

User's guide for Rosetta alter_spec mode.

Author: Brian Kuhlman, August 2004, bkuhlman@email.unc.edu

This protocol is an automated version of the second-site suppressor strategy recently used by Kortemme et al. (NSB, 2004) to create orthogonal binding interactions.

Goal: redesign two interacting proteins so that:

$\text{Binding Energy}_{\text{Mut-Mut}} \sim \text{Binding Energy}_{\text{WT-WT}} \ll \text{Binding Energy}_{\text{WT-Mut}} \sim \text{Binding Energy}_{\text{Mut-WT}}$

Protocol:

1. Loop through all possible point mutants at the interface and identify mutations that weaken binding. Neighboring residues are allowed to repack to accommodate the point mutations.
2. For each destabilizing mutation redesign the surrounding residues to better accommodate the mutation. Output a file (mutlist) that contains all the point mutations and the compensating mutations.
3. Calculate the binding energies of WT-WT, WT-Mut, Mut-WT and Mut-Mut for each redesign using interface mode with the alter_spec flag. The results are sorted so the most promising redesigns are at the top.

Sample command lines, this protocol requires 2 steps:

1) `rosetta.gcc -s 1c4z.pdb -design -alter_spec -pmut pmut63 -alter_spec_mutlist mut63c -fix fix63n`

`-alter_spec_mutlist [file name]`, output file with list of point mutants and redesigns

`-fix [file name]`, input file specifying residues that are aren't allowed to change identity. Each line in the file should contain a residue number and a chain id. Spacing can vary.

`-pmut [file name]`, input file specifying which residues to try point mutants at (the default is all interface residues). Each line in the file should contain a residue number and a chain id. Spacing can vary.

2) `rosetta.gcc -s 1c4z.pdb -interface -repack_neighbors -alter_spec_format -mutlist mut63c -intout E63c -output_structure -Wpack_only`

`-mutlist [file name]` input file generated by the previous step

-intout [file name] output file that contains energies for the redesigns specified in the mutlist

-output_structure: instructs interface mode to output structures

-Wpack_only: controls which weights are used, check the users guide for interface mode

-repack_neighbors: repack the residues that surround the mutations

-interface: use the interface mode of rosetta that calculates ddG values

-alter_spec_format: tells interface mode that the redesigns come in threes, WT-WT, WT-Mut, Mut-WT

Note: the standard flags to design with extra rotamers (ex1, ex2 ...) can be used at either command line.

Sample output:

For each point mutant and the resulting redesign that surrounds that point mutant the binding energies of Mut:Mut, Mut:WT and WT:Mut are displayed. In addition, the energy of the monomers is compared to that of the WT monomers (Gmut-Gwt). The structure numbers refer to the pdb files output by the routine. They refer to Mut-Mut, Mut-WT and WT-Mut respectively. The column titled Eres shows the total energies for each design.

```
point_mut_number = 4 structure_number= 10 , 11 , 12
      Eatr      Erep      Esol      Eaa      Edun      Eres
Gmut-Gwt Partner1      5.4      -2.4      0.0      -0.7      -2.2      -1.1
Gmut-Gwt Partner2      1.4      -0.1      -0.2      -0.4      0.3      -1.0
ddGbind  MUT:MUT      -4.8      -4.7      2.8      0.0      4.0      -5.3
ddGbind  MUT:WT      -5.0      4.7      1.3      0.0      2.0      4.9
ddGbind  WT:MUT      1.4      -0.4      2.5      0.0      0.7      1.6
Mutations Partner 1  S638A I655W F690Y Y694A
Mutations Partner 2  F63E

point_mut_number = 3 structure_number= 7 , 8 , 9
      Eatr      Erep      Esol      Eaa      Edun      Eres
Gmut-Gwt Partner1      1.9      -2.0      3.9      -0.6      1.1      1.9
Gmut-Gwt Partner2      1.1      -0.1      0.3      -0.4      -0.1      -0.9
ddGbind  MUT:MUT      -1.3      -6.4      2.2      0.0      0.9      -9.0
ddGbind  MUT:WT      -4.7      -3.2      1.6      0.0      0.5      -3.6
ddGbind  WT:MUT      1.5      -0.6      2.0      0.0      -0.5      0.9
Mutations Partner 1  S638A L642R I655W Y694A
Mutations Partner 2  F63D
```

