Forschungszentrum Karlsruhe
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Protein Folding and Structure Prediction of Proteins Containing Disulfide Bridges

Overview

We used the all-atom protein force field PFF02 to calculate the internal free energy of the protein and search for the global free-energy minimum using of a stochastic optimization methods, in particular the basin hopping method. Various constraining potentials were employed to describe the covalent disulfide bonds between cysteine residues in following proteins: the β-hairpin 1KVG, the potassium channel blocker 1WQE, the antimicrobial peptide protegrin-1 (1PG1), and the pheromone 1HD6. Prediction accuracy of the results was assessed by comparing the simulated structures with experimental NMR structures.

PFF02
A free-energy force field

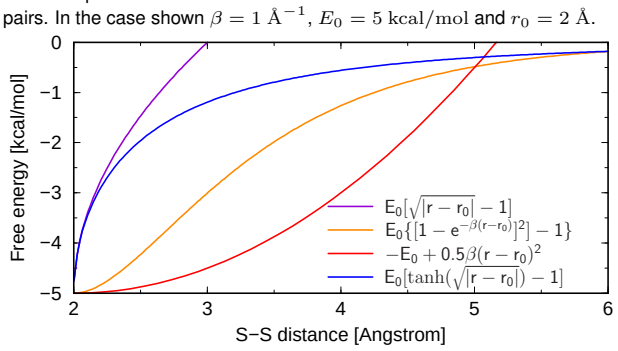
The native three-dimensional structure of a protein is assumed to occupy the global free energy minimum. We employ stochastic optimization methods to perform the search for the global minimum of the free-energy. The free-energy within the forcefield PFF02 of the state $[\vec{r}]$ is partitioned into several contributions:

$$G([\vec{r}]) = \sum_{ij} V_{ij}\left[\left(\frac{R_{ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{ij}}{r_{ij}}\right)^{6}\right] + \sum_{ij}\frac{q_i q_j}{\epsilon_{g_i g_j} r_{ij}} + \sum_i \sigma_i \cdot A_i + \sum_{\text{Hbonds}} V_{\text{hb}}$$

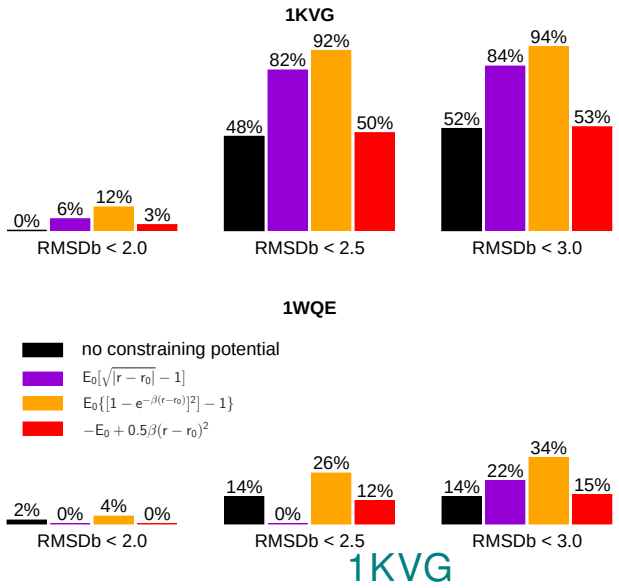
- Lennard–Jones Potential, V_{ij} and R_{ij} mean potential depth and equilibrium distance
- Electrostatic interaction, q_i and q_j are the partial charges of two atoms, $\epsilon_{g_i g_j}$ the group-specific dielectric constants, depending on the aminoacid type of atoms i and j
- Implicit solvent interaction by minimal accessible surface area, σ_i gives the free-energy per area unit, A_i is the surface area
- Short-range contributions to hydrogen bonding interactions, V_{hb}
- Torsional potential for backbone dihedral angles (not shown)
- Specific backbone electrostatics in segments with different secondary structure (not shown)

Constraining potential

This is a potential which favors the formation of disulfide bonds between predefined cysteine pairs. In the case shown $\beta = 1$ Å^{-1}, $E_0 = 5$ kcal/mol and $r_0 = 2$ Å.



Performance analysis
Occurrence of near-natural conformations

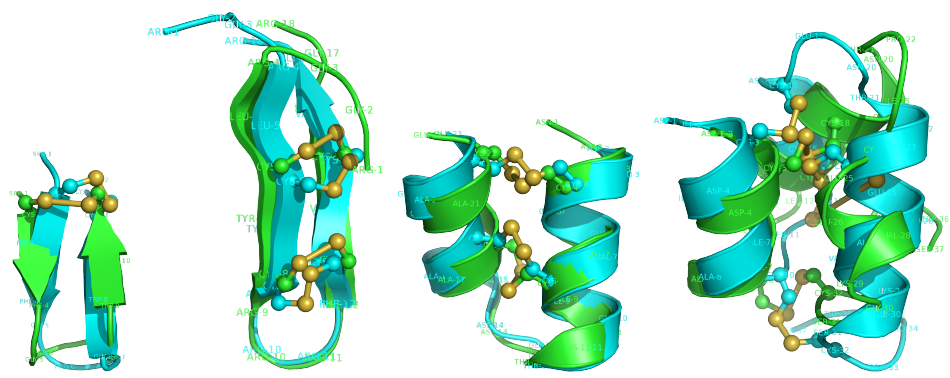


1KVG

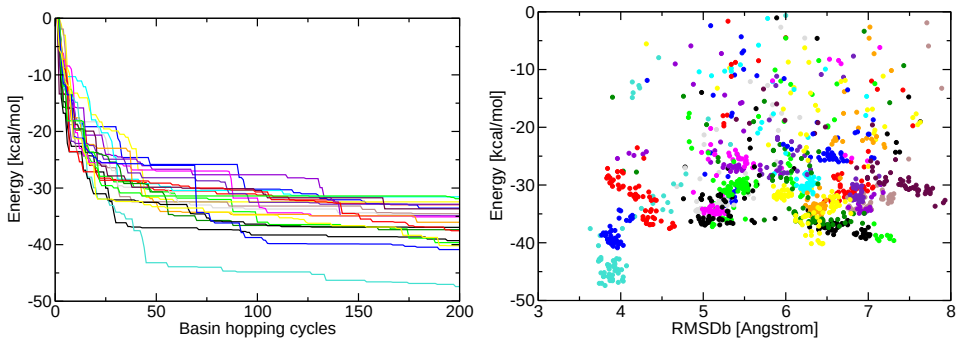
method	energy	RMSDb	r_{SS}, Å	Sequence and secondary structure
			2-11	SCHFGPLGWVCK
natural				CEEEETTEEEEC
no constr ext	-8.7	2.12	3.3	CBCBTTTBSCBC
Morse 2 ext	-8.2	2.12	2.9	CEEEETTEEEEC
Morse 5 ext	-9.9	2.25	2.7	CEEESSSSEEEC
sqrt 5 ext	-7.1	2.11	2.6	CEEESSSSEEEC

1WQE

method	energy	RMSDb	r_{SS}, Å		Sequence and secondary structure
			4–22	8–18	NDPCEEVCIQHTGDVKACEEACQ
natural					CCHHHHHHHHTCCHHHHHHHHC
no constr ext	-42.6	2.10	3.3	5.5	CHHHHHHHHHTCCHHHHHHHHC
Morse 2 ext	-43.6	1.94	2.8	2.9	CHHHHHHHHHTCCHHHHHHHHC
Morse 5 ext	-47.8	4.43	2.7	2.7	CHHHHHHSCSSTTTCHHHHHHHC
sqrt 2 ext	-41.9	6.04	11.8	6.2	CHHHHHHHHHTCCSSSCHHHHHC
sqrt 5 ext	-39.9	6.28	10.7	7.3	CCSBCSSSBSSCCSCSBCSSBCC

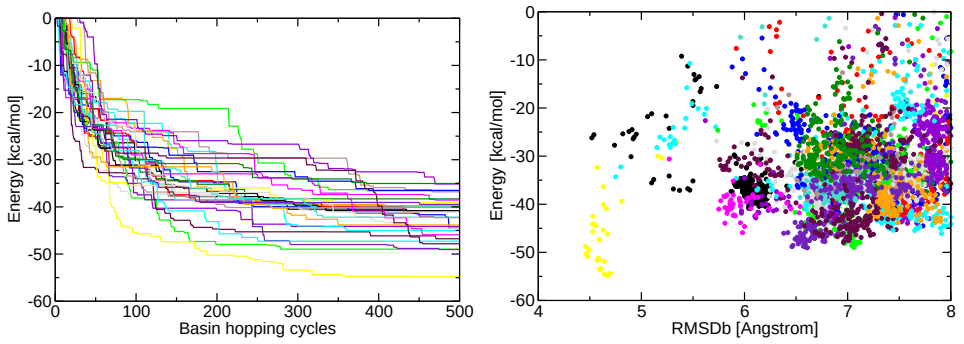


1PG1



method	energy	RMSDb	r_{SS}, Å		Sequence and secondary structure
			6-15	8–13	RGGRLCYCRRRFCVCVGR
natural					CCSEEEEEETTEEEEEEC
no constr ext	-38.5	7.24	8.8	4.7	CTTCHHHHHHTCCSSSCC
Morse 2 ext	-38.9	4.57	5.0	2.9	CCSEEEEECSSCEEEECC
Morse 5 ext	-47.4	3.81	2.7	2.7	CCSSEEEEETTEEEEECC
sqrt 5 ext	-40.1	4.89	2.6	2.6	CCSEEEEETTEEEEESCC

1HD6



method	energy	RMSDb	r_{SS}, Å			Sequence and secondary structure
			3–18	10–32	15–24	DICDIAIAQCSLTLCQDCENTPICELAVKGSCPPPWS
natural						CHHHHHHHHTCHHHHTTSTTHHHHHHHHHHSCSSCC
no constr ext	-50.8	5.26	14.9	5.3	13.0	CHHHHHHHHHHCCSTTCCCHHHHHHHHTSSSTTTC
no constr nat	-59.7	2.15	3.5	5.6	4.6	CHHHHHHHHTCHHHHHHHSCHHHHHHHHHTSSCTTTC
Morse 5 ext	-59.4	6.31	2.6	2.6	2.7	CHHHHHHHHSCCHHHHHTSSHHHHHHHHHHCCCCCCC
Morse 5 nat	-62.8	1.53	2.6	2.7	2.7	CHHHHHHHHTCHHHHHHSSCHHHHHHHHHHHSSTTTC
Morse 2 ext	-54.8	3.60	3.2	2.9	6.9	CHHHHHHHHTCHHHHHHHHHSSSCHHHHHTCCCTTTC
Morse 2 nat	-57.7	0.94	2.8	2.8	2.9	CHHHHHHHHTCHHHHHHSTTHHHHHHHHHHHSSTTTC
tanh ext	-53.1	7.50	2.8	6.5	8.6	CCSEEEEEECCHHHHHHHHHSSBEEEEESSSCCCSBC
tanh nat	-59.8	2.38	3.0	11.3	3.0	CHHHHHHHHTCHHHHHHSSSHHHHHHHHHTSSCTTTC

Conclusions

- For all proteins studied, inclusion of the constraining potential resulted in improved RMSDb values compared to constraint-free simulations.
- The potassium channel blocker 1WQE can be folded to near-native conformation without constraining potential; however, this is computationally much less efficient than folding with constraining potential.
- All proteins folded qualitatively correctly from extended to native conformations with inclusion of the constraining potential.
- For 1PG1 β-sheet secondary structure is more favorable than the α-helical structure in presence of constraining potential.
- On the example of 1HD6 the interplay between formation of helical regions and closure of disulfide bridges can be studied.
- The Morse potential exhibits better efficiency and accuracy compared to other constraining potentials.

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HELMHOLTZ GEMEINSCHAFT

Karlsruhe Institute of Technology