Rosetta Scoring Function

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Leipzig Rosetta Workshop
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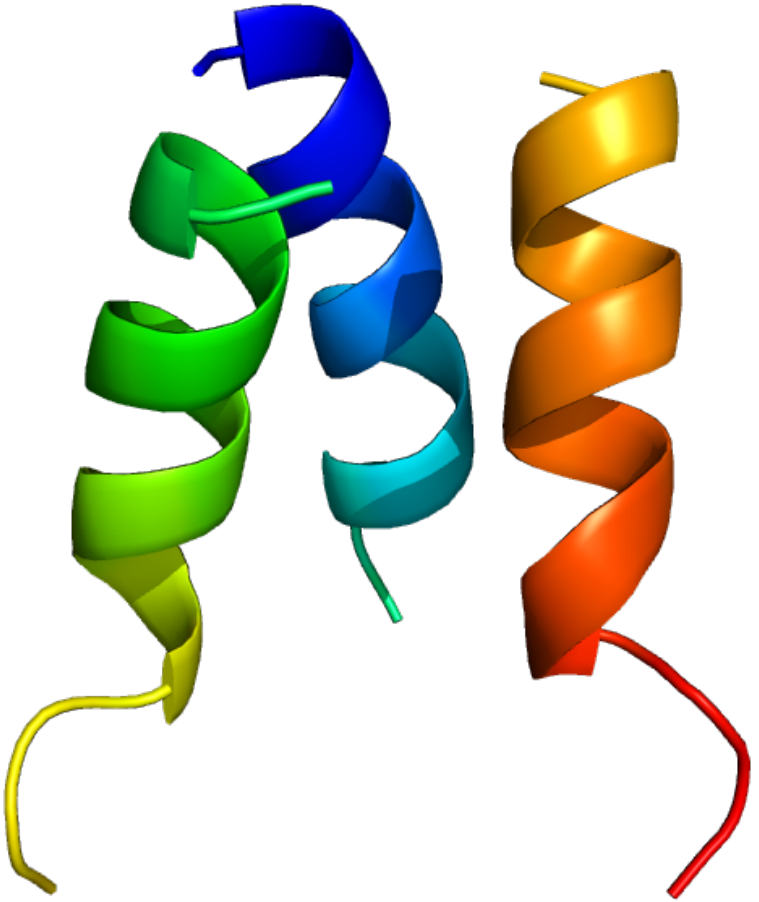
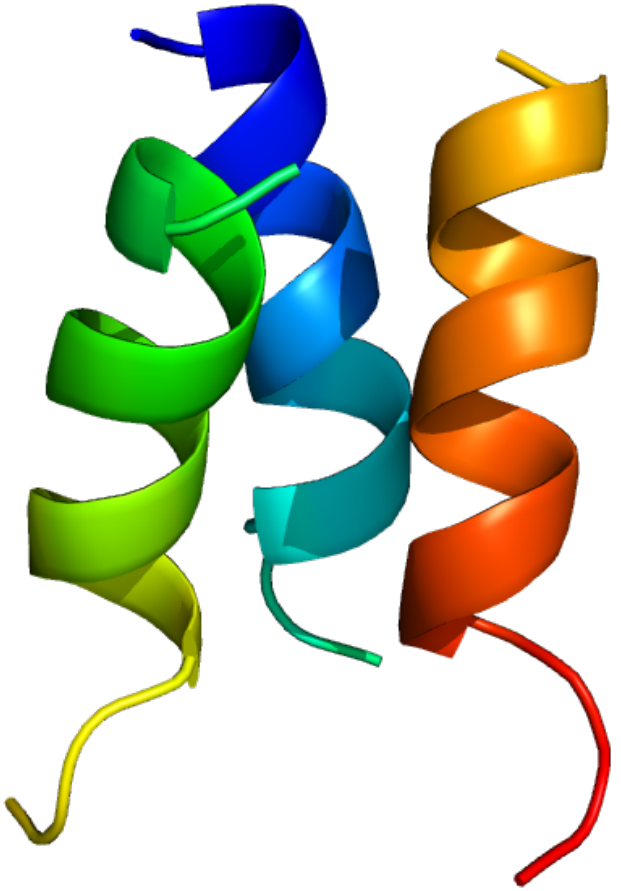
Why we need a scoring function?

Conformational changes

Conformation A

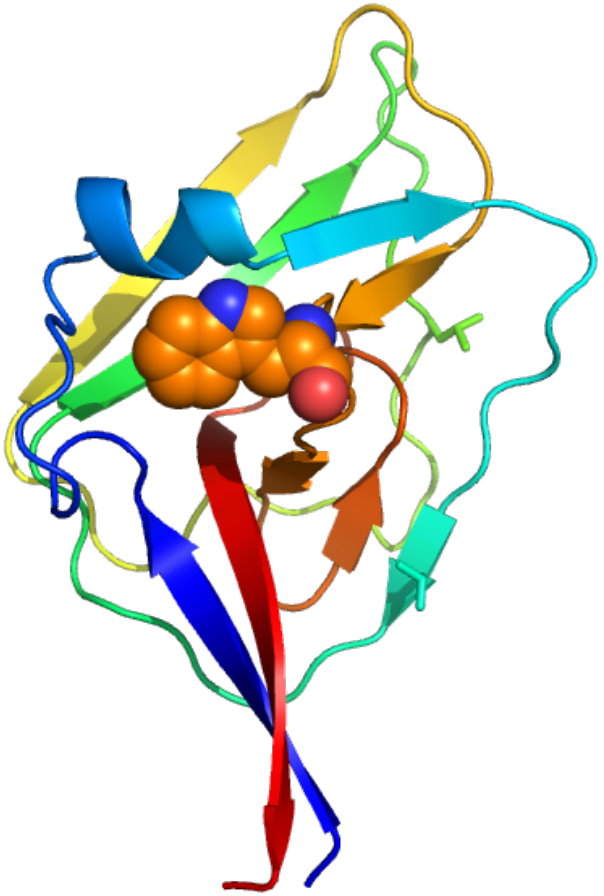


Conformation B

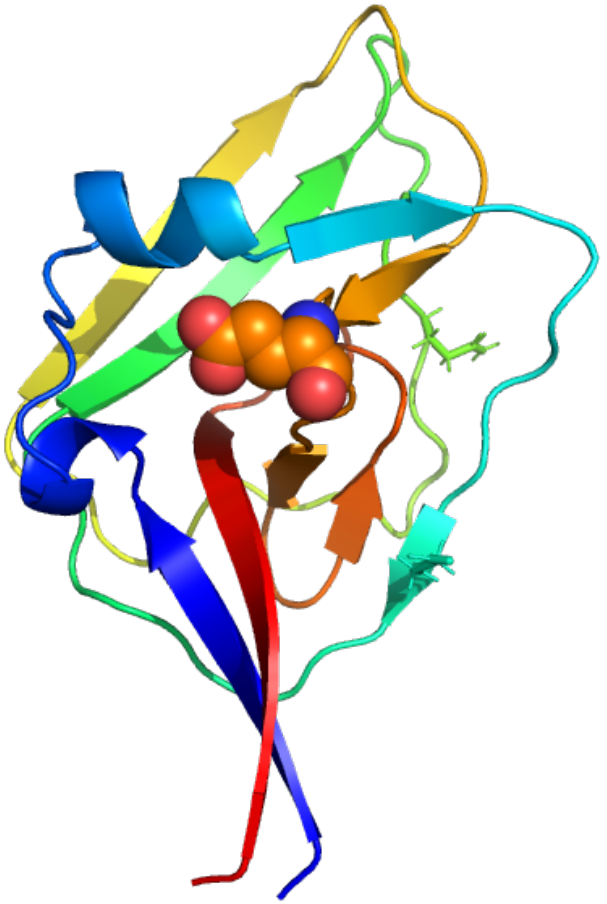


Mutations

Mutation A



Mutation B



How discriminate among A and B?

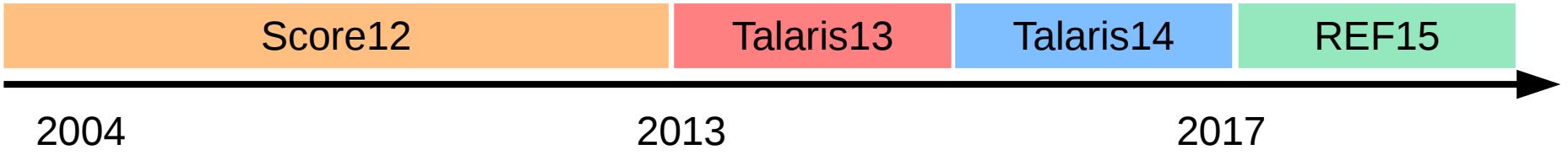
Scoring Function
(or Energy Function)

$$\Delta E_{\text{total}} = \sum_i w_i E_i(\Theta_i, \text{aa}_i)$$

The total energy (ΔE_{total}) is the sum of weighted energy terms ($\Sigma_i w_i E_i$) that are calculated as functions of geometric degrees of freedom (Θ_i) and chemical identities (aa_i).

(Alford RF, 2017)

Default scoring functions in Rosetta:

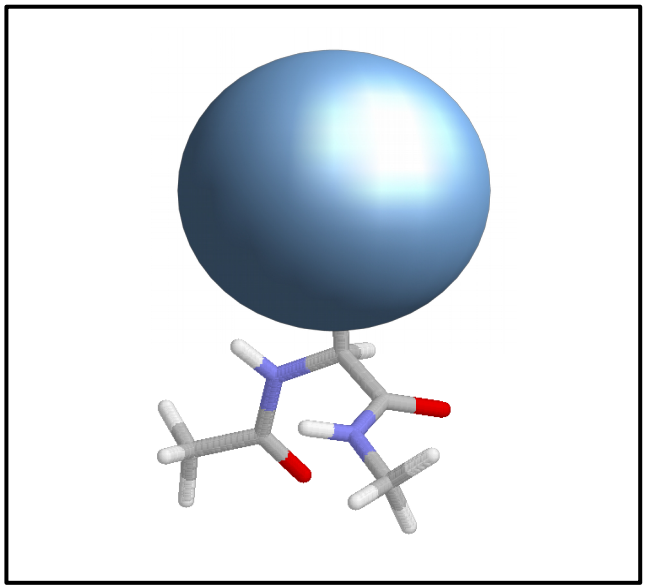


Historically the total energy was given in Rosetta Energy Units, (**REU**)

The newest scoring function (REF15) is given in **kcal/mol**

Types of scoring functions

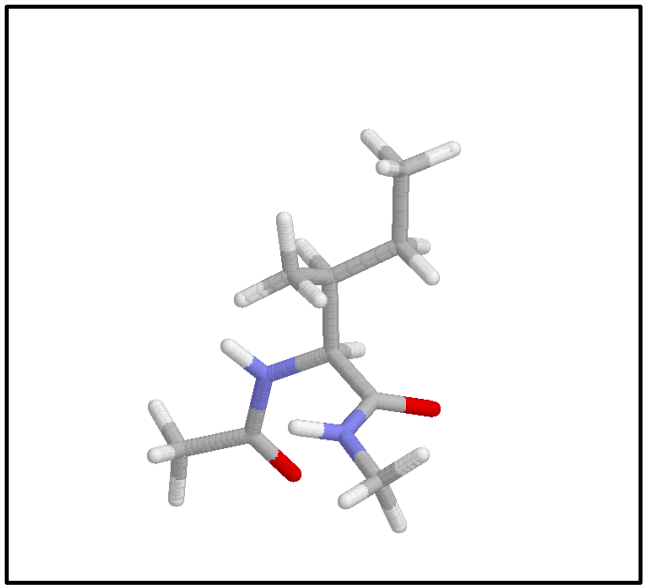
Low Resolution



Centroid
Simple energy function
Faster

Global search of conformational space
(Ab-initio folding, loop modeling)

High Resolution



Full-atom
More accurate energy function
Time consuming

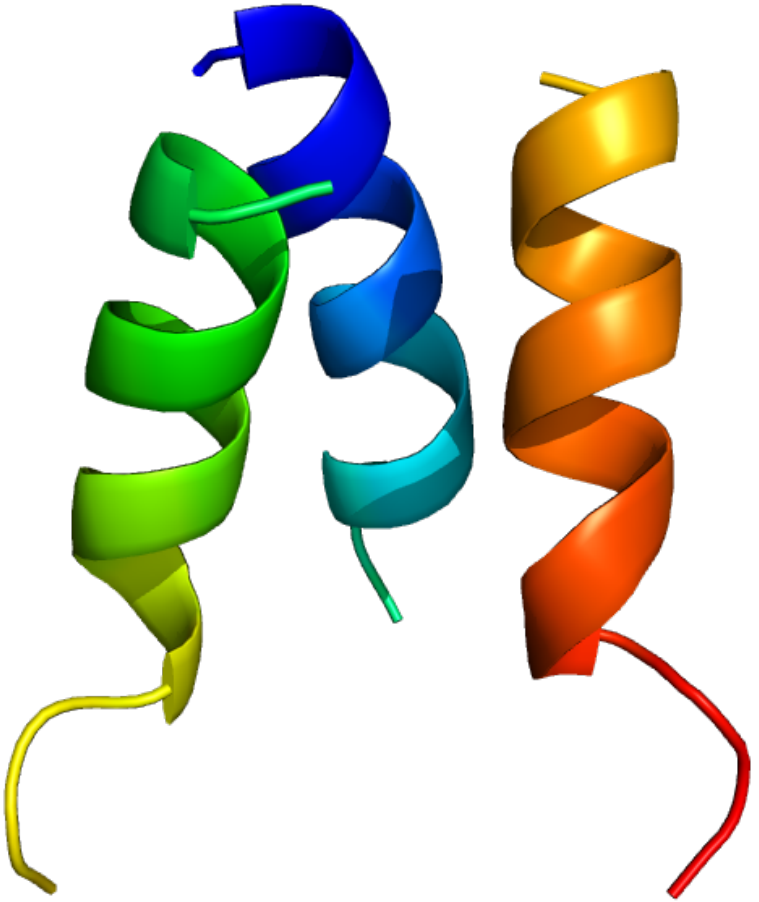
Local search of conformational space

Scoring in Rosetta

The lower the better!

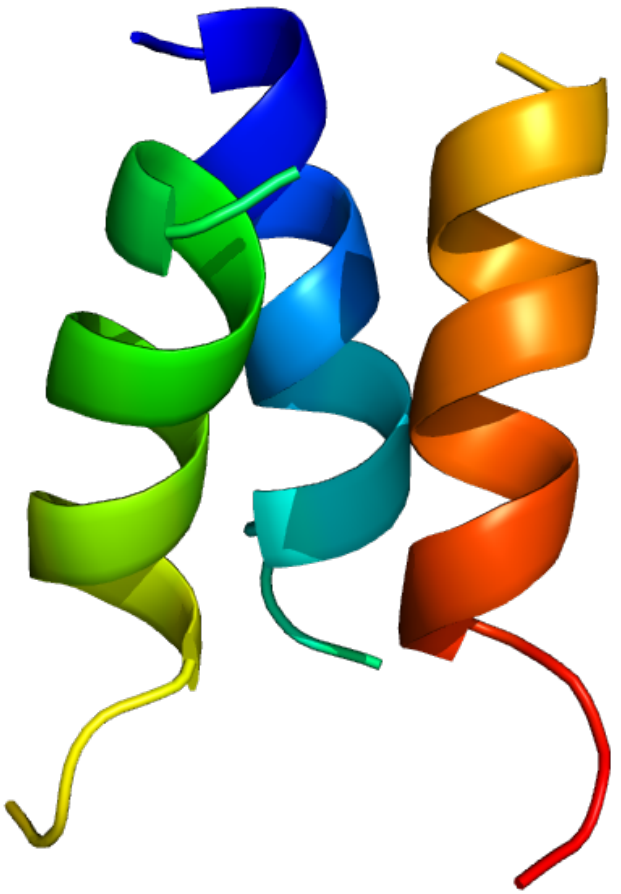
Total Energy

Conformation A



Total Energy = 253 REU

Conformation B

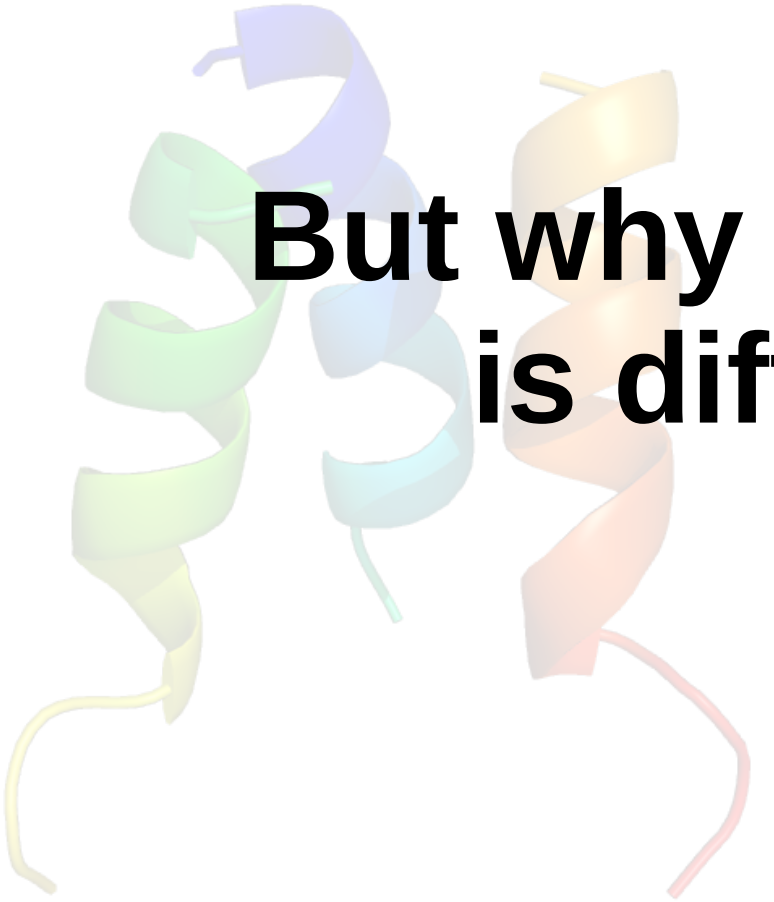


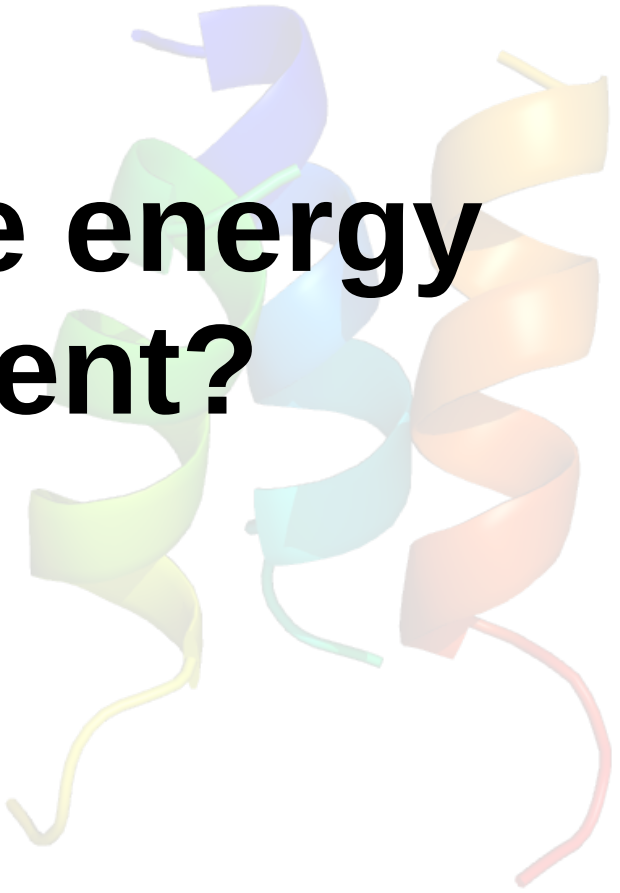
Total Energy = -82 REU

Total Energy

Conformation A

Conformation B





But why the energy is different?

Total Energy = 253 REU

Total Energy = -82 REU

Energy terms

$$\Delta E_{total} = w_1 E_1 + w_2 E_2 + w_3 E_3 + w_4 E_4 + \ldots + w_n E_n$$

$$E_{fa\_sol} = \sum_{i,j} w_{i,j}^{conn} [g_{desolv}(i, j) + g_{desolv}(j, i)]$$

$$E_{fa\_rep}(i, j) = \sum_{i,j} w_{i,j}^{conn} \begin{cases} m_{i,j} d_{i,j} + b_{i,j} & d_{i,j} \leq 0.6\sigma_{i,j} \\ \varepsilon_{i,j} \left[\left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{12} - 2 \left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{6} + 1 \right] & 0.6\sigma_{i,j} < d_{i,j} \leq \sigma_{i,j} \\ 0 & \sigma_{i,j} < d_{i,j} \end{cases}$$

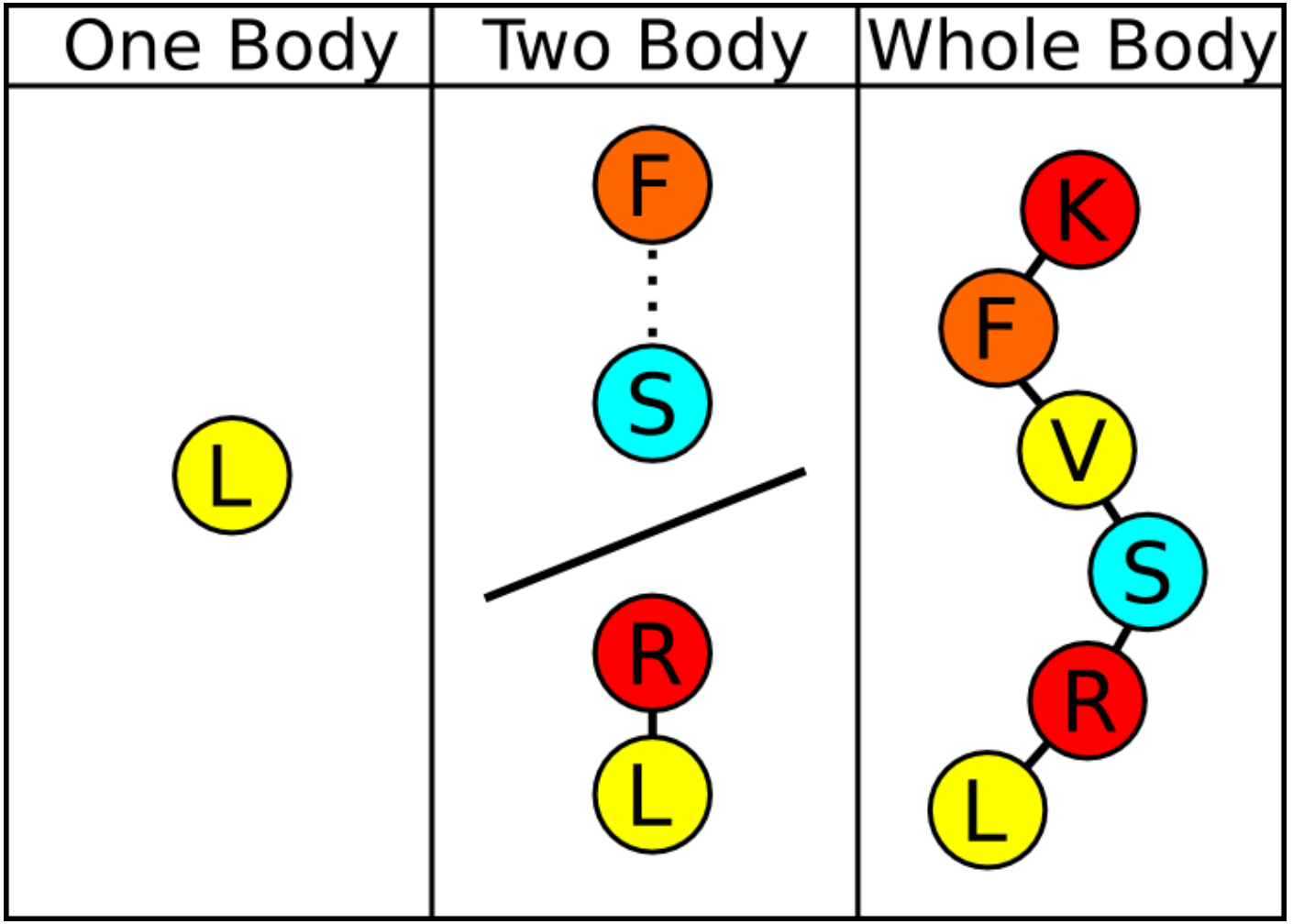
$$E_{fa\_atr} = \sum_{i,j} w_{i,j}^{conn} \begin{cases} -\varepsilon_{i,j} & d_{i,j} \leq \sigma_{ij} \\ \varepsilon_{i,j} \left[\left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{12} - 2 \left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{6} \right] & \sigma_{i,j} < d_{i,j} \leq 4.5 \text{ Å} \\ f_{poly}(d_{i,j}) & 4.5 \text{ Å} < d_{i,j} \leq 6.0 \text{ Å} \\ 0 & 6.0 \text{ Å} < d_{i,j} \end{cases}$$

$$E_{elec}(i, j, d_{i,j}) = \frac{C_0 q_i q_j}{\epsilon(d_{i,j})} \begin{cases} \frac{1}{d_{i,j}^{\,2}} - \frac{1}{d_{max}^{\,2}} & d_{i,j} \leq d_{max} \\ 0 & d_{max} < d_{i,j} \end{cases}$$

$$E_{ref} = \sum_{i} \Delta G_i^{ref}(aa_i)$$

(*Alford RF, 2017*)

Energy terms



Energy terms

One Body	Two Body	Whole Body
Backbone - p_aa_pp - rama_prepro Side Chain - fa_dun - yhh_planarity Reference - ref	Lennard-Jones - fa_atr - fa_rep Solvation - fa_sol Hydrogen Bond - hbond_lr_bb - hbond_sr_bb - hbond_bb_sc - hbond_sc	Radius of Gyration - rg Contact Order - co Structure Alignment - hs_pair - ss_pair - sheet

Energy terms - Full-Atom

term	description	weight	units	ref(s)
fa_atr	attractive energy between two atoms on different residues separated by a distance d	1.0	kcal/mol	5, 6
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fa_intra_rep	repulsive energy between two atoms on the same residue separated by a distance d	0.005	kcal/mol	5, 6
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fa_dun	probability that a chosen rotamer is native-like given backbone ϕ, ψ angles	(0.7 kcal/mol)/kT	kT	52
omega	backbone-dependent penalty for cis ω dihedrals that deviate from 0° and trans ω dihedrals that deviate from 180°	(0.6 kcal/mol)/AU	AU[a]	72
pro_close	penalty for an open proline ring and proline ω bonding energy	(1.25 kcal/mol)/AU	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	(0.625 kcal/mol)/AU	AU	49
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(*Alford RF, 2017*)

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Physics-Based

Knowledge-Based

(*Alford RF, 2017*)

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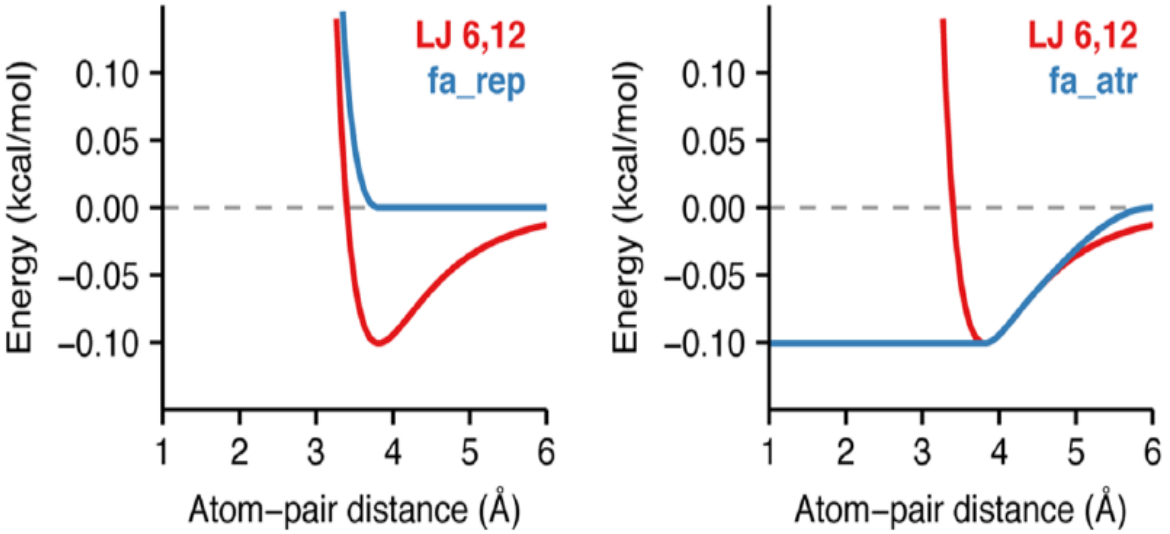
Van der Waals interactions



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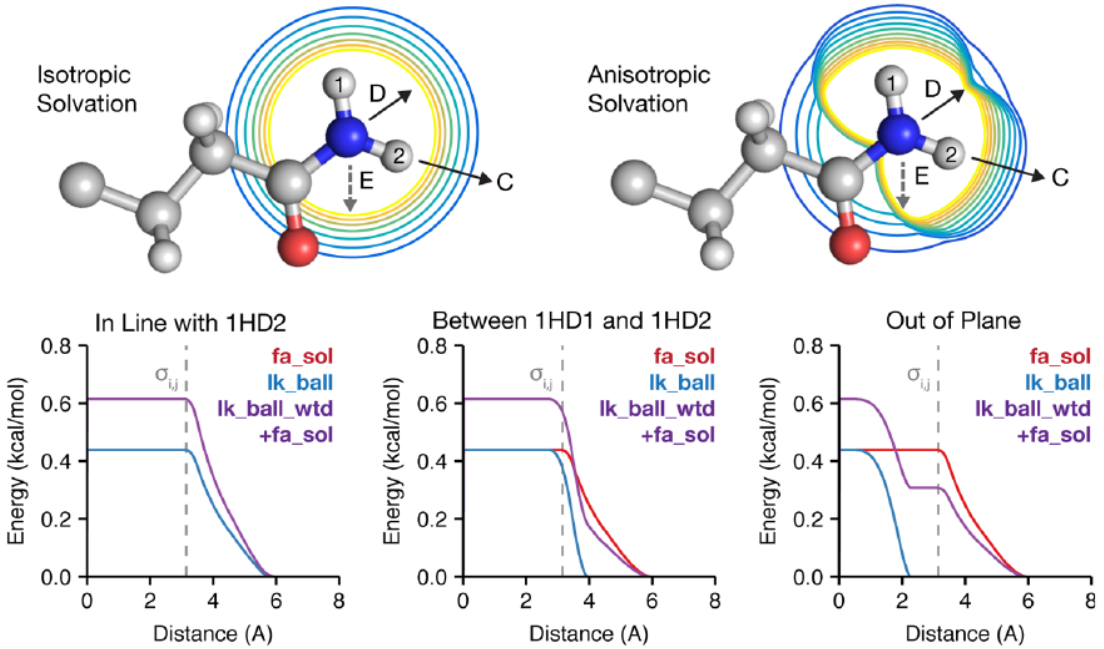
Implicit solvation



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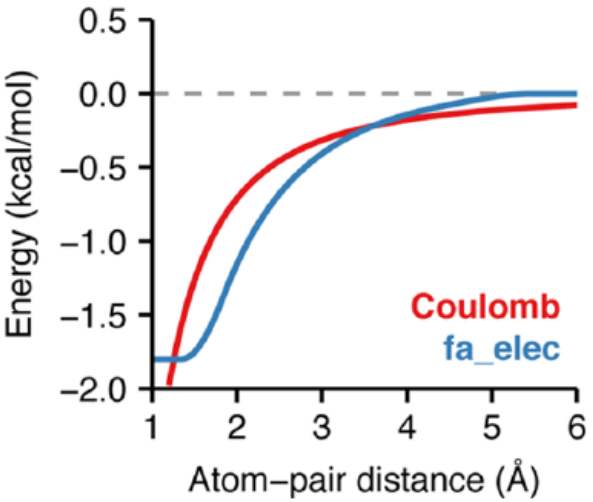
Electrostatics



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Hydrogen Bonds

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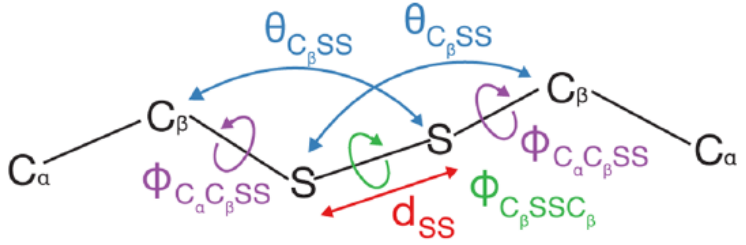
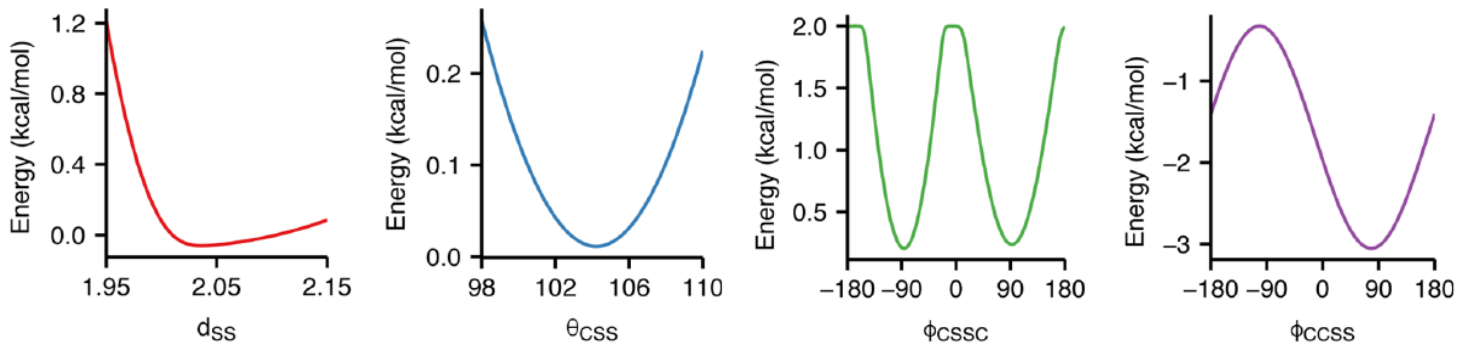
Disulfide Bonds





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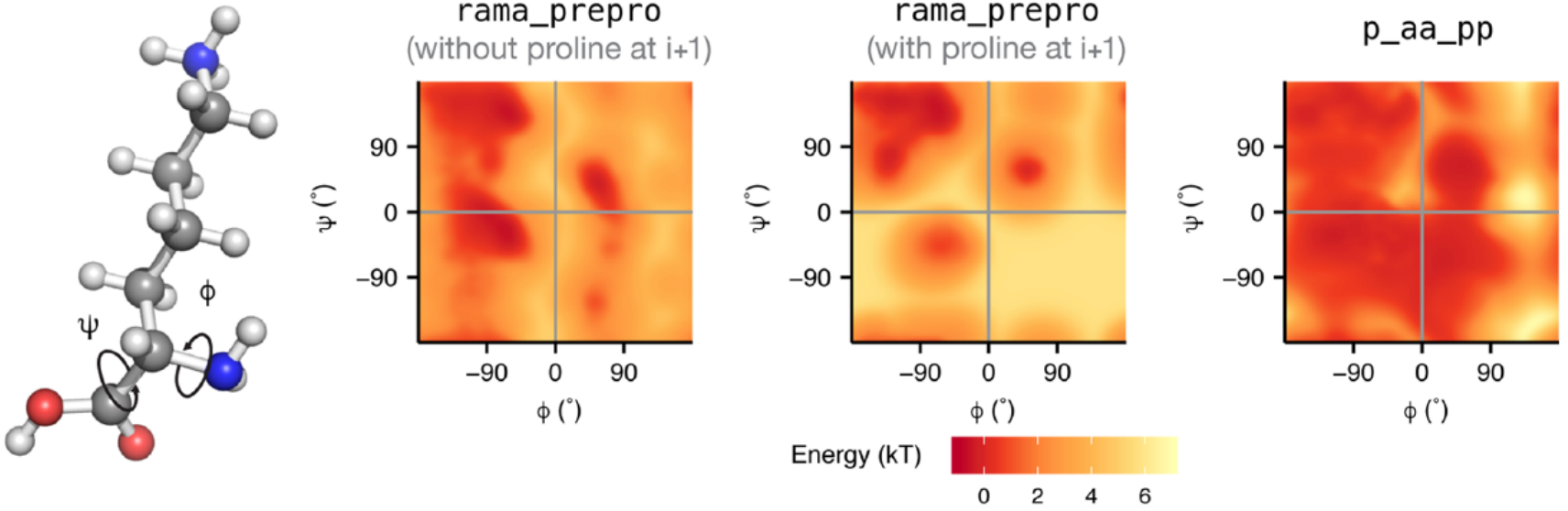
Protein Backbone



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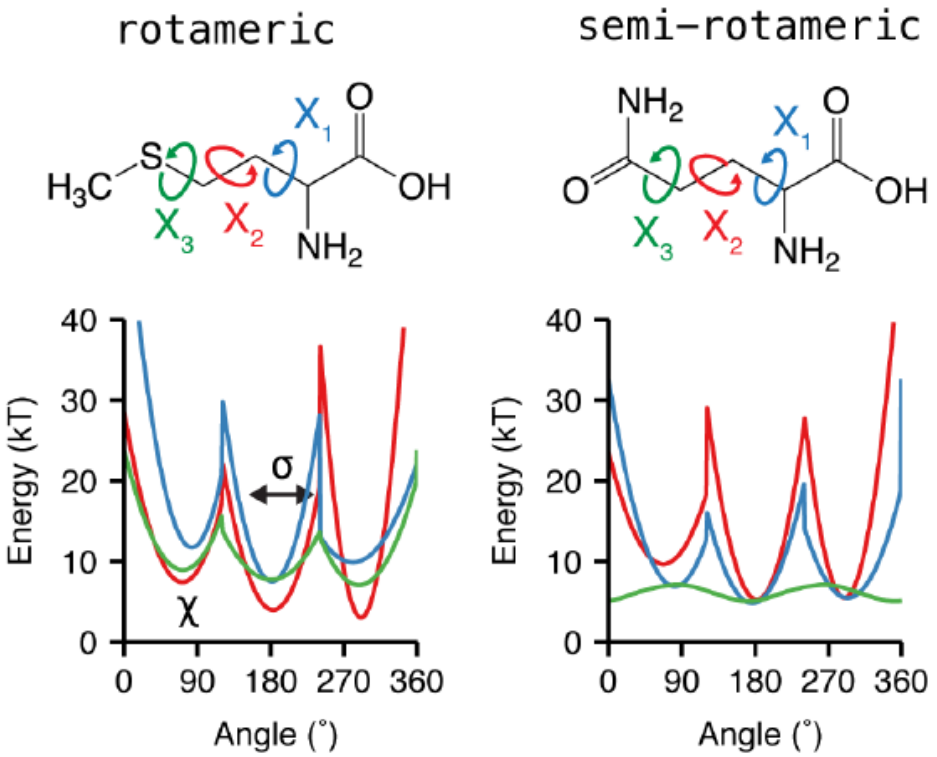
p_aa_pp	probability of amino acid identity given backbone ϕ, ψ angles	(0.4 kcal/mol)/kT	kT	51
fa_dun	probability that a chosen rotamer is native-like given backbone ϕ, ψ angles	(0.7 kcal/mol)/kT	kT	52
omega	backbone-dependent penalty for cis ω dihedrals that deviate from 0° and trans ω dihedrals that deviate from 180°	(0.6 kcal/mol)/AU	AU[a]	72
pro_close	penalty for an open proline ring and proline ω bonding energy	(1.25 kcal/mol)/AU	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	(0.625 kcal/mol)/AU	AU	49
ref	reference energies for amino acid types	(1.0 kcal/mol)/AU	AU	1, 51

Protein Side-Chains

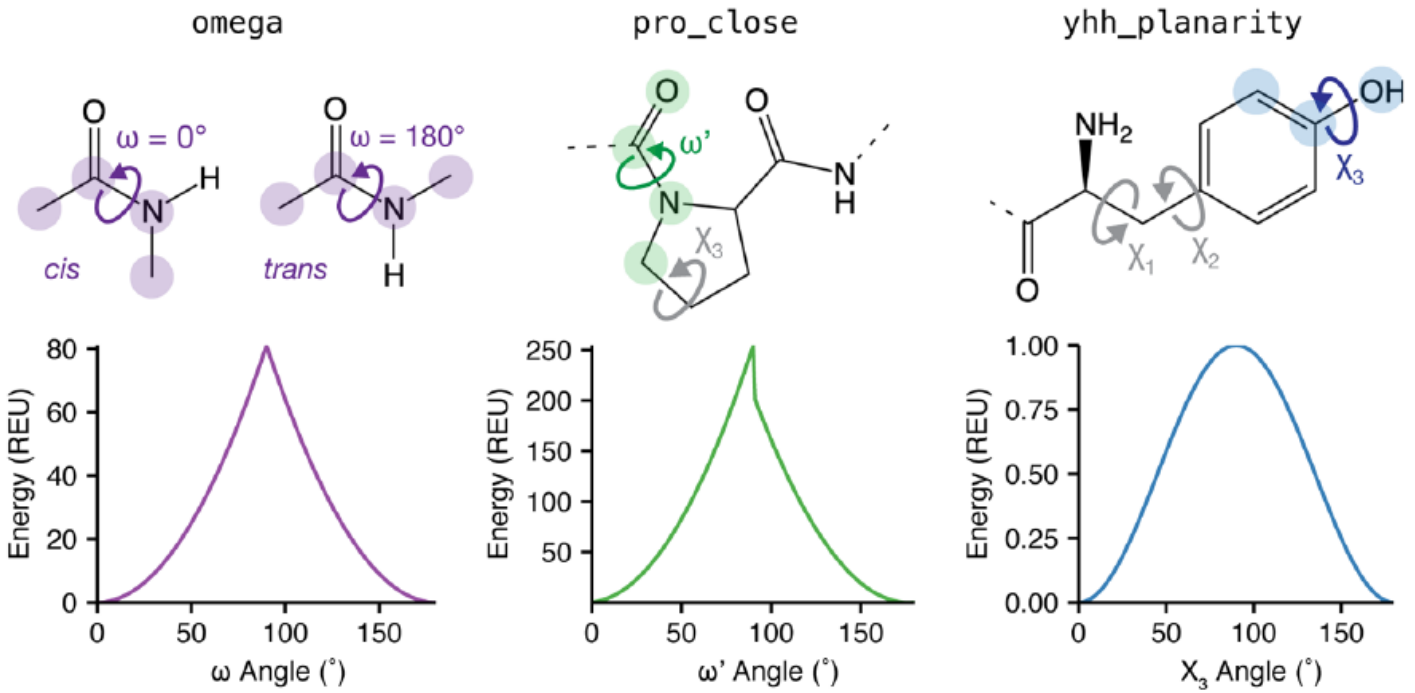


(*Alford RF, 2017*)

Knowledge-Based Energy terms

term	description	weight	units	ref(s)
rama_prepro	probability of backbone ϕ, ψ angles given the amino acid type	(0.45 kcal/mol)/kT	kT	50, 51
p_aa_pp	probability of amino acid identity given backbone ϕ, ψ angles	(0.4 kcal/mol)/kT	kT	51
fa_dun	probability that a chosen rotamer is native-like given backbone ϕ, ψ angles	(0.7 kcal/mol)/kT	kT	52
omega	backbone-dependent penalty for cis ω dihedrals that deviate from 0° and trans ω dihedrals that deviate from 180°	(0.6 kcal/mol)/AU	AU[a]	72
pro_close	penalty for an open proline ring and proline ω bonding energy	(1.25 kcal/mol)/AU	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	(0.625 kcal/mol)/AU	AU	49
ref	reference energies for amino acid types	(1.0 kcal/mol)/AU	AU	1, 51

Special-Case Torsions



(*Alford RF, 2017*)

Knowledge-Based Energy terms

term	description	weight	units	ref(s)
rama_prepro	probability of backbone ϕ, ψ angles given the amino acid type	(0.45 kcal/mol)/kT	kT	50, 51
p_aa_pp	probability of amino acid identity given backbone ϕ, ψ angles	(0.4 kcal/mol)/kT	kT	51
fa_dun	probability that a chosen rotamer is native-like given backbone ϕ, ψ angles	(0.7 kcal/mol)/kT	kT	52
omega	backbone-dependent penalty for cis ω dihedrals that deviate from 0° and trans ω dihedrals that deviate from 180°	(0.6 kcal/mol)/AU	AU[a]	72
pro_close	penalty for an open proline ring and proline ω bonding energy	(1.25 kcal/mol)/AU	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	(0.625 kcal/mol)/AU	AU	49
ref	reference energies for amino acid types	(1.0 kcal/mol)/AU	AU	1, 51

Special-Case, Design

Takes in account the unfolding energy of the amino acids

Adjusts amino acids frequencies during protein design

(*Alford RF, 2017*)

Weights

$$\Delta E_{total} = w_1 E_1 + w_2 E_2 + w_3 E_3 + w_4 E_4 + \ldots + w_n E_n$$

$$E_{fa\_sol} = \sum_{i,j} w_{i,j}^{conn} [g_{desolv}(i, j) + g_{desolv}(j, i)]$$

$$E_{fa\_rep}(i, j) = \sum_{i,j} w_{i,j}^{conn} \begin{cases} m_{i,j} d_{i,j} + b_{i,j} & d_{i,j} \leq 0.6\sigma_{i,j} \\ \varepsilon_{i,j} \left[\left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{12} - 2 \left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{6} + 1 \right] & 0.6\sigma_{i,j} < d_{i,j} \leq \sigma_{i,j} \\ 0 & \sigma_{i,j} < d_{i,j} \end{cases}$$

$$E_{fa\_atr} = \sum_{i,j} w_{i,j}^{conn} \begin{cases} -\varepsilon_{i,j} & d_{i,j} \leq \sigma_{ij} \\ \varepsilon_{i,j} \left[\left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{12} - 2 \left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{6} \right] & \sigma_{i,j} < d_{i,j} \leq 4.5 \text{ Å} \\ f_{poly}(d_{i,j}) & 4.5 \text{ Å} < d_{i,j} \leq 6.0 \text{ Å} \\ 0 & 6.0 \text{ Å} < d_{i,j} \end{cases}$$

$$E_{elec}(i, j, d_{i,j}) = \frac{C_0 q_i q_j}{\epsilon(d_{i,j})} \begin{cases} \frac{1}{d_{i,j}^{\,2}} - \frac{1}{d_{max}^{\,2}} & d_{i,j} \leq d_{max} \\ 0 & d_{max} < d_{i,j} \end{cases}$$

$$E_{ref} = \sum_{i} \Delta G_i^{ref}(aa_i)$$

(*Alford RF, 2017*)

Weights

term	description	weight	units	ref(s)
fa_atr	attractive energy between two atoms on different residues separated by a distance d	1.0	kcal/mol	5, 6
fa_rep	repulsive energy between two atoms on different residues separated by a distance d	0.55	kcal/mol	5, 6
fa_intra_rep	repulsive energy between two atoms on the same residue separated by a distance d	0.005	kcal/mol	5, 6
fa_sol	Gaussian exclusion implicit solvation energy between protein atoms in different residues	1.0	kcal/mol	36
lk_ball_wtd	orientation-dependent solvation of polar atoms assuming ideal water geometry	1.0	kcal/mol	50, 71
fa_intra_sol	Gaussian exclusion implicit solvation energy between protein atoms in the same residue	1.0	kcal/mol	36
fa_elec	energy of interaction between two nonbonded charged atoms separated by a distance d	1.0	kcal/mol	50
hbond_lr_bb	energy of short-range hydrogen bonds	1.0	kcal/mol	38, 49
hbond_sr_bb	energy of long-range hydrogen bonds	1.0	kcal/mol	38, 49
hbond_bb_sc	energy of backbone–side-chain hydrogen bonds	1.0	kcal/mol	38, 49
hbond_sc	energy of side-chain–side-chain hydrogen bonds	1.0	kcal/mol	38, 49
dslf_fa13	energy of disulfide bridges	1.25	kcal/mol	49
rama_prepro	probability of backbone ϕ, ψ angles given the amino acid type	(0.45 kcal/mol)/kT	kT	50, 51
p_aa_pp	probability of amino acid identity given backbone ϕ, ψ angles	(0.4 kcal/mol)/kT	kT	51
fa_dun	probability that a chosen rotamer is native-like given backbone ϕ, ψ angles	(0.7 kcal/mol)/kT	kT	52
omega	backbone-dependent penalty for cis ω dihedrals that deviate from 0° and trans ω dihedrals that deviate from 180°	(0.6 kcal/mol)/AU	AU[a]	72
pro_close	penalty for an open proline ring and proline ω bonding energy	(1.25 kcal/mol)/AU	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	(0.625 kcal/mol)/AU	AU	49
ref	reference energies for amino acid types	(1.0 kcal/mol)/AU	AU	1, 51

[a]AU = arbitrary units.

Some weights for specific energy terms are set to zero!

(*Alford RF, 2017*)

Weights

term	description	weight	units	ref(s)
fa_atr	attractive energy between two atoms on different residues separated by a distance d	1.0	kcal/mol	5, 6
fa_rep	repulsive energy between two atoms on different residues separated by a distance d	0.55	kcal/mol	5, 6
fa_intra_rep	repulsive energy between two atoms on the same residue separated by a distance d	0.005	kcal/mol	5, 6
fa_sol	Gaussian exclusion implicit solvation energy between protein atoms in different residues	1.0	kcal/mol	36
lk_ball_wtd	orientation-dependent solvation of polar atoms assuming ideal water geometry	1.0	kcal/mol	50, 71
fa_intra_sol	Gaussian exclusion implicit solvation energy between protein atoms in the same residue	1.0	kcal/mol	36
fa_elec	energy of interaction between two nonbonded charged atoms separated by a distance d	1.0	kcal/mol	50
hbond_lr_bb	energy of short-range hydrogen bonds	1.0	kcal/mol	38, 49
hbond_sr_bb	energy of long-range hydrogen bonds	1.0	kcal/mol	38, 49
hbond_bb_sc	energy of backbone–side-chain hydrogen bonds	1.0	kcal/mol	38, 49
hbond_sc	energy of side-chain–side-chain hydrogen bonds	1.0	kcal/mol	38, 49
dslf_fa13	energy of disulfide bridges	1.25	kcal/mol	49
rama_prepro	probability of backbone ϕ, ψ angles given the amino acid type	(0.45 kcal/mol)/kT	kT	50, 51
p_aa_pp	probability of amino acid identity given backbone ϕ, ψ angles	(0.4 kcal/mol)/kT	kT	51
fa_dun	probability that a chosen rotamer is native-like given backbone ϕ, ψ angles	(0.7 kcal/mol)/kT	kT	52
omega	backbone-dependent penalty for cis ω dihedrals that deviate from 0° and trans ω dihedrals that deviate from 180°	(0.6 kcal/mol)/AU	AU[a]	72
pro_close	penalty for an open proline ring and proline ω bonding energy	(1.25 kcal/mol)/AU	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	(0.625 kcal/mol)/AU	AU	49
ref	reference energies for amino acid types	(1.0 kcal/mol)/AU	AU	1, 51

[a]AU = arbitrary units.

You can modify the weights if needed in special applications!

(*Alford RF, 2017*)

Modifying Weights:

Using the command line:

1 - score:weights <filename>

2 - score:set_weight <scoreterm$_1$> <wt$_1$> <scoreterm$_2$> <wt$_2$>

3 - score:patch <patchfile>

```
fa_atr = 0.423
fa_rep = 0.100
```

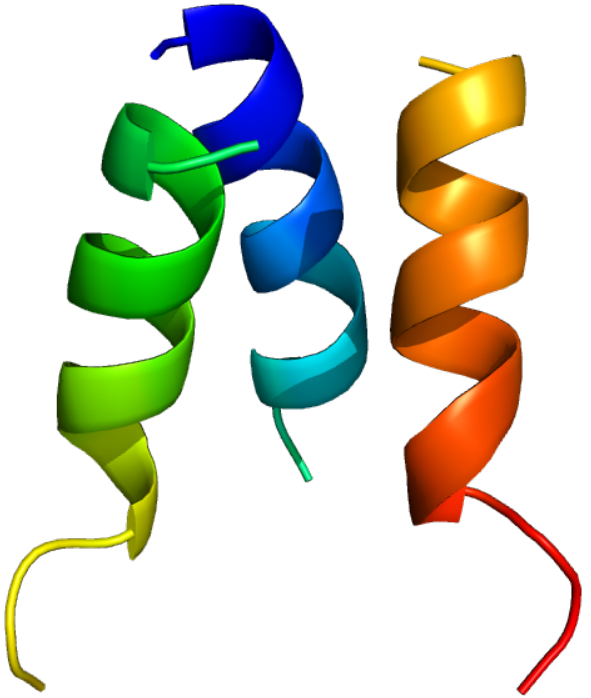
Using xml script file:

```
<ROSETTASCRIPTS>
    <SCOREFXNS>
        <ScoreFunction name="ligand_soft_rep" weights="ligand_soft_rep">
            <Reweight scoretype="fa_elec" weight="0.42" />
        </ScoreFunction>
        <ScoreFunction name="hard_rep" weights="ligandprime"/>
    </SCOREFXNS>
    <OUTPUT scorefxn="hard_rep" />
</ROSETTASCRIPTS>
```

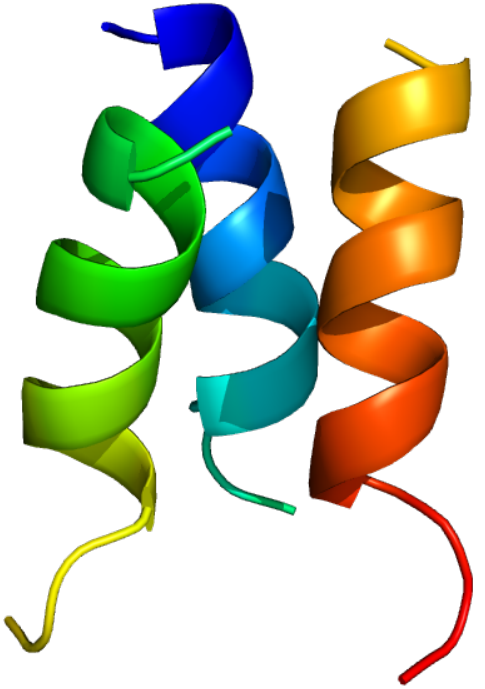
(*Alford RF, 2017*)

Total Energy

Conformation A (253 REU)



Conformation B (-82 REU)



Score File (score.sc):

```
SCORE:  score   fa_atr          fa_rep  fa_sol  fa_intra_rep
fa_intra_sol_xover4     lk_ball_wtd     fa_elec pro_close       hbond_sr_bb
hbond_lr_bb     hbond_bb_sc     hbond_sc        omega   fa_dun  p_aa_pp
yhh_planarity   ref     rama_prepro     description
SCORE:  253.063 -181.52         45.561  149.367 0.702
17.998                  -7.402          -1.488  5.473           -12.482
0               0               0               7.578   236.014 2.073
0               -5.466  -3.345          Conformation_A
SCORE:  -82.141 -217.874        26.366  130.127 0.591
9.342                   -10.943         -36.763 1.125           -20.668
0               -3.441          0               0.491   54.899  -2.846
0               -5.466  -7.08           Conformation_B
```

Score File (score.sc):

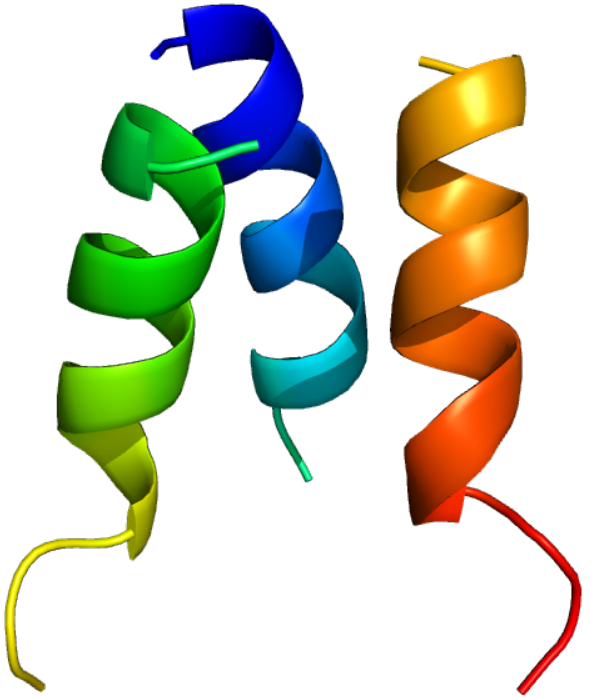
```
SCORE:  score   fa_atr          fa_rep  fa_sol  fa_intra_rep
fa_intra_sol_xover4     lk_ball_wtd     fa_elec pro_close       hbond_sr_bb
hbond_lr_bb     hbond_bb_sc     hbond_sc        omega   fa_dun  p_aa_pp
yhh_planarity   ref     rama_prepro     description
SCORE:  253.063 -181.52         45.561  149.367 0.702
17.998                  -7.402          -1.488  5.473           -12.482
0               0               0               7.578   236.014 2.073
0               -5.466  -3.345          Conformation_A
SCORE:  -82.141 -217.874        26.366  130.127 0.591
9.342                   -10.943         -36.763 1.125           -20.668
0               -3.441          0               0.491   54.899  -2.846
0               -5.466  -7.08           Conformation_B
```

Score File (score.sc):

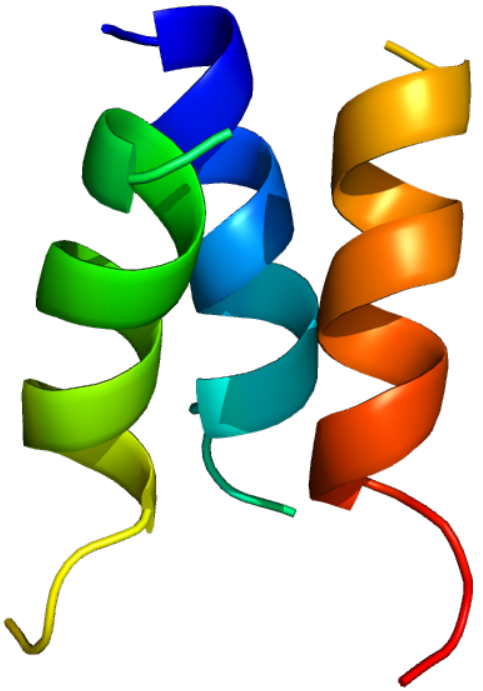
```
SCORE:  score    fa_atr          fa_rep   fa_sol   fa_intra_rep
fa_intra_sol_xover4     lk_ball_wtd     fa_elec pro_close        hbond_sr_bb
hbond_lr_bb     hbond_bb_sc     hbond_sc        omega   fa_dun   p_aa_pp
yhh_planarity   ref     rama_prepro     description
SCORE:  253.063 -181.52         45.561   149.367 0.702
17.998                  -7.402          -1.488  5.473           -12.482
0               0               0               7.578   236.014 2.073
0               -5.466  -3.345          Conformation_A
SCORE:  -82.141 -217.874        26.366   130.127 0.591
9.342                   -10.943         -36.763 1.125           -20.668
0               -3.441          0               0.491   54.899  -2.846
0               -5.466  -7.08           Conformation_B
```

Total Energy

Conformation A (253 REU)



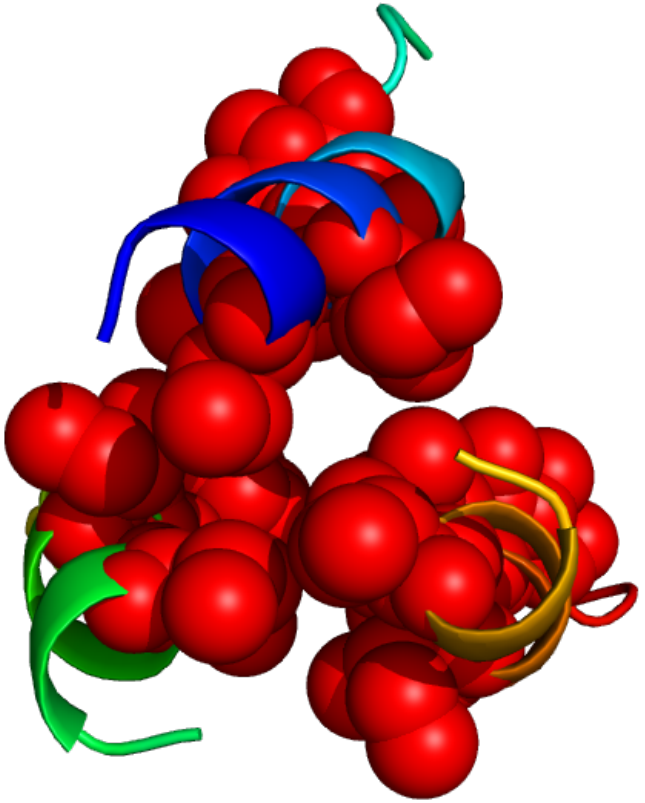
Conformation B (-82 REU)



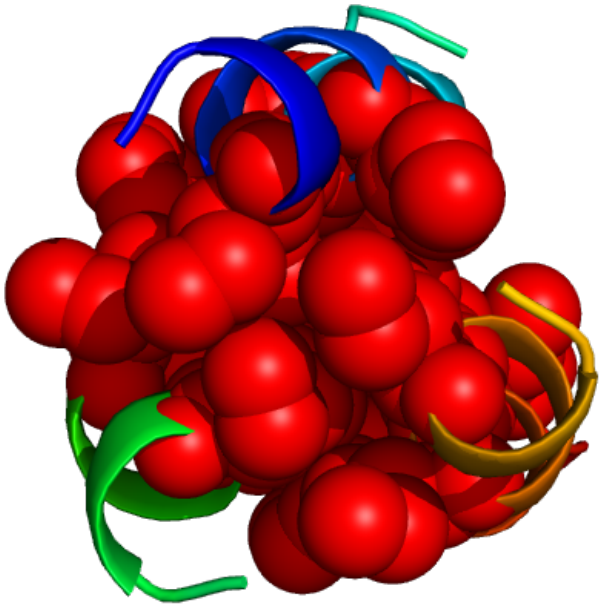
	score	fa_atr	fa_rep
A	253.1	-181.5	45.6
B	-82.1	-217.9	26.4
Delta (A-B)	335.2	36.4	19.2

Total Energy

Conformation A (253 REU)



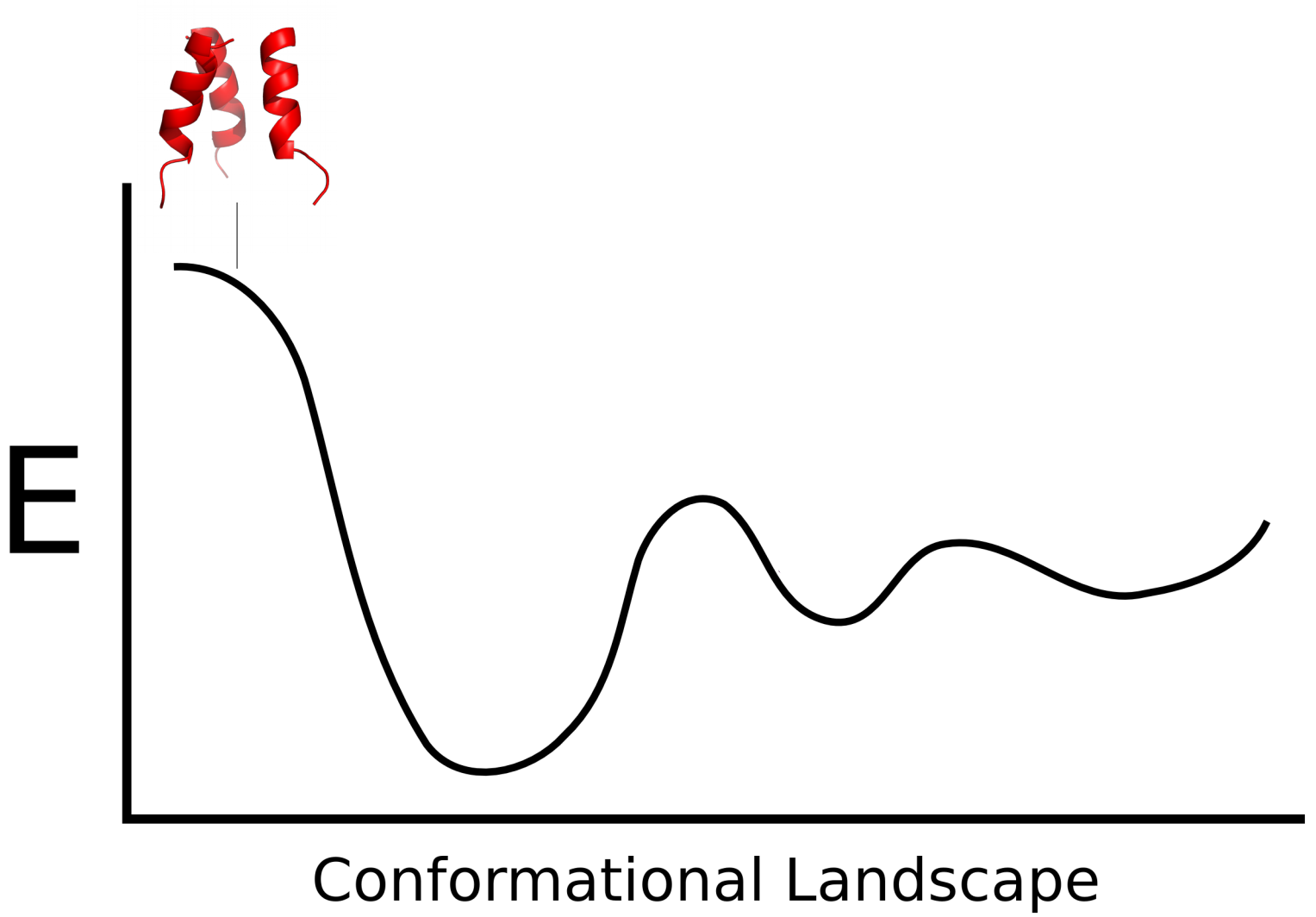
Conformation B (-82 REU)



	score	fa_atr	fa_rep
A	253.1	-181.5	45.6
B	-82.1	-217.9	26.4
Delta (A-B)	335.2	36.4	19.2

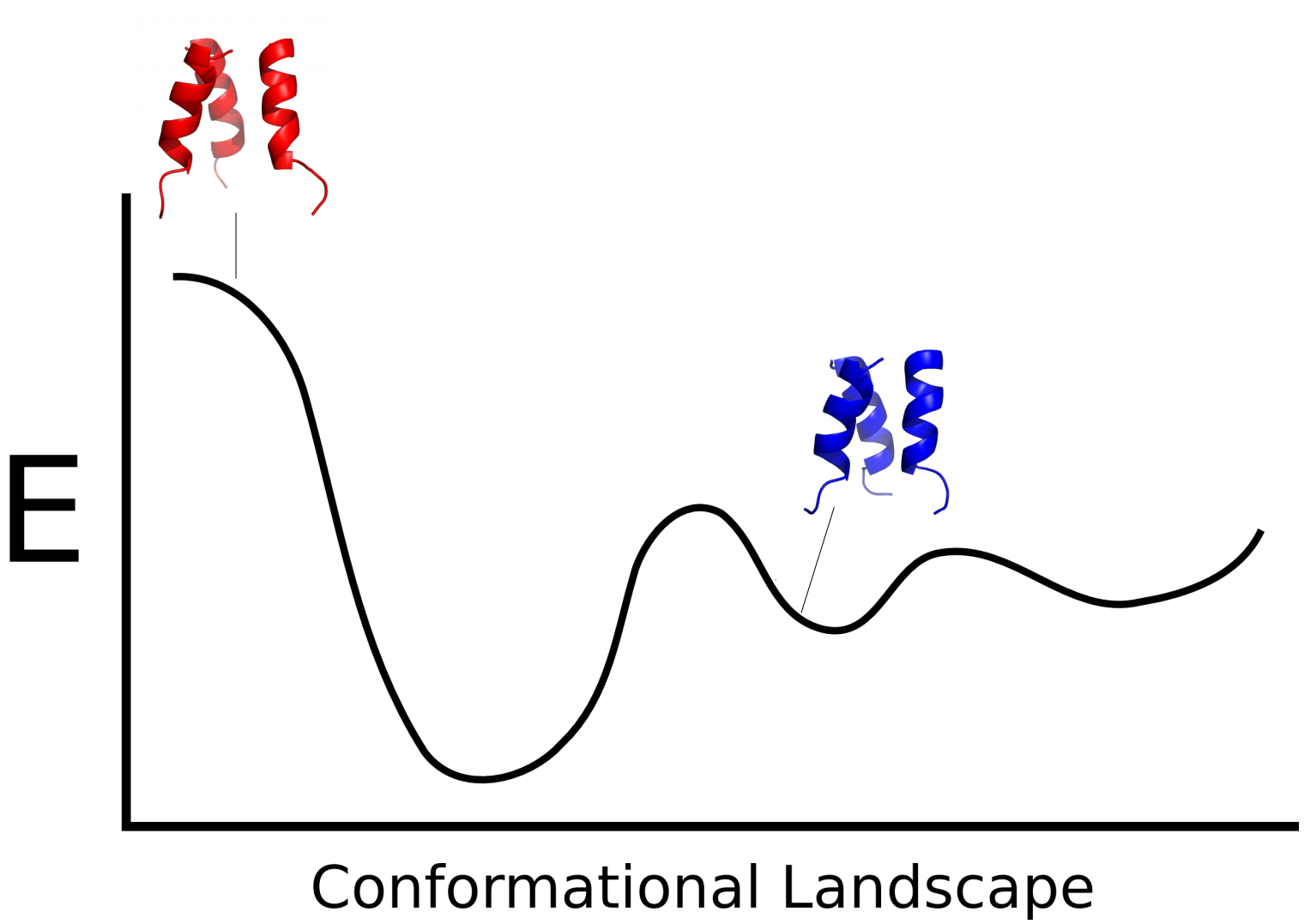
Sampling in Rosetta

Metropolis Monte Carlo Algorithm



Metropolis Monte Carlo Algorithm

If $E_{new} < E_{old}$: Accept structure

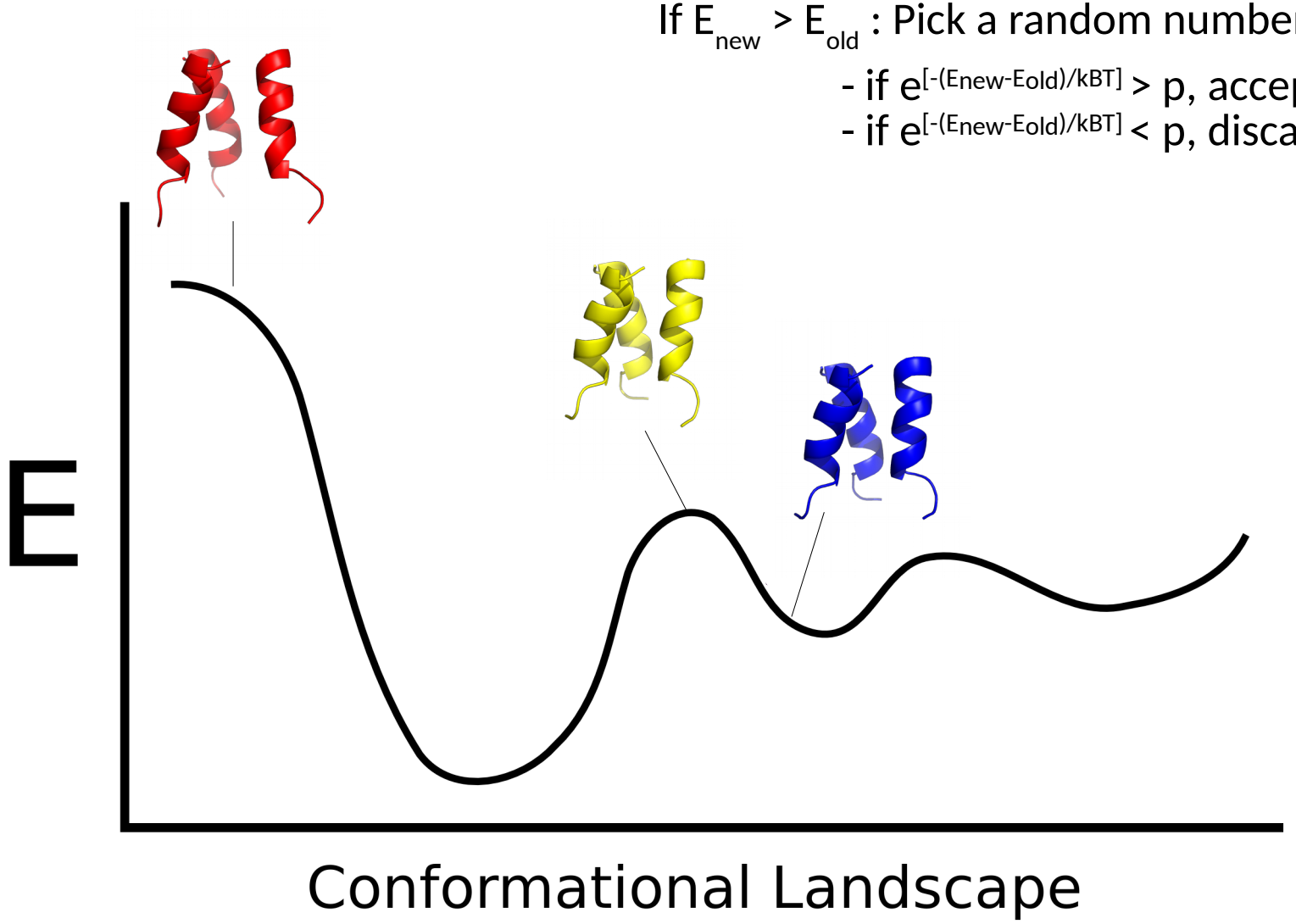


Metropolis Monte Carlo Algorithm

If $E_{new} < E_{old}$: Accept structure

If $E_{new} > E_{old}$: Pick a random number p(0, 1)

- if $e^{[-(E_{new}-E_{old})/kBT]} > p$, accept structure
- if $e^{[-(E_{new}-E_{old})/kBT]} < p$, discard structure

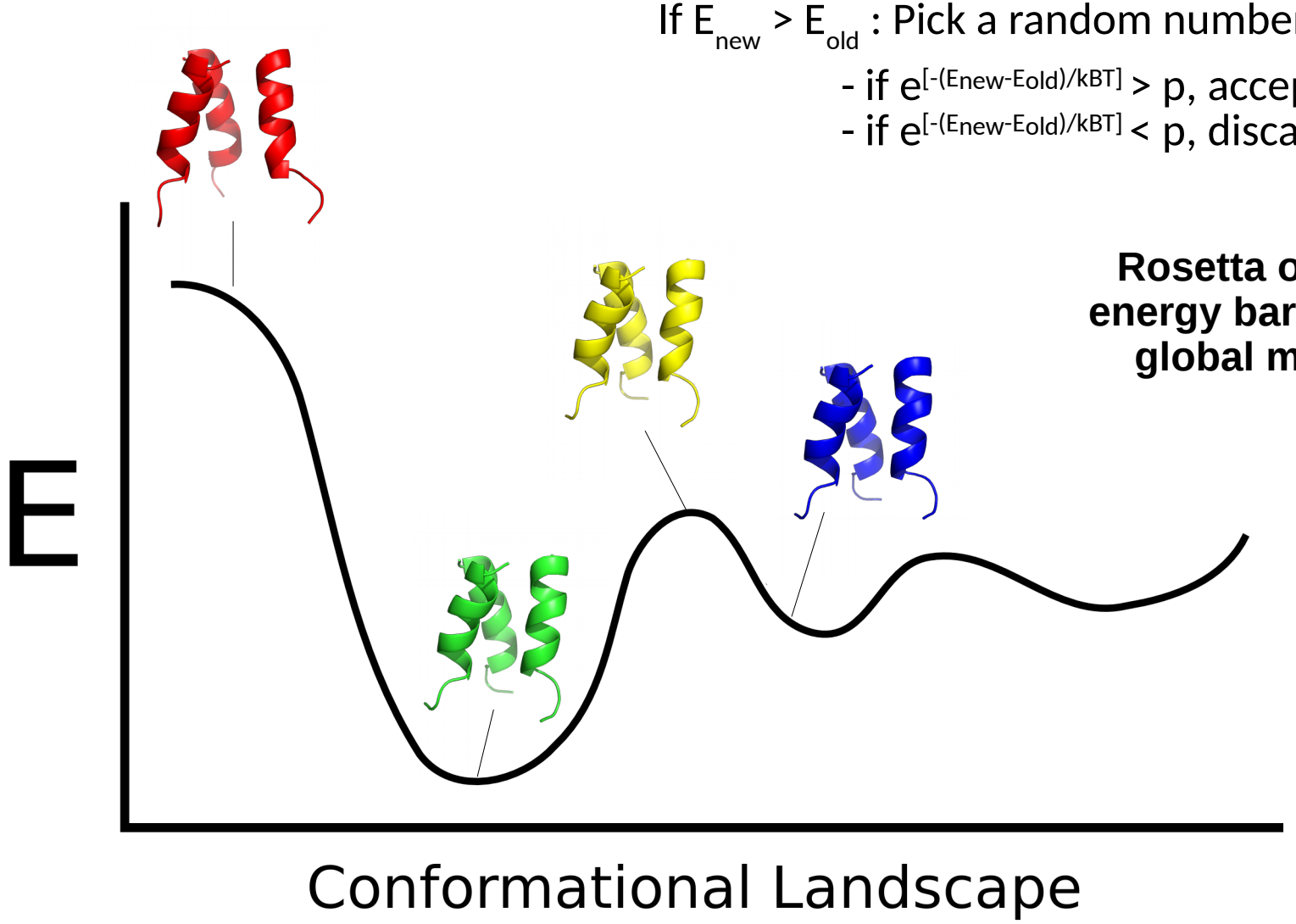


Metropolis Monte Carlo Algorithm

If $E_{new} < E_{old}$: Accept structure

If $E_{new} > E_{old}$: Pick a random number p(0, 1)

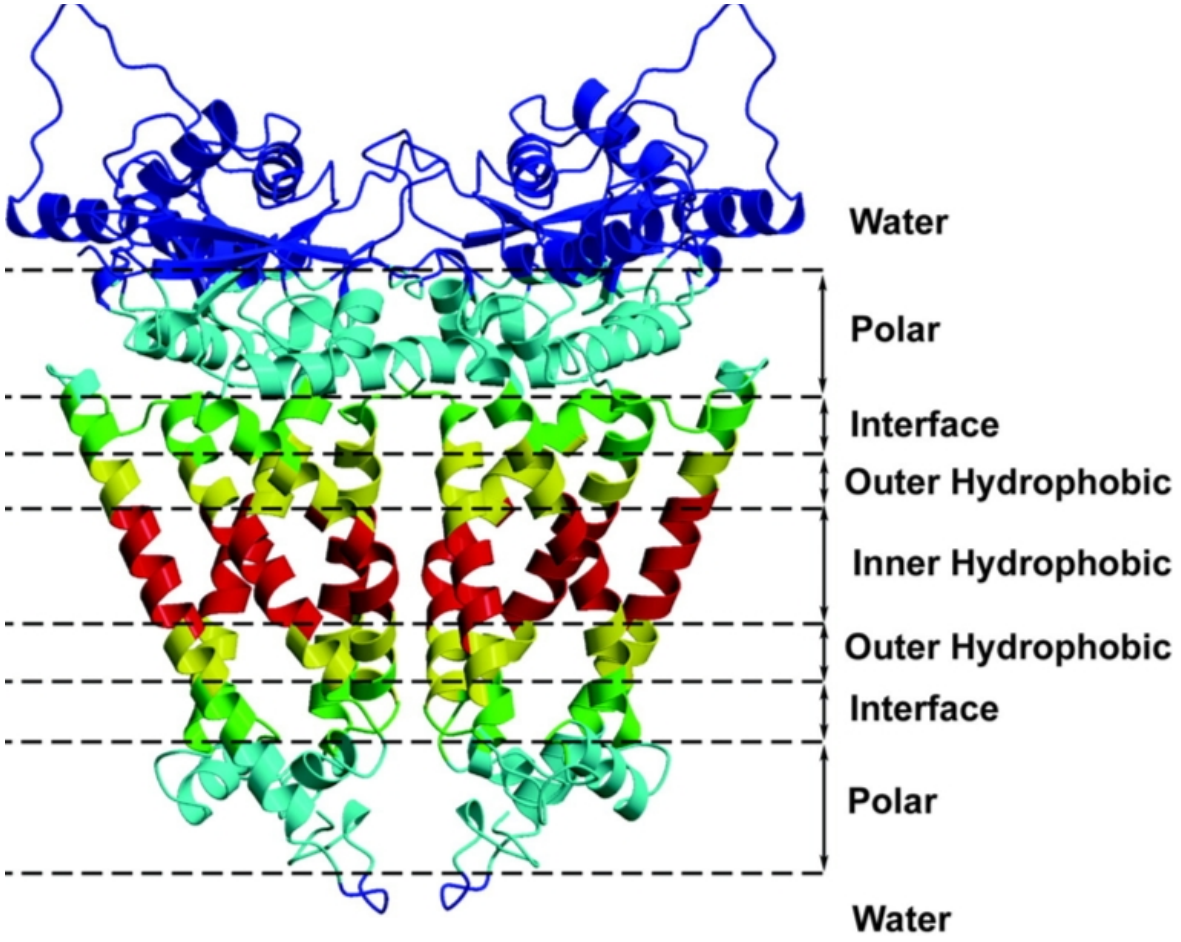
- if $e^{[-(Enew-Eold)/kBT]} > p$, accept structure
- if $e^{[-(Enew-Eold)/kBT]} < p$, discard structure



Rosetta overcomes energy barriers to find global minimum!!

Other score terms and functions

Membrane proteins:



Score functions for membrane proteins:

- mpframework_smooth_fa_2012
- ref2015_memb
- franklin2019

Constraints:

(Usually obtained from experimental data)

Atom Pair	Restrains a distance between Atom1 and Atom2
Dihedral	Restrains a dihedral angle
Angle	Restrains an angle
Coordinate	Restrains an atom to a fix XYZ position
Site	Restrains a residue to interact with another chain
...	

Score terms for other biomolecules:

biomolecule	term	description	unit	ref
noncanonical amino acids	mm_lj_intra_rep	repulsive van der Waals energy between two atoms from the same residue	kcal/mol	67
	mm_lj_intra_atr	attractive van der Waals energy between two atoms from the same residue	kcal/mol	67
	mm_twist	molecular mechanics derived torsion term for all proper torsions	kcal/mol	67
	unfolded	energy of the unfolded state based on explicit unfolded state model	AU[a]	67
	split_unfolded_1b	one-body component of the two-component reference energy, lowest energy of a side chain in a dipeptide model system	AU	in the SI
	split_unfolded_2b	two-body component of the two-component reference energy, median two-body interaction energy based on atom-type composition	AU	in the SI
carbohydrates	sugar_bb	energy for glycosidic torsions	kcal/mol	70
DNA	gb_elec	generalized Born model of the electrostatics energy	kcal/mol	107
RNA	fa_stack	$\pi-\pi$ stacking energy for RNA bases	kT	113
	stack_elec	electrostatic energy for stacked RNA bases	kT	114
	fa_elec_rna_phos	electrostatic energy (fa_elec) between RNA phosphate atoms	kT	62
	rna_torsion	knowledge-based torsional potential for RNA	kT	62
	rna_sugar_close	penalty for opening an RNA sugar	kT	62

[a]AU = arbitrary units.

(*Alford RF, 2017*)

Score terms for experimental data:

- Electron density
- Residual dipolar coupling (RDC)
- SAXs

References:

REF2015:

- Alford RF, et. al, **The Rosetta All-Atom Energy Function for Macromolecular Modeling and Design**. *Journal of Chemical Theory and Computation,* **2017.** *13* (6), 3031-3048
- Park H, et. al **Simultaneous Optimization of Biomolecular Energy Functions on Features from Small Molecules and Macromolecules** *Journal of Chemical Theory and Computation,* **2016.** *12* (12), 6201-6212

Old Scorefxn (Talaris):

- O'Meara MJ, et. al, **A Combined Covalent-Electrostatic Model of Hydrogen Bonding Improves Structure Prediction with Rosetta.** *Journal of Chemical Theory and Computation*, **2015**.
- Leaver-Fay A, et. al **Scientific benchmarks for guiding macromolecular energy function improvement.** *Methods in enzymology*, **2013**. 523: p. 109.

Links:

https://www.rosettacommons.org/docs/latest/rosetta_basics/scoring/score-types

https://www.rosettacommons.org/docs/latest/rosetta_basics/scoring/scoring-explained