

Rosetta Scoring Function



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Why we need a scoring function?

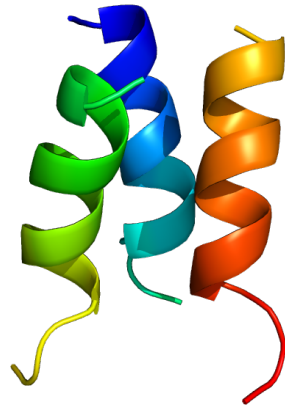
Example 1:

Same amino acid sequence, different conformations. Which one is the more stable?

Conformation A



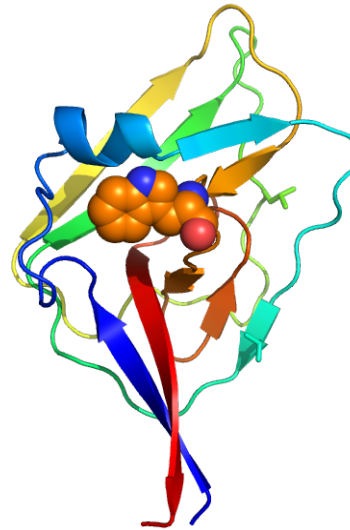
Conformation B



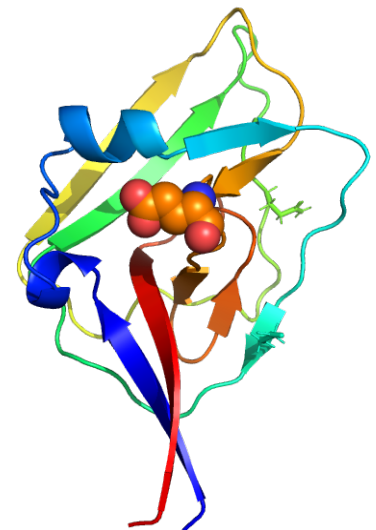
Example 2:

Different single point mutations (or multiple mutations). Which residue is the best one?

Mutation A



Mutation B



A scoring function is able to distinguish and rank different models.



How discriminate among A and B?

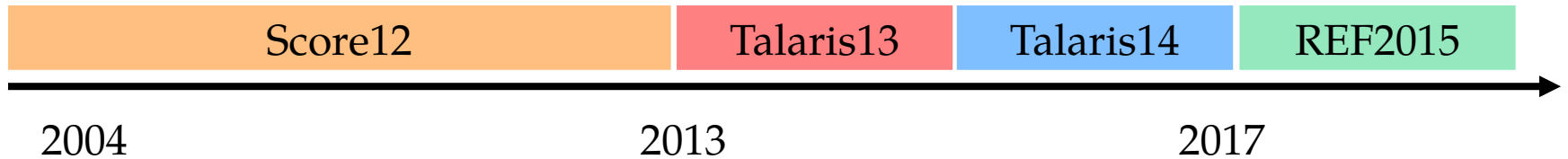
Scoring Function (or Energy Function)

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

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



Scoring Functions in Rosetta:



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

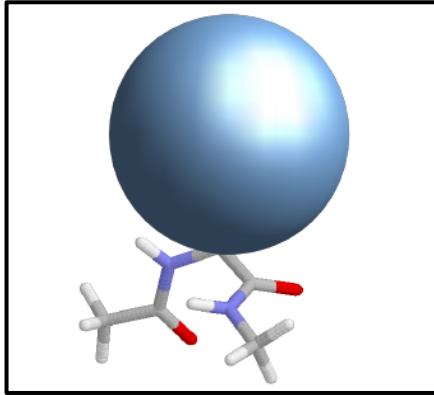
The newest scoring function (REF2015) can be converted to kcal/mol



Rosetta updates its scoring functions to improve the accuracy of the scoring.

Types of Scoring Functions:

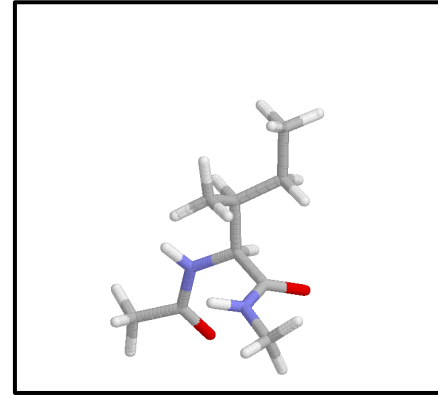
Low Resolution



Centroid-mode or Coarse-Grain
Simple energy function
Faster

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

High Resolution



Full-Atom or All-Atom
More accurate energy function
Time consuming

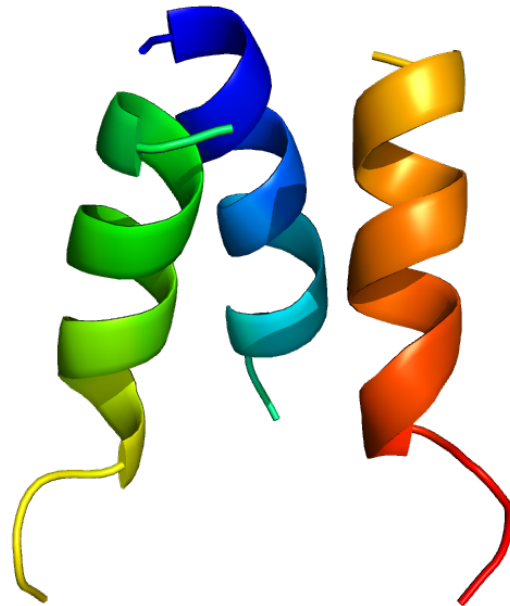
Local search of conformational space

Rosetta has multiple types of scoring function, depending on the needs.



Rosetta Scoring: The lower the better!

Conformation A



Total Energy = 253 REU

Conformation B



Total Energy = -82 REU

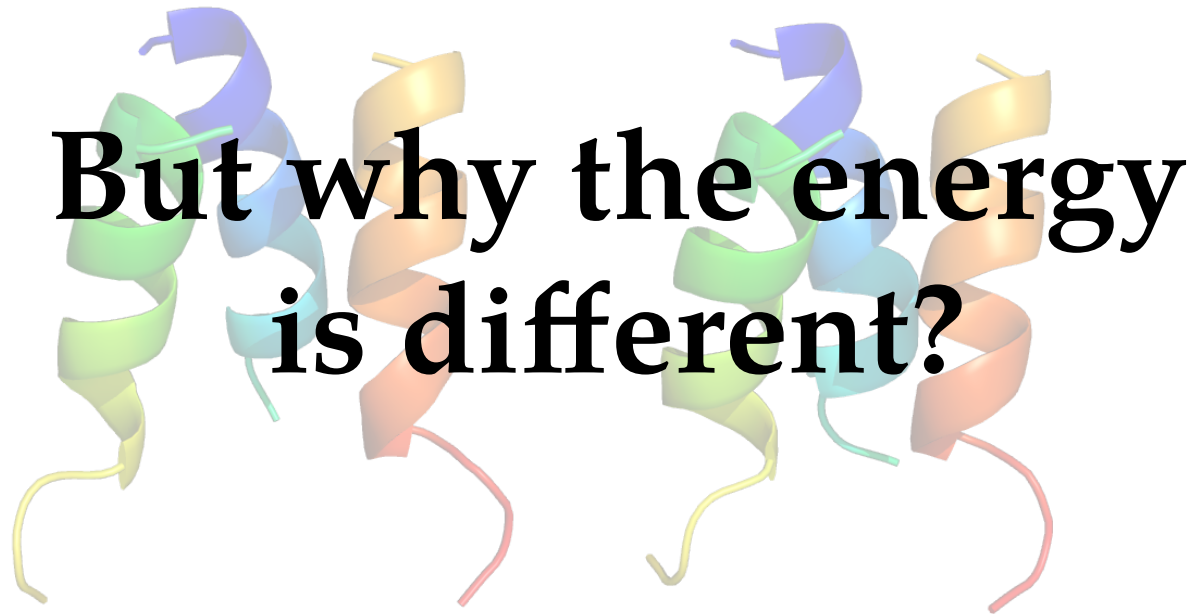
Conformation B is the more stable because it has the lower energy (-82 REU).



Rosetta Scoring: The lower the better!

Conformation A

Conformation B



Total Energy = 253 REU

Total Energy = -82 REU



Energy Terms

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

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

$$E_{\text{ref}} = \sum_i \Delta G_i^{\text{ref}}(\text{aa}_i)$$

$$E_{\text{fa_rep}}(i, j) =$$

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

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

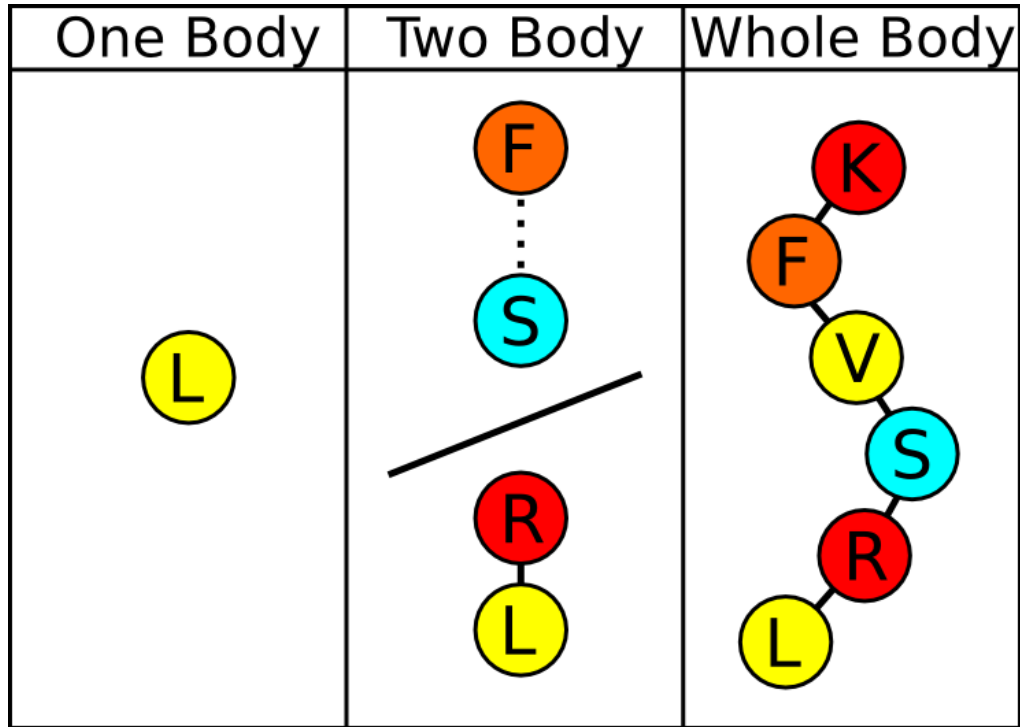
$$E_{\text{fa_atr}} =$$

$$\sum_{i,j} w_{i,j}^{\text{conn}} \begin{cases} -\epsilon_{i,j} & d_{i,j} \leq \sigma_j \\ \epsilon_{i,j} \left[\left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^{12} - 2 \left(\frac{\sigma_{i,j}}{d_{i,j}} \right)^6 \right] & \sigma_{i,j} < d_{i,j} \leq 4.5 \text{ \AA} \\ f_{\text{poly}}(d_{i,j}) & 4.5 \text{ \AA} < d_{i,j} \leq 6.0 \text{ \AA} \\ 0 & 6.0 \text{ \AA} < d_{i,j} \end{cases}$$

(Alford RF, 2017)



Energy Terms



Energy Terms

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



Energy Terms: Full-Atom

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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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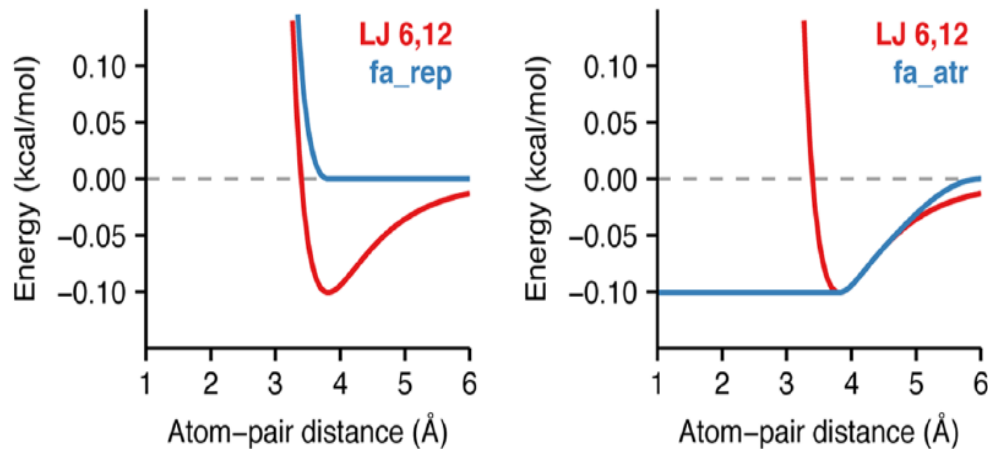
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Van der Waals Interactions



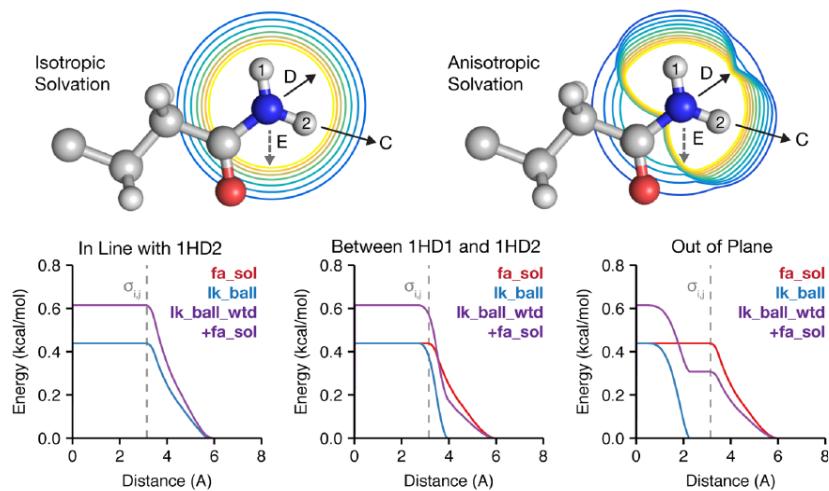
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Implicit Solvation



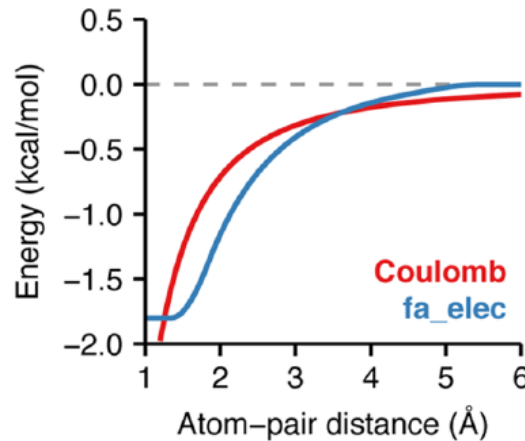
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Electrostatics



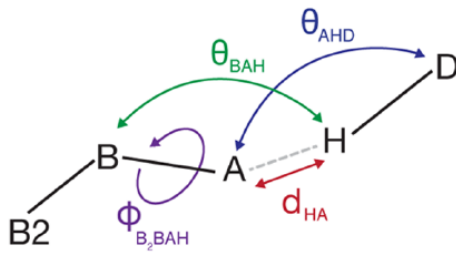
(Alford *et al.*, 2017)



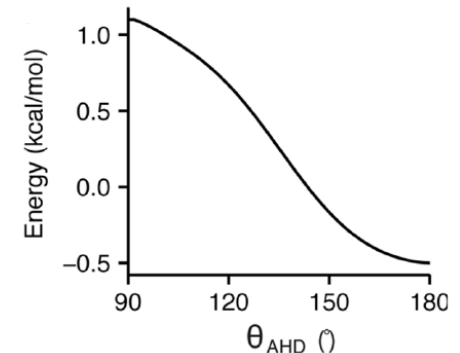
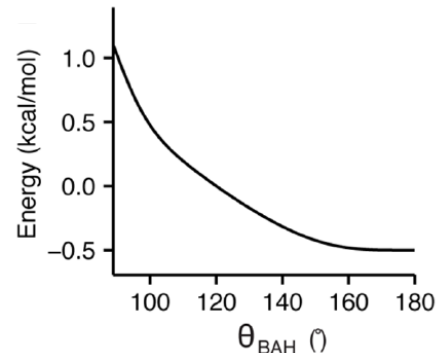
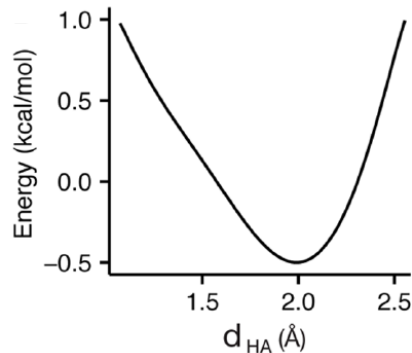
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Hydrogen Bonds



A = Acceptor H = Hydrogen
D = Donor B = Base B2 = Parent



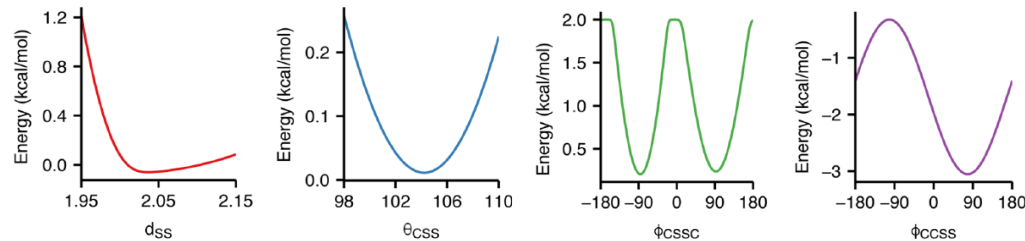
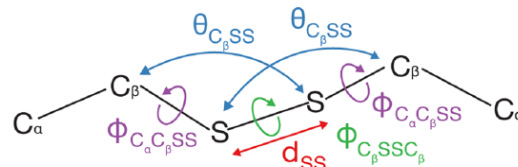
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Disulfide Bond



(Alford *et al.*, 2017)



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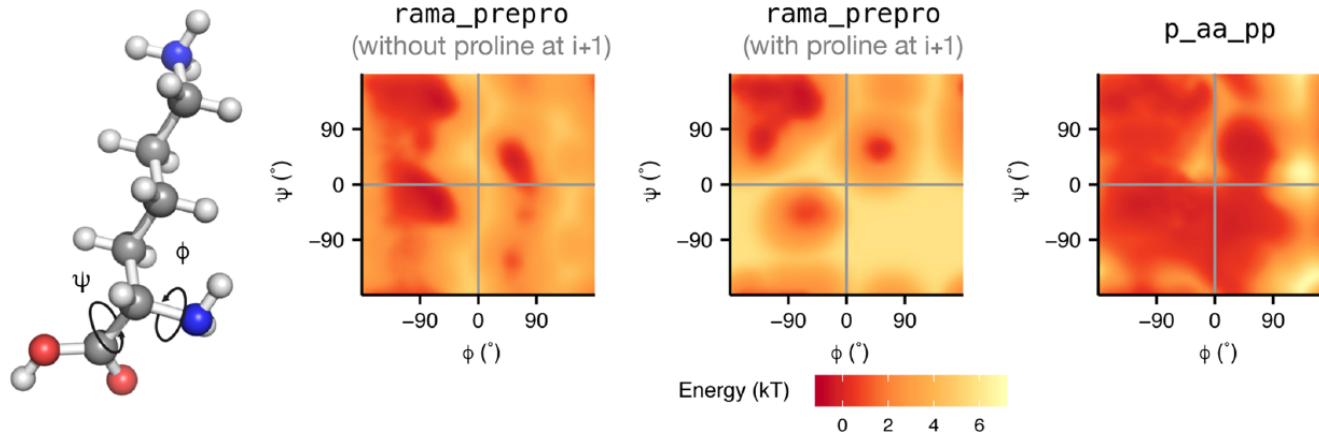
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Protein Backbone



(Alford RF, 2017)

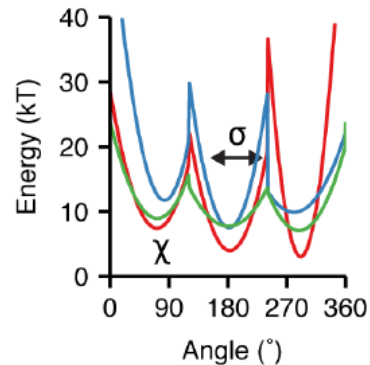
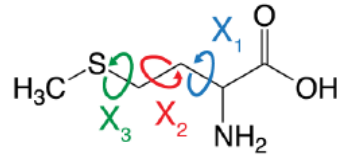


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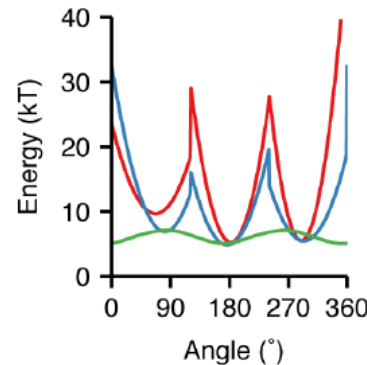
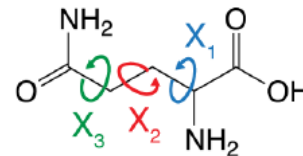
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Protein Side-Chains

rotameric



semi-rotameric



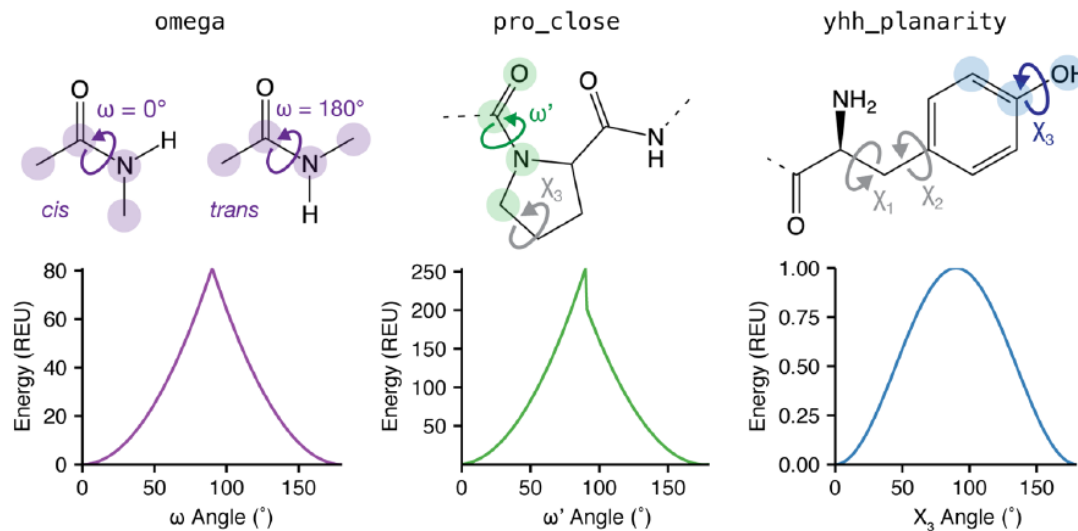
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rama_prepro	probability of backbone ϕ , ψ angles given the amino acid type	(0.45 kcal/mol)/kT	kT	50, 51
p_aa_pp	probability of amino acid identity given backbone ϕ , ψ angles	(0.4 kcal/mol)/kT	kT	51
fa_dun	probability that a chosen rotamer is native-like 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 ^α	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:

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

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

$$E_{\text{ref}} = \sum_i \Delta G_i^{\text{ref}}(\text{aa}_i)$$

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

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

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

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

^aAU = arbitrary units.

(Alford *et al.*, 2017)

Some weights for specific energy terms are set to zero!



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	$(0.45 \text{ kcal/mol})/kT$	kT	50, 51
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pro_close	penalty for an open proline ring and proline ω bonding energy	$(1.25 \text{ kcal/mol})/AU$	AU	51
yhh_planarity	sinusoidal penalty for nonplanar tyrosine χ_3 dihedral angle	$(0.625 \text{ kcal/mol})/AU$	AU	49
ref	reference energies for amino acid types	$(1.0 \text{ kcal/mol})/AU$	AU	1, 51

^aAU = arbitrary units.

(Alford RE, 2017)

You can modify the weights, if needed in special applications!



Modifying Weights:

Using the command line:

- 1 - score:weights <filename>
- 2 - score:set_weight <scoreterm₁> <wt₁> <scoreterm₂> <wt₂>
- 3 - score:patch <patchfile>

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

Using xml script file:

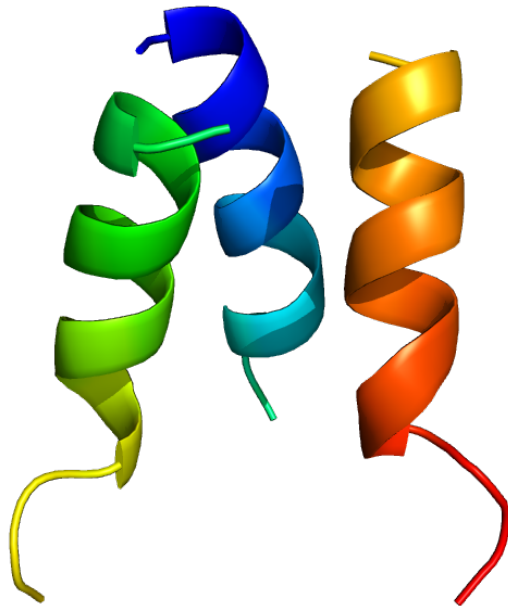
```
<ROSETTASCRIPTS>  
  <SCOREFXNS>  
    <ScoreFunction name="ligand_soft_rep" weights="ligand_soft_rep">  
      <Reweight scoretype="fa_elec" weight="0.42" />  
    </ScoreFunction>  
    <ScoreFunction name="hard_rep" weights="ligandprime" />  
  </SCOREFXNS>  
  <OUTPUT scorefxn="hard_rep" /> 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

(Alford RF, 2017)

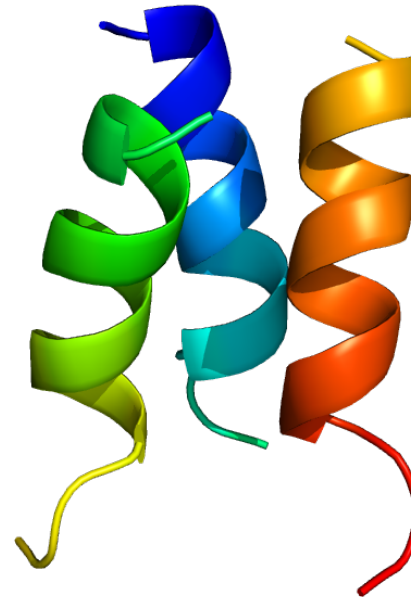


Total Energy:

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  0  -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  0  -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):

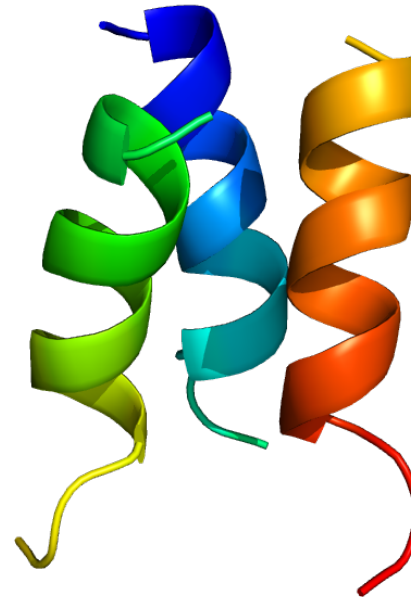
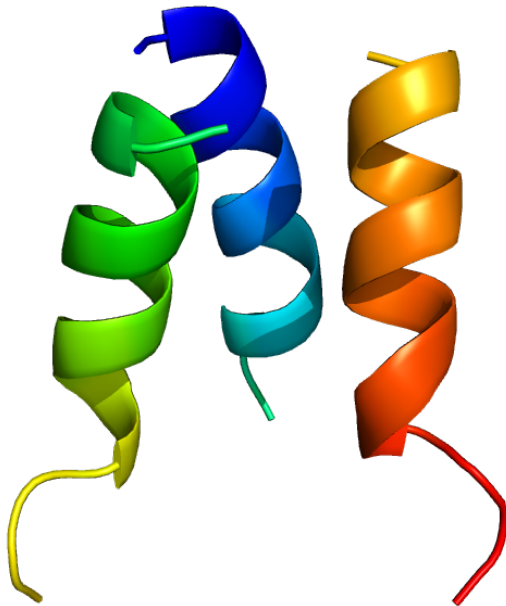
```
SCORE:  score  fa_atr      fa_rep  fa_sol  fa_intra_rep
fa_intra_sol_xover4  lk_ball_wtd  fa_elec  pro_close  hbond_sr_bb
hbond_lr_bb  hbond_bb_sc  hbond_sc  omega  fa_dun  p_aa_pp
yhh_planarity  ref  rama_prepro  description
SCORE:  253.063  -181.52  45.561  149.367  0.702
17.998  -7.402  -1.488  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)

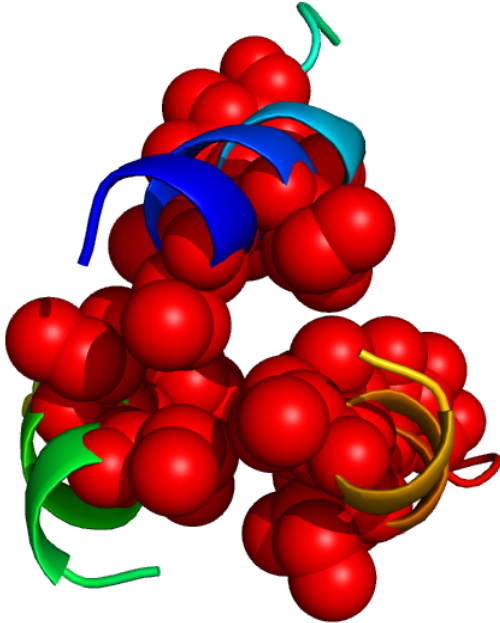


	score	fa_atr	fa_rep
A	253.1	-181.5	45.6
B	-82.1	-217.9	26.4

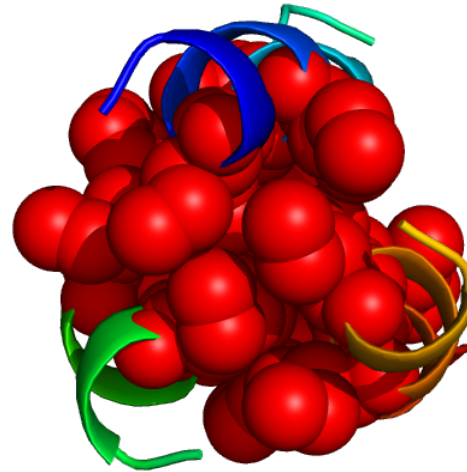


Total Energy:

Conformation A (253 REU)



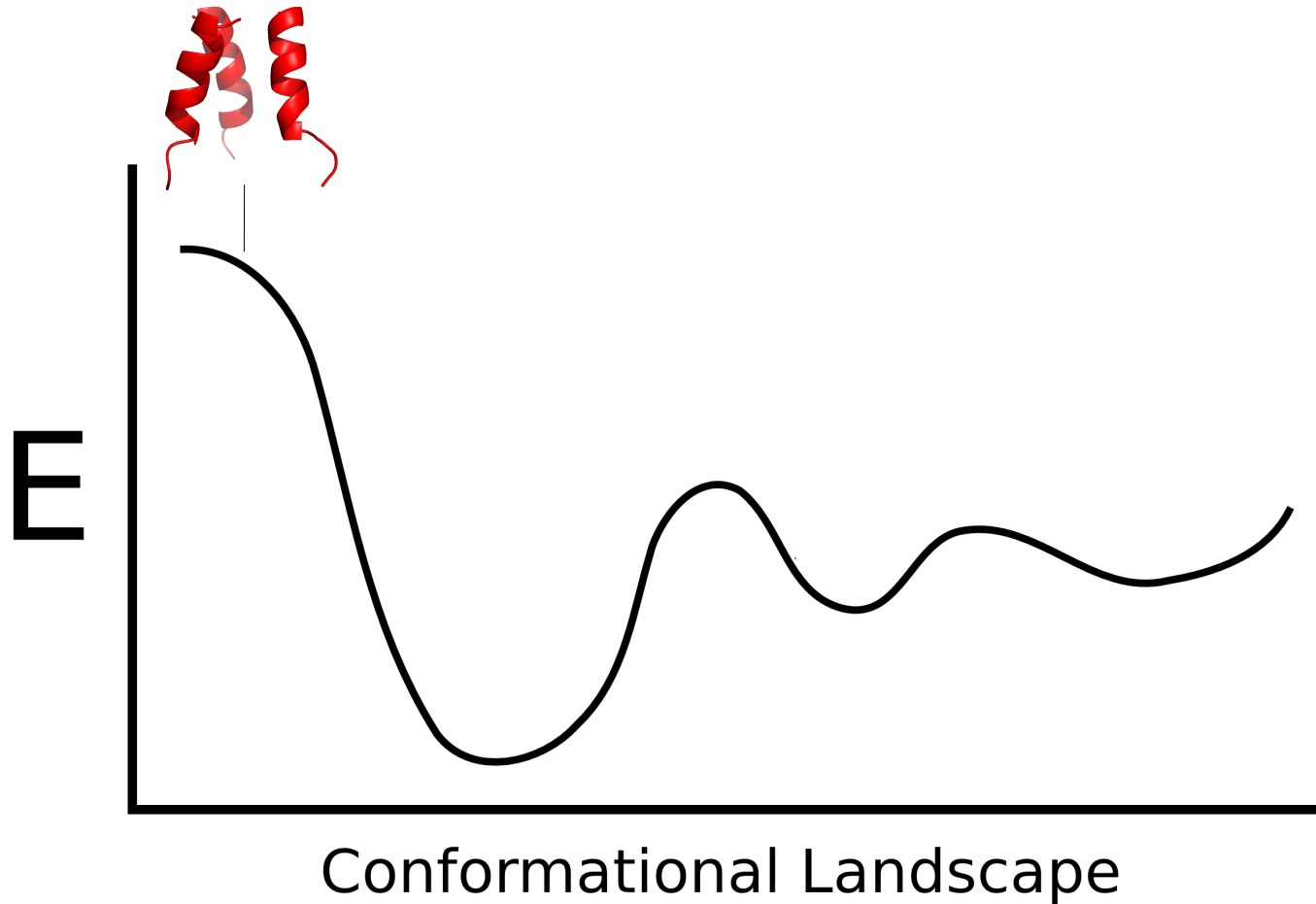
Conformation B (-82 REU)



	score	fa_atr	fa_rep
A	253.1	-181.5	45.6
B	-82.1	-217.9	26.4

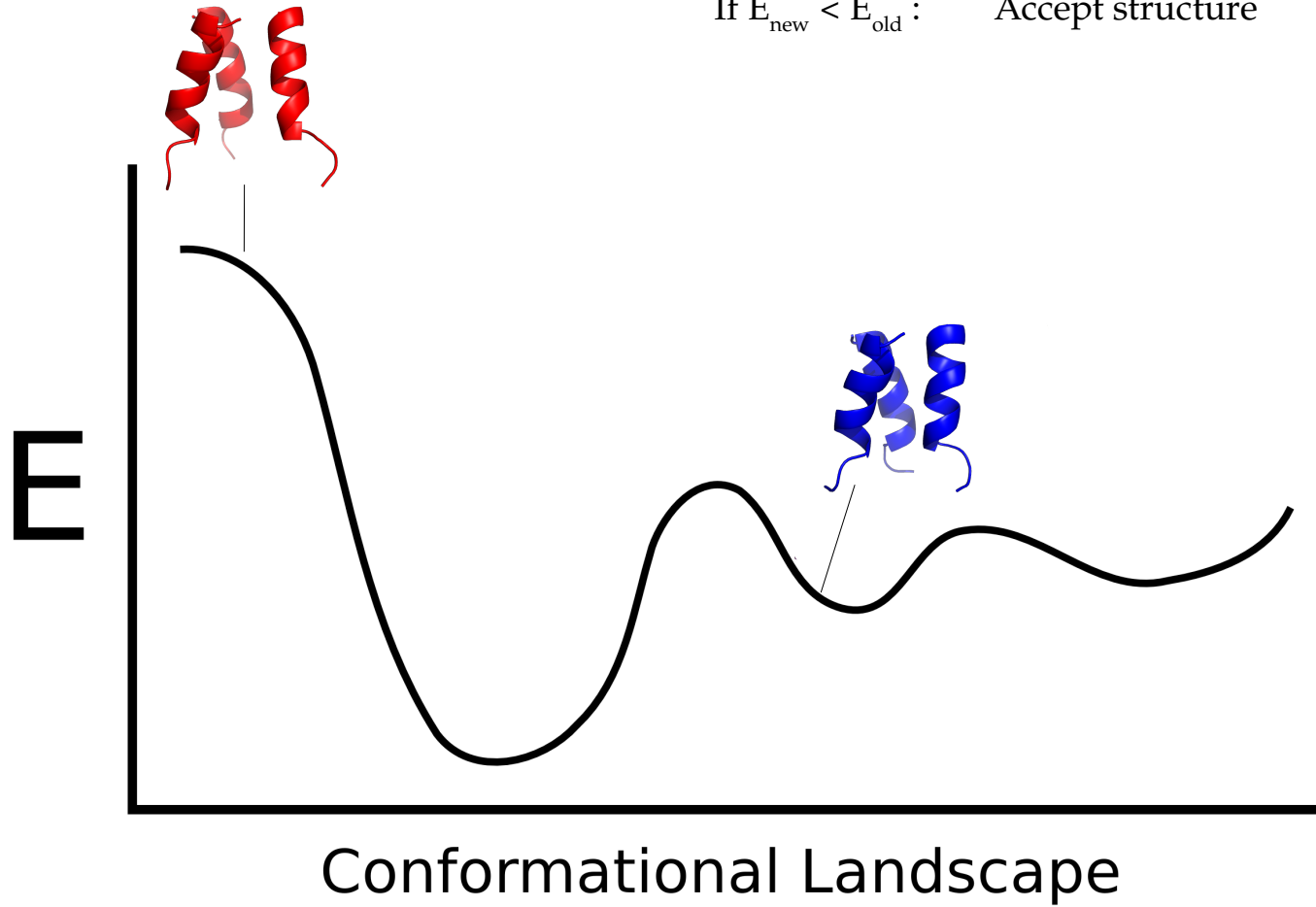


Monte-Carlo Sampling in Rosetta:

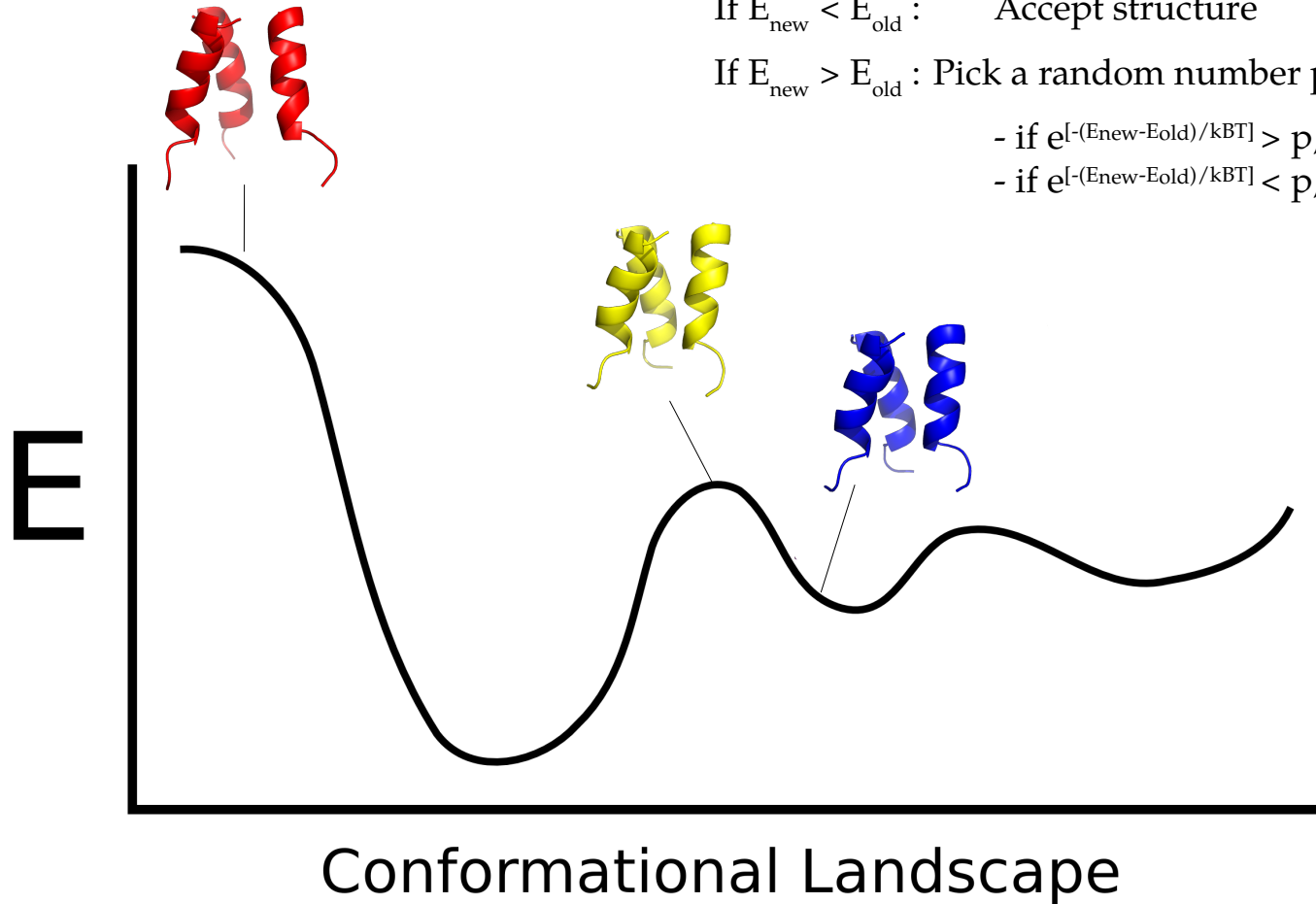


Sampling in Rosetta:

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



Sampling in Rosetta:



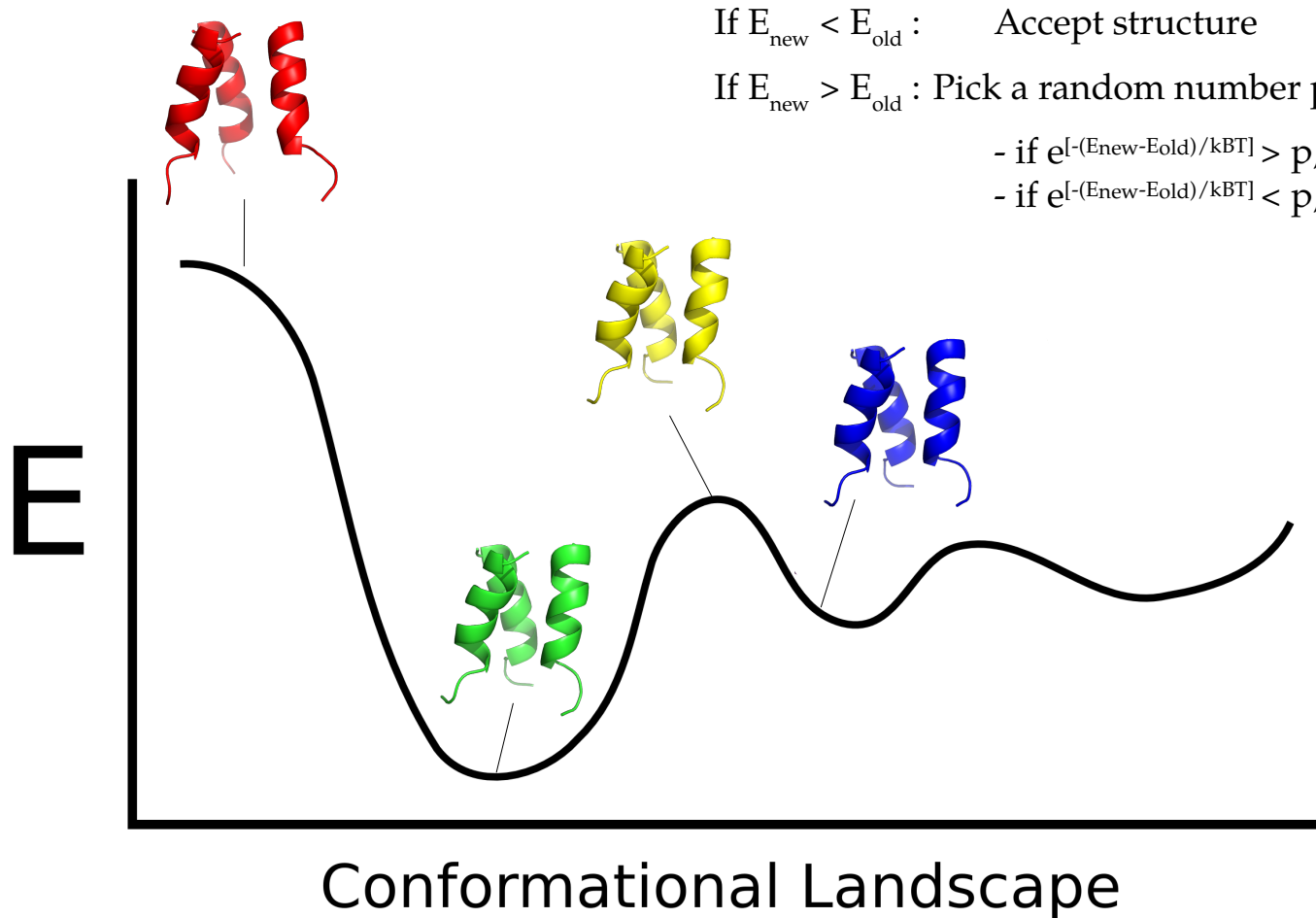
If $E_{\text{new}} < E_{\text{old}}$: Accept structure

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

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



Sampling in Rosetta:



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

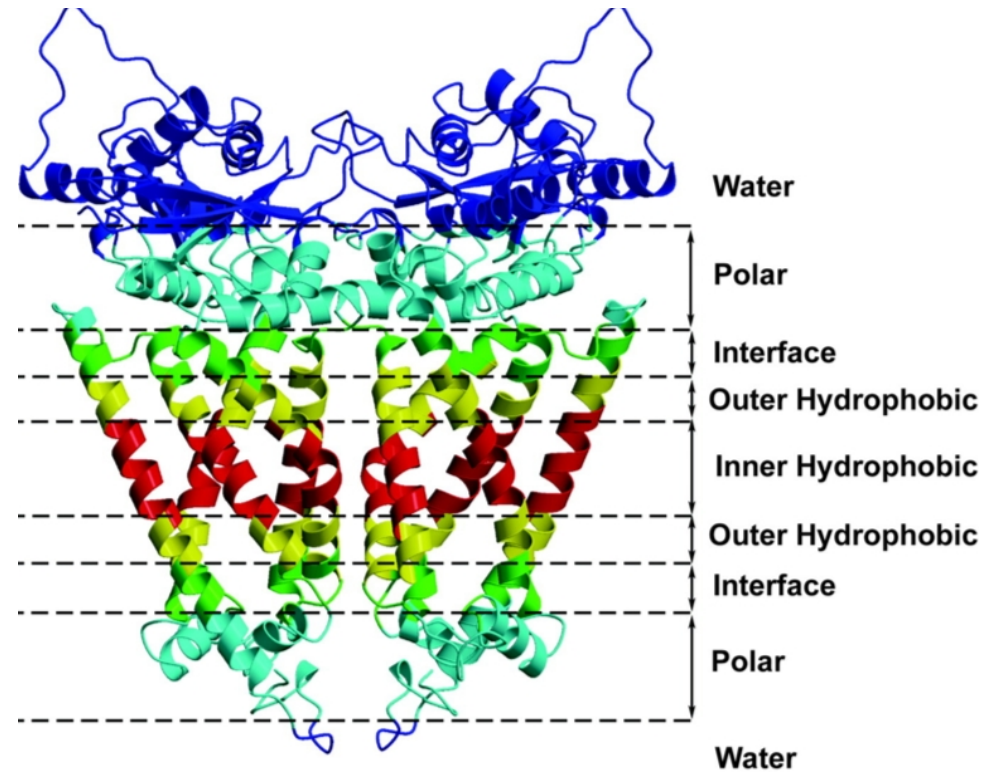
If $E_{\text{new}} > E_{\text{old}}$: Pick a random number $p(0, 1)$

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

Rosetta overcomes energy barriers to find global minimum.



Other functions: Membrane Proteins



Score functions for membrane proteins:

- `mpframework_smooth_fa_2012`
- `ref2015_memb`
- `franklin2019`



Other terms: 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
...	



Other terms: 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	π - π 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

^aAU = arbitrary units.

(Alford *et al.*, 2017)



Other terms: Experimental Data

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



Bibliography - Score Functions:

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

