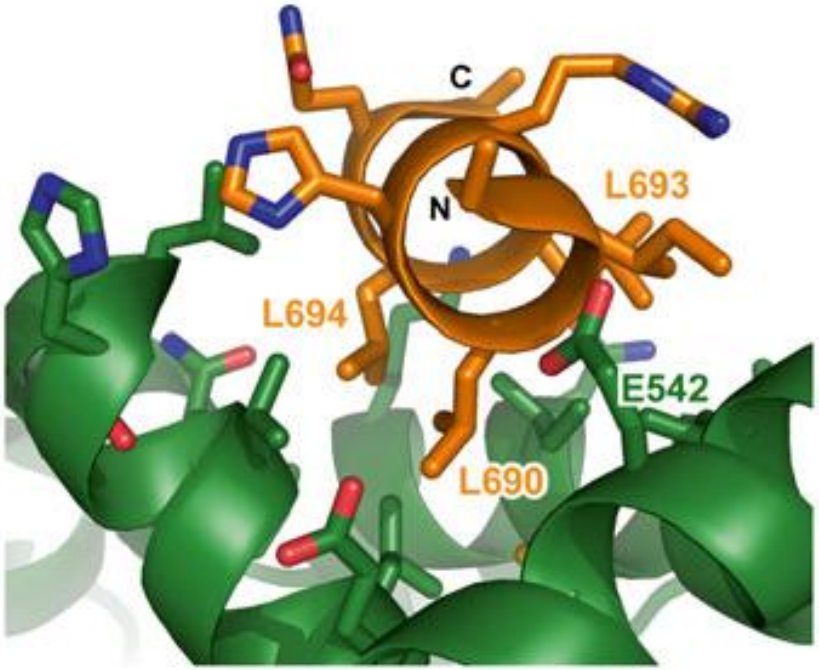
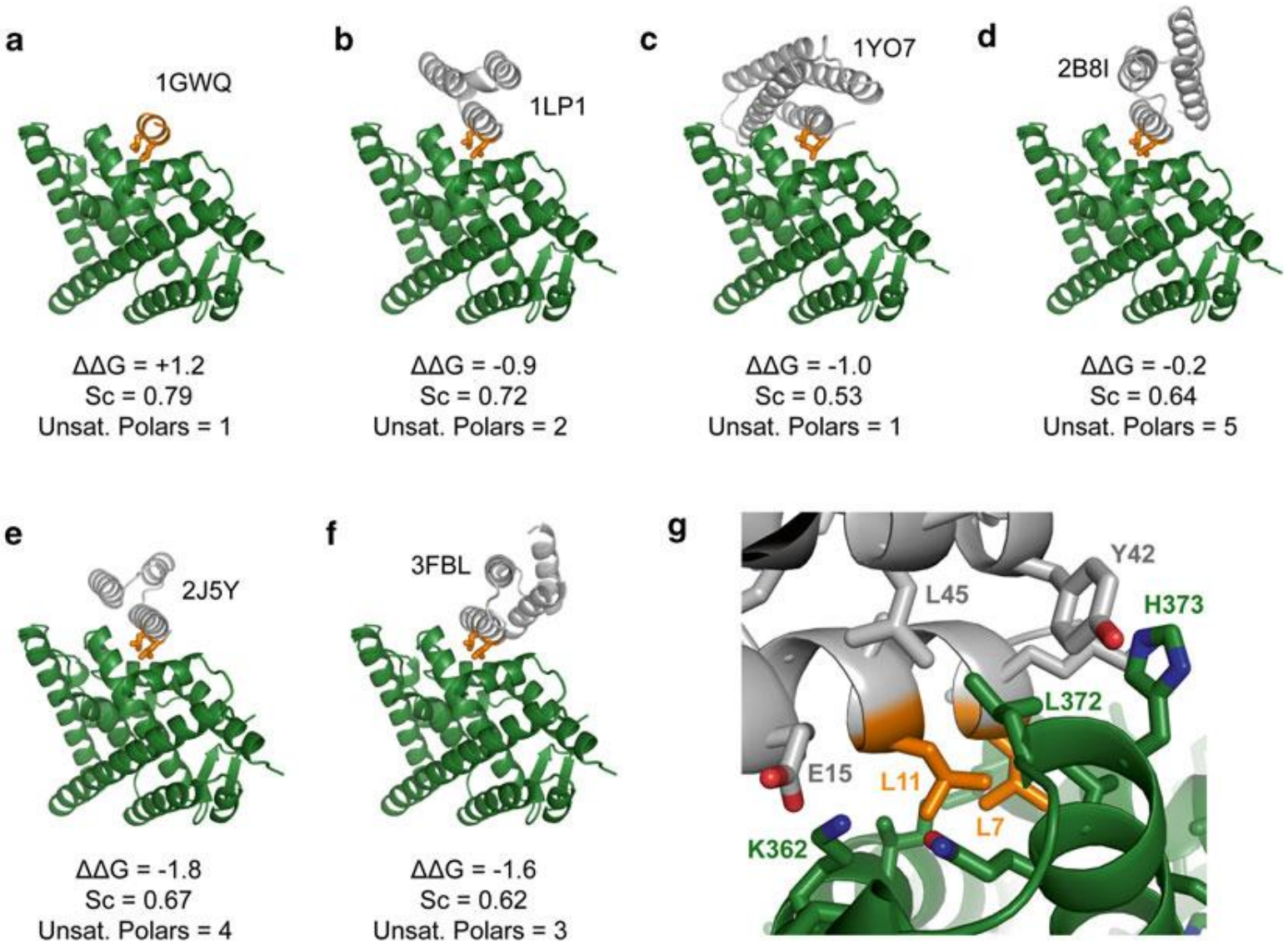


Peptide structure contains LXXLL motif; necessary for interaction.

Side Chain Grafting

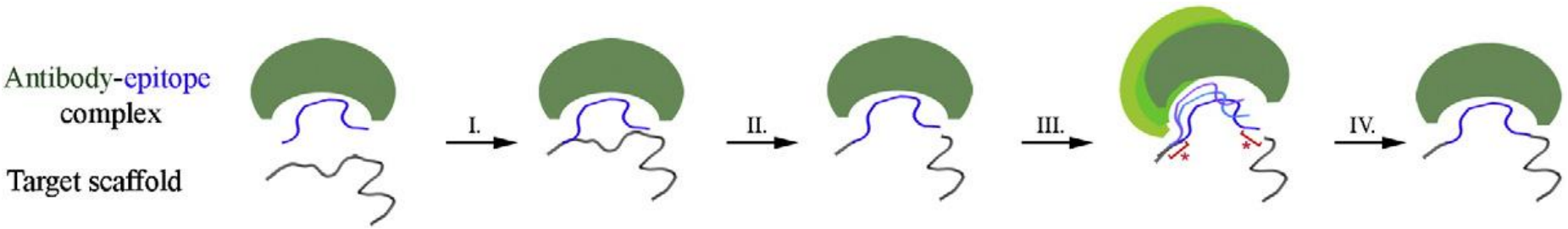


Backbone Grafting

- 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 scaffold; redesign the hydrophobic core along with interface Introduces too many mutations to the scaffold Careful filtering of designs

Backbone Grafting



J. Mol. Biol. (2012) 415, 175-192.

Backbone Grafting

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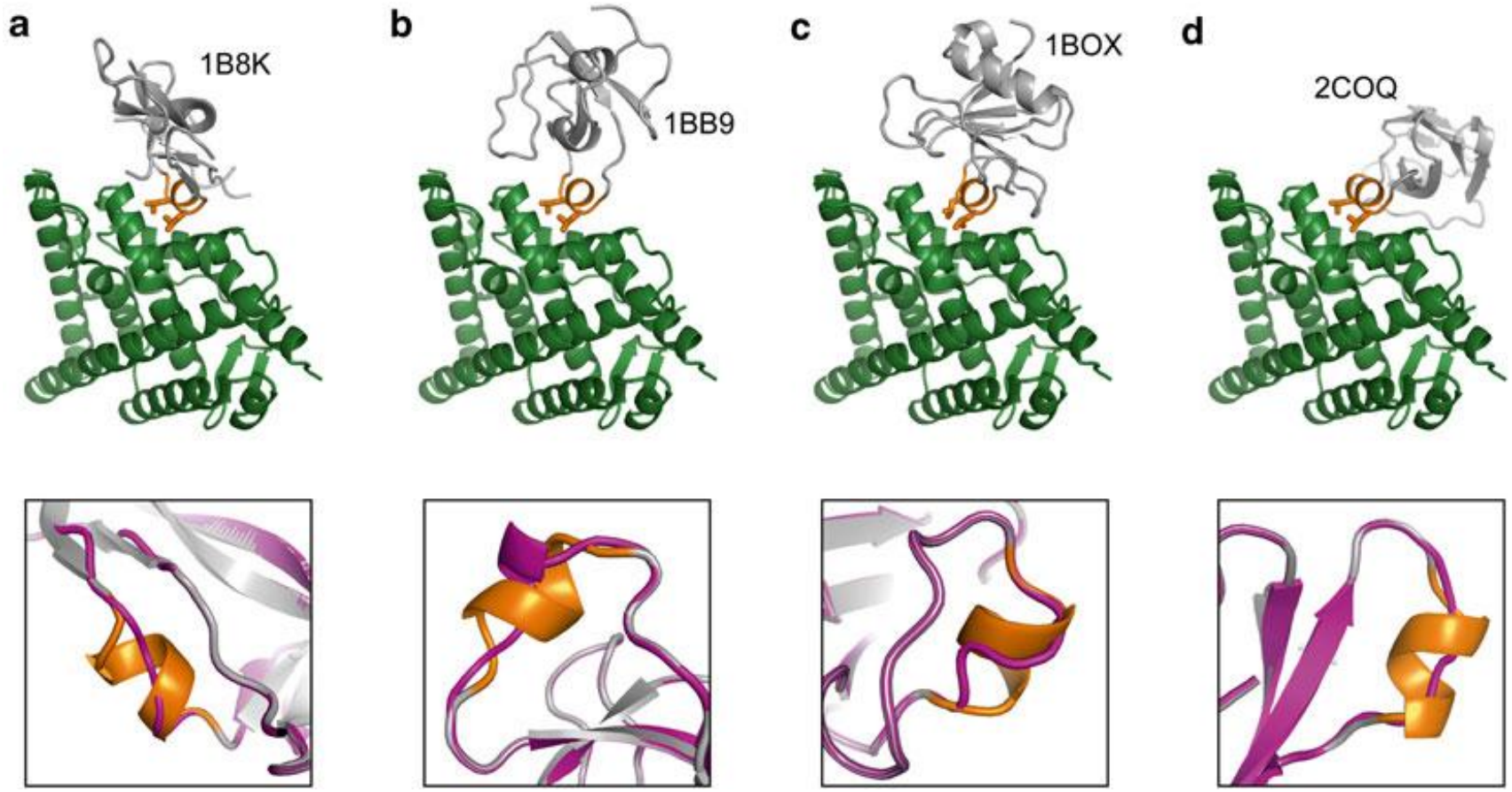
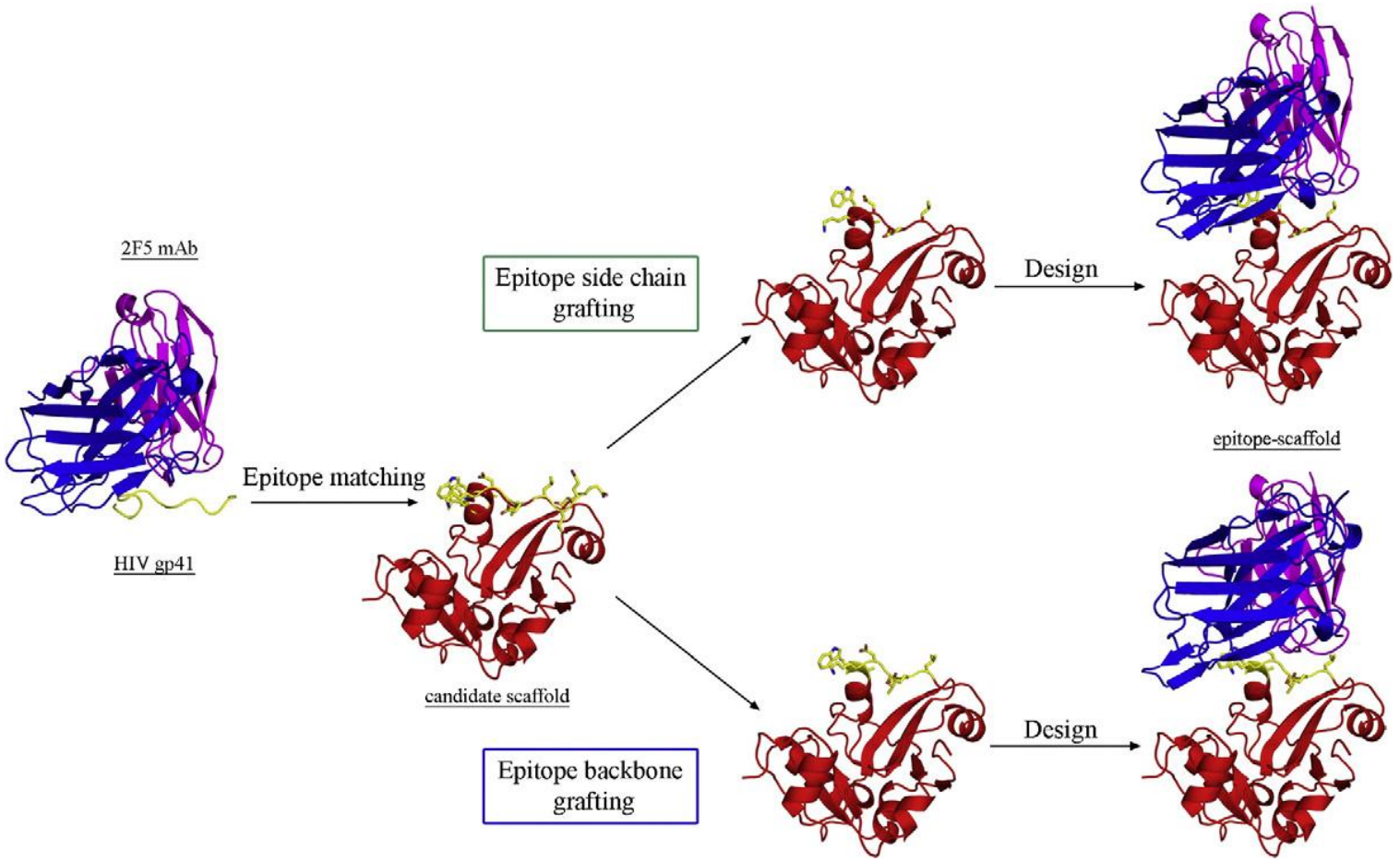
Backbone Grafting



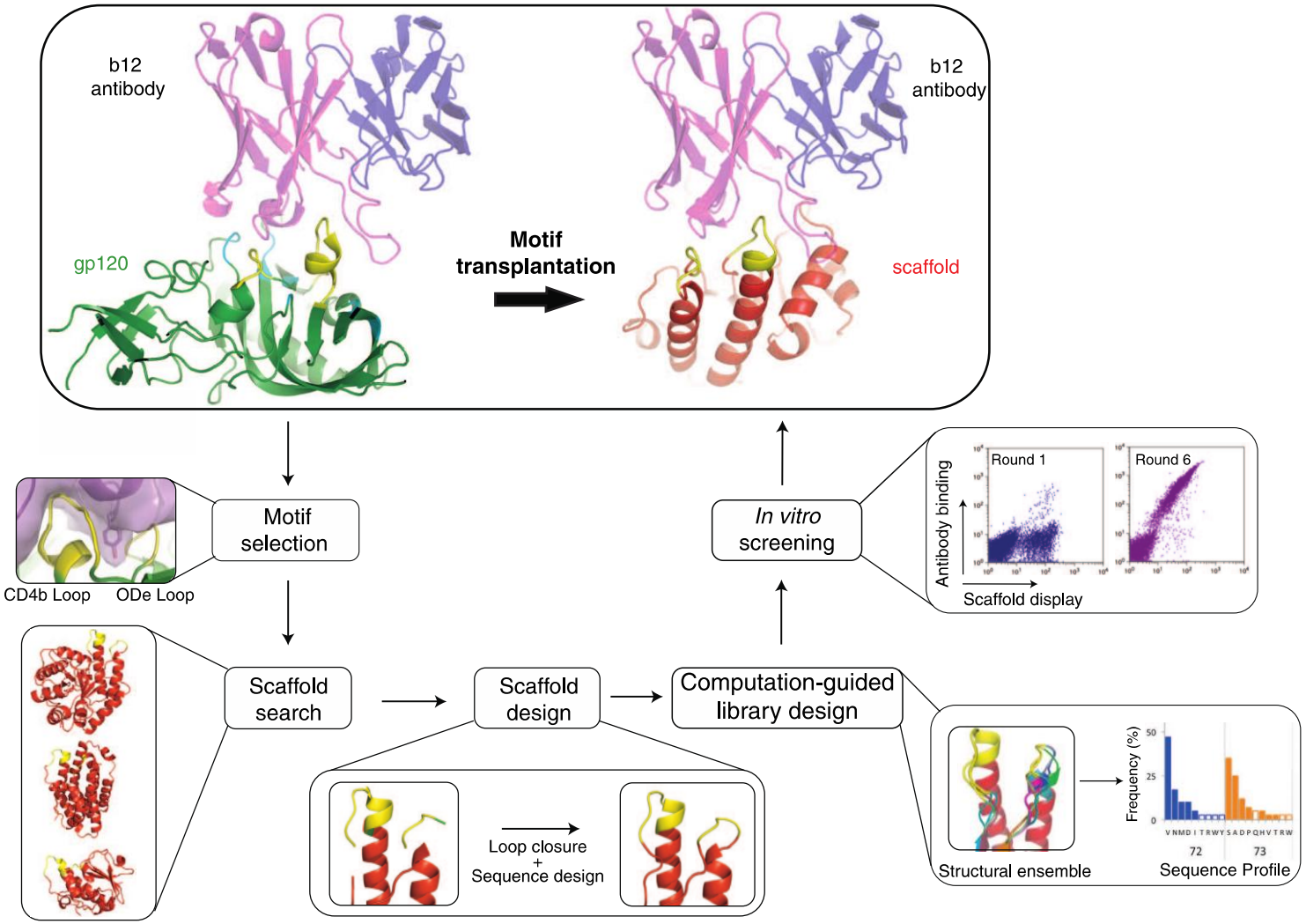
Side Chain *vs* Backbone Grafting



J. Mol. Biol. (2012) 415, 175-192.

But most of the antibody epitopes are discontinuous (involving two or more antigen segments)

Grafting a discontinuous motif using Backbone Grafting



Science (2011), 334, 373-376.

Selection of Designs and Optimization

- Favorable binding energy ddG
- High shape complementarity
- Low number of buried unsatisfied H-bonding atoms

Defects

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

Selection of Designs and Optimization

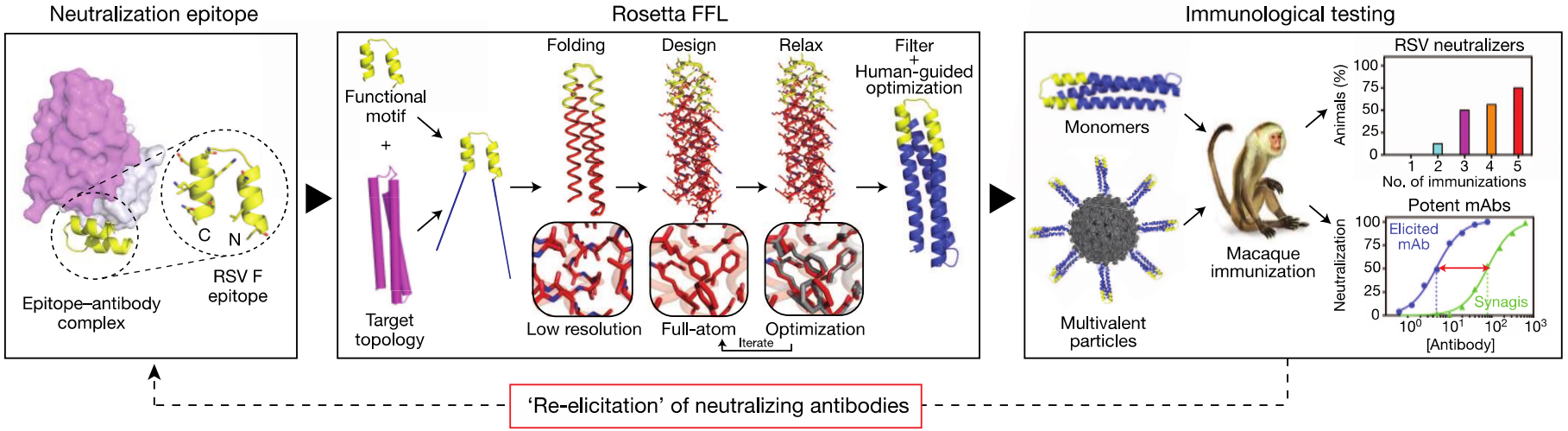
- Insert as few mutations as possible because probability of a designed sequence to properly fold is inversely correlated with the number of mutations imposed on the scaffold during design process
- Check if the design is “stable” using energy funnel.
- Manually adjust designs

Fold From Loops (FFL)

- Generate motif scaffolds in the presence of a binder to have configuration that better suits the interaction. If you don't have a binder, no problem!
- Multi-segment or discontinuous motif can be put into a scaffold using multi-loop file.
- Motif does not have to be of same length as segment being replaced in the scaffold protein.

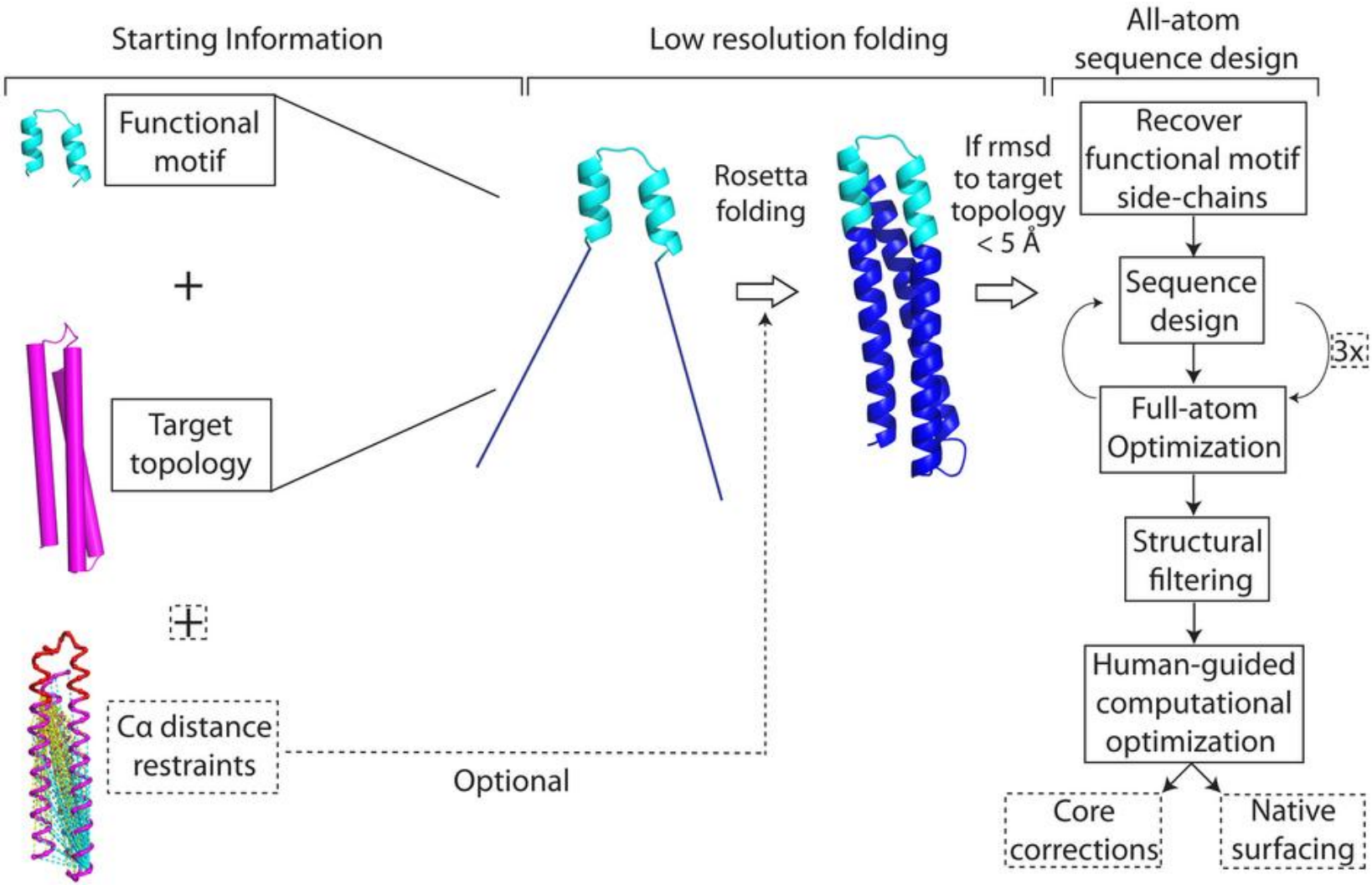
- RosettaScripts not available yet!
- Not in the master branch yet!

FFL Example: RSV Vaccine Design



Nature (2014), 507, 201-206.

FFL Example: RSV Vaccine Design



Rosetta Remodel

RosettaRemodel: A Generalized Framework for Flexible Backbone Protein Design

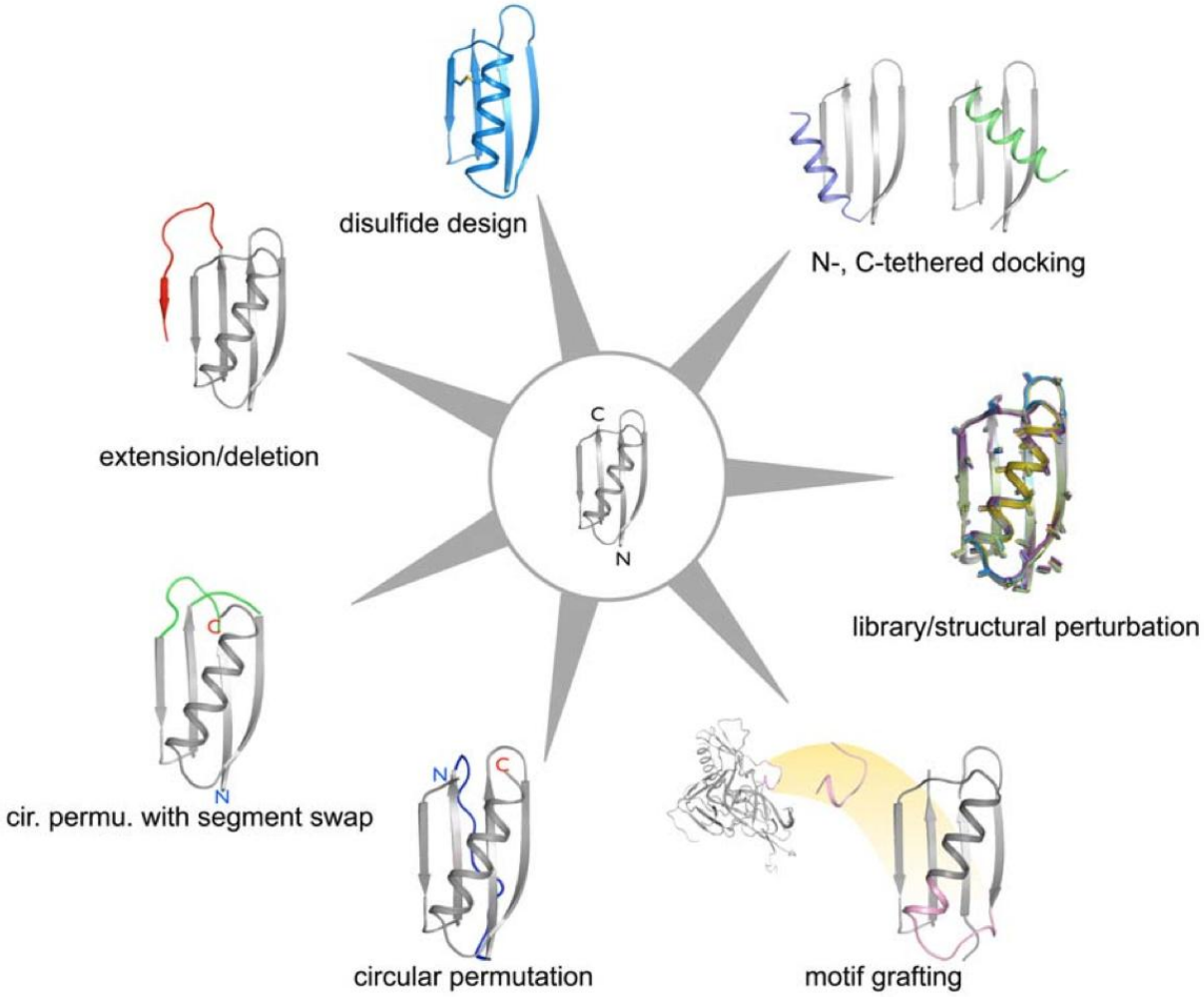
Po-Ssu Huang[1], Yih-En Andrew Ban[1¤a], Florian Richter[1,2], Ingemar Andre[3], Robert Vernon[4], William R. Schief[1*¤b], David Baker[1,5*]

Abstract

We describe RosettaRemodel, a generalized framework for flexible protein design that provides a versatile and convenient interface to the Rosetta modeling suite. RosettaRemodel employs a unified interface, called a blueprint, which allows detailed control over many aspects of flexible backbone protein design calculations. RosettaRemodel allows the construction and elaboration of customized protocols for a wide range of design problems ranging from loop insertion and deletion, disulfide engineering, domain assembly, loop remodeling, motif grafting, symmetrical units, to *de novo* structure modeling.

doi:10.1371/journal.pone.0024109.g001

Rosetta Remodel



doi:10.1371/journal.pone.0024109.g001