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Reduced Protein Model as a Tool in the Homology Modeling

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Abstract

Motivation. Theoretical prediction of protein structures is important because the number of sequenced proteins grows much faster than the number of experimentally determined 3D structures. Among theoretical methods, homology or comparative modeling of unknown 3D protein structures (targets) has been established. It is based on experimental structures of proteins (templates) with sequence similarity to the target, taking advantage of the assumption that similarity between sequences implies also structural similarity. The method is, however, limited by the degree of sequence identity. Frequently, the target- template sequence alignment is non-uniform along the sequence. In order to improve the method in regions of low target- template similarity, we have developed a reduced protein modeling approach. It allows us to generate a large number of putative conformations by energy minimization and subsequently to pre-select the most favorable conformations. The force field is based on the concept of residue- residue contact energies. Reduced structures can be translated to atomic resolution, and further evaluated.

Method. Randomly generated protein structures are subjected to energy minimization employing a reduced protein model and using positional restraints for conserved parts of the protein structure as well as distance constraints to enforce a preset secondary structure. The alpha-helical test protein results are compared to the experimental protein structure.

Results. There is a correlation between energy of a reduced protein structure, and its similarity to the experimentally known structure, evaluated by the root mean square deviation of corresponding atoms. Low energy structures can be pre-selected for further refinement.

Conclusions. Our reduced protein model can be a tool to improve homology modeling in regions of low target-template sequence similarity.

Keywords. Homology modeling; reduced protein model; energy minimization; residue-residue contact energies;

Abbreviations and notations

rmsd, root mean square deviation

1 INTRODUCTION

The formation of the three-dimensional structure of a protein from a given sequence of amino acids is one of the most important problems in molecular biology, in particular in molecular modeling. There are two basic theoretical approaches: *ab initio* methods have been developed in order to predict the 3D protein structure from scratch, whereas comparative modeling of protein structures is based on recognition of homology (in most cases sequence similarity) to a template of an experimentally known structure. The latter is limited by the degree of the target-template

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sequence identity. Frequently, the quality of the target- template sequence alignment is non-uniform along the sequence: parts can be modeled with a high confidence, whereas other parts differ strongly from the template. Segments of the target sequence that have no equivalent regions in the template structure (insertions or loops) are the most difficult regions to model [1]. They are often larger than small loop segments. Since at atomic resolution the accurate loop prediction is limited to short loops of up to 9 residues [1, 2], one needs to evaluate a large number of possible conformations.

For pre-selection of possible protein segment 3D topologies, we propose an application of a reduced protein model. It allows a very rapid generation of protein segment conformations, compatible with the boundaries imposed by those parts of the protein chain, that can be accurately modeled based on the template structure. In contrast to threading based fold recognition approaches, the present method allows in principle the generation of partially new topologies that are derivatives of existing protein fold topologies. The idea is schematically presented in Fig. 1, and outlined in the following.

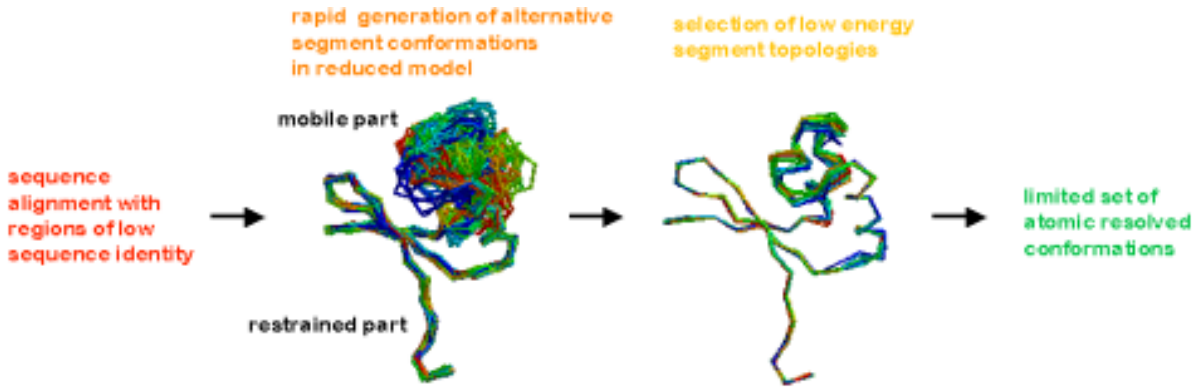


Fig. 1 Schematic presentation of the reduced model approach to homology modeling.

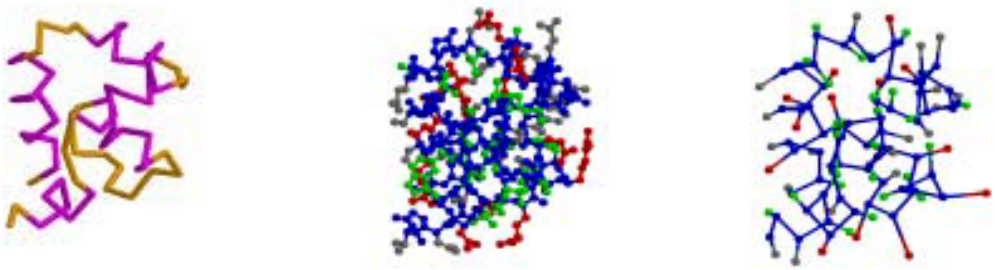
A number of reduced protein models have been developed over the years [3-9] in most cases meaningful only for particular systems, or particular properties. The present model is not intended to reproduce 3D protein structure accurately *ab initio*. It is a supporting tool in homology modeling, as in fact the majority of the target structures is modeled according to principles of the technique, applied e.g. in software Modeller [10]. Since some segments are, indeed, predicted from scratch, the present method can be thought of as a kind of bridge, joining the two basic approaches of theoretical protein structure determination.

In the present study, we want to report test results for a small alpha-helical gene regulation

protein, amino terminal domain of phage 434 repressor, PDB- entry 1r69 [11]. It contains 63 amino acid residues with 5 alpha helices joined by loops (Fig. 2a). Pairs of consecutive helices (1-2, 2-3, 3-4, 4-5) including loops between them are our mobile segments for test purposes, which leads to 4 tests in total. In each case, the rest of the protein was restrained to experimental positions, so the protein mobility pattern can be described as R(estrained)-M(obile)-R(estrained) in each of the four cases. Several hundreds of energy minimized conformations were generated for each case. On the basis of the reduced model energy function the favorable conformations were generally relatively close in rmsd to the experimental structure.

2 MATERIALS AND METHODS

The phage 434 repressor (further referred to as 1R69) was chosen as a test example to evaluate the reduced model performance in segments containing alpha helices and one loop. Although the chain is relatively short (63 residues), it contains 16 of 20 amino acids types (exceptions are CYS, HIS, MET and TYR; parameters for those residues are given in the force field description as well). Our test protein is presented in Fig. 2a-c. The alpha- helical type of 1R69 can be recognized from Fig. 2a. In Figs. 2 the difference between atomic resolution (2b) and reduced representation (2c) is demonstrated.



a b c

Fig.2 Crystal structure of 1R69: a) backbone with helical segments indicated magenta, b) atomic resolution, c) reduced representation. In b) and c) protein backbone is in blue, hydrophobic side-chains in green, charged side chains in red, others in grey.

The protocol for reduced structure generation and evaluation is as follows:

1. For a given protein sequence the topology file is generated. Parameters for particular residues depend on the residue type and the known (as in our test case) or predicted secondary structure of the protein.

2. The initial structure, based on the original pdb file, is prepared. For each residue, positions of two pseudo atoms, CA and CB, are needed. CA positions are simply original C-alpha atom coordinates. The equilibrium CB atom positions for each residue are given by the average distance of the center of geometry of each side chain with respect to the CA position of the residue (r_B stored in the topology file and given in Table 1).

3. The next step is the division of the protein into segments that will be treated as restrained (R) or mobile (M). As mentioned above, experiences so far are based on R-M-R structure scheme, but in principle the number of M's separated by R's can be greater than 1. Such cases will be investigated in the future. According to the R-M-R division, restraint data file for R segments is prepared. If the M segment contains regular secondary structures (alpha helices or beta strands), distance constraints files are additionally prepared, so that this conformation could be retained during energy minimization.

4. The pseudo atoms of the M segment are randomly placed initially and the energy minimization of the whole structure follows. Finally obtained energy minima are subjected to evaluation, according to the total energy calculated for each of them.

The energy minimization is performed with the use of the conjugate gradient algorithm. The interactions are defined for each of the terms in the following expression for the total energy:

$$E_{tot} = E_{bonds} + E_{bondangles} + E_{torsions} + E_{impropers} + E_{nonbonded} + E_{restr} + E_{constr} \quad (1)$$

The first three terms have the standard form of molecular mechanics force fields with quadratic bond length and bond angle terms between consecutive pseudo atoms of the chain and cosine terms to describe the dihedral angle energy for the reduced model chain. The parameters are based on the statistical evaluation of experimental protein structures. In addition an improper dihedral between three consecutive CA atoms and a CB pseudo side chain atom was used to control the chirality of the side chain placement. The bonded interactions provide the integrity of a reduced chain representation. In folded structures contacts between residues close to each other in space are of special importance. They are described by pairwise non-bonded interactions:

$$E_{nonbonded} = \sum_{i<j} E_{CAiCAj} + E_{CAiCBj} + E_{CBiCAj} + E_{CBiCBj} \quad (2)$$

"CA" terms in (2) are residue type independent and are given by a soft van der Waals type 6-8 expression:

$$E_{ij} = \begin{cases} \dfrac{A}{r_{ij}^{8}} - \dfrac{B}{r_{ij}^{6}} & ; \quad 0 < r_{ij} < r_0 \\ 0 & ; \quad r_{ij} \geq r_0 \end{cases} \quad (3)$$

where B=0.001 [kJmol^{-1}nm^{6}] and A=0.0022 [kJmol^{-1}nm^{8}] for CA-CB interactions, and 0.001 for CA-CA ones. r_0 is the "cut-off" value of 0.8 nm.

The residue-specific non-bonded interactions are parameterized as CB-CB contacts. Miyazawa and Jernigan [12] provide a list of pairwise contact energies, obtained on the basis of experimental folded protein structures. Some of these values are positive, some negative, and some equal to 0; since the last case is not desirable for van der Waals type parameterization, we replace it by a value of –0.001 in RT units; this does not lead to significant changes but is more convenient from mathematical point of view. Two cases should be regarded:

I.

$\varepsilon_{ij} < 0$

in this "normal" van der Waals type case, the energy is defined as:

$$E_{ij} = \begin{cases} \dfrac{A_{ij}}{r_{ij}^{8}} - \dfrac{B_{ij}}{r_{ij}^{6}} & ; \quad 0 < r_{ij} < r_{ij}^{e} \\ RT\varepsilon_{ij} & ; \quad r_{ij}^{e} \leq r_{ij} < r_{ij}^{e} + \Delta r \\ \dfrac{A_{ij}}{(r_{ij} - \Delta r)^{8}} - \dfrac{B_{ij}}{(r_{ij} - \Delta r)^{6}} & ; \quad r_{ij}^{e} + \Delta r \leq r_{ij} \leq r_{cutoff} \\ 0 & ; \quad r_{ij} > r_{cutoff} \end{cases}$$

where $r^{e}_{ij} = r^{e}_{1/2}(i) + r^{e}_{1/2}(j)$ (i.e. it is the equilibrium or minimum energy distance between pseudo atoms CB_i and CB_j; see Table 1), ε_{ij} denotes contact energy CB_i-CB_j taken from [12], Δr=0.2 nm and r_{cutoff}=50 nm. B_{ij} and A_{ij} are related to ε_{ij} and r^{e}_{ij} by expressions:

$$B_{ij} = -4(r_{ij}^{e})^{6} RT\varepsilon_{ij} \quad (4)$$

$$A_{ij} = 3(r_{ij}^{e})^{2} B_{ij} \quad (5)$$

II

$\varepsilon_{ij} > 0$

the procedure is as follows: primarily, for given values of r^{e}_{ij} and ε_{ij}, A_{ij} and B_{ij} are calculated as in eqs. (4) and (5), with the negative value $-\varepsilon_{ij}$ instead of ε_{ij}. Then, r^{t}_{ij} is defined as the value of r_{ij}, for which the energy function (3), with the minimum value $-\varepsilon_{ij}$, takes the opposite value of ε_{ij}. Due to the nature of the potential, this is only slightly less than r^{e}_{ij}, obtained analogically as in the case I, for the opposite value of ε_{ij}. Finally, we have:

$$E_{ij} = \begin{cases} \dfrac{A_{ij}}{r_{ij}^{8}} - \dfrac{B_{ij}}{r_{ij}^{6}} & ; \quad 0 < r_{ij} < r_{ij}^{t} \\ RT\,\varepsilon_{ij} & ; \quad r_{ij}^{t} \le r_{ij} < r_{ij}^{t} + \Delta r \\ -\dfrac{A_{ij}}{(r_{ij} - \Delta r)^{8}} + \dfrac{B_{ij}}{(r_{ij} - \Delta r)^{6}} & ; \quad r^{t} + \Delta r \le r_{ij} \le r_{cutoff} \\ 0 & ; \quad r_{ij} > r_{cutoff} \end{cases}$$

parameters Δr and r_{cutoff} are same as in case I. To illustrate non-bonded energy functions in both cases, examples are presented in Fig. 3. All other interaction functions are similar to one of the two types.

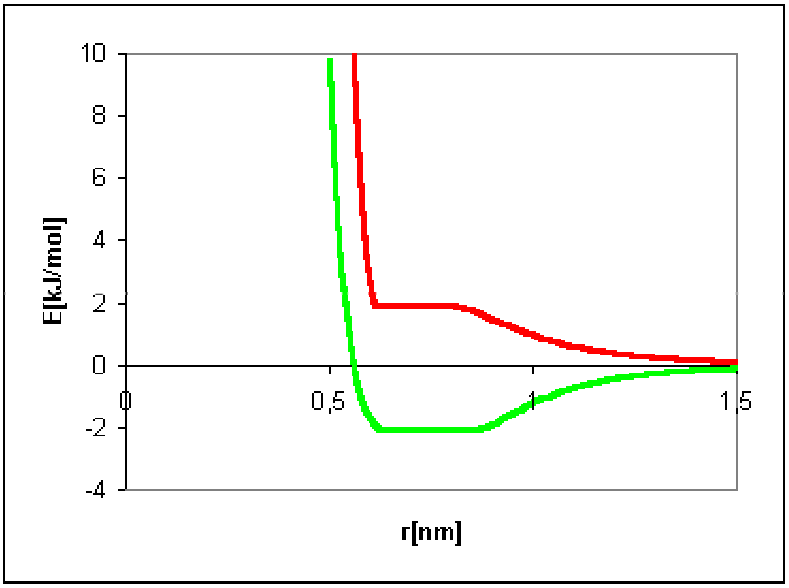


Fig. 3 Potential energy function for LEU-LEU (green) and LYS-LYS (red) pairwise contacts.

The division into segments implies the use of positional restraints for restrained parts of the protein. If the initial coordinates of a given pseudo atom *i* are x_{0i}, y_{0i} and z_{0i}, and during energy minimization it moves to x_i,y_i,z_i, the restrained energy is given by:

$$E_{restr} = \frac{1}{2} k_{restr} \sum_i (x_i - x_{0i})^2 + (y_i - y_{0i})^2 + (z_i - z_{0i})^2 \qquad (6)$$

the sum in (6) is over all restrained atoms. For the mobile parts in the reduced representation and with the use of the described force field alone, regular secondary structures, like alpha helices or beta sheets, are only weakly stabilized. In the present test cases, we include information on the secondary structure of the mobile part by employing secondary structure specific distance constraints during energy minimization. That is we assume that it is possible to predict the secondary structure of the mobile segment accurately. If there are M alpha helices 1,2,...M, and the length of each of them is L_1, L_2, ..., L_M, L_i>3, then the constraint energy, E_{constr}, is given by:

$$E_{constr} = \frac{1}{2} \sum_{i=1}^{M} [\sum_{j=1}^{L_i - 2} k_{13} (r_{CA_j CA_{j+2}} - r_{13})^2 + \sum_{j=1}^{L_i - 3} k_{14} (r_{CA_j CA_{j+3}} - r_{14})^2] \qquad (7)$$

i.e. constraints are imposed on CA pairs of type 1-3 and 1-4. Values of parameters k_{13}, r_{13}, k_{14} and r_{14} from eqs. (6) and (7) are collected in Table 2.

residue type	r_B[nm]	$r^e_{1/2}$[nm]
ALA	0.1621	0.1964
ARG	0.4824	0.3535
ASN	0.2616	0.2719
ASP	0.2579	0.2732
CYS	0.2328	0.2438
GLN	0.3528	0.3024
GLU	0.3535	0.3015
GLY	0.1000	0.1710
HIS	0.3151	0.3011
ILE	0.2558	0.3023
LEU	0.2715	0.2906
LYS	0.3945	0.3272
MET	0.3589	0.2940
PHE	0.3468	0.3212
PRO	0.1929	0.2746
SER	0.1998	0.2413
THR	0.2104	0.2723
TRP	0.3804	0.3481
TYR	0.3936	0.3389
VAL	0.2125	0.2788

Table 1 Residue type-dependent force field parameters

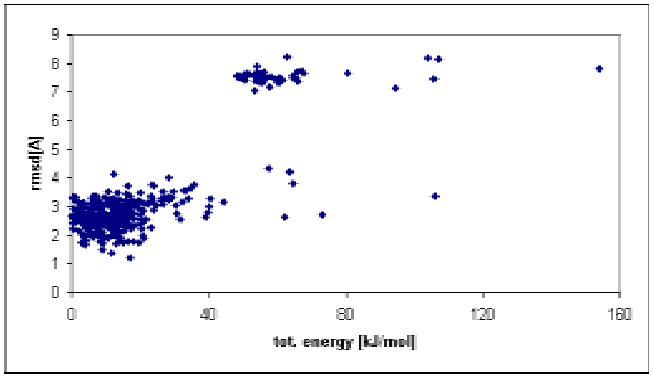
parameter [unit]	value
$k_{restr}[kJmol^{-1}nm^{-2}]$	2000
$k_{13}[kJmol^{-1}nm^{-2}]$	5000
$r_{13}[nm]$	0.545
$k_{14}[kJmol^{-1}nm^{-2}]$	5000
$r_{14}[nm]$	0.515

Table 2 Restrained/constrained force field parameters.

3 RESULTS AND DISCUSSION

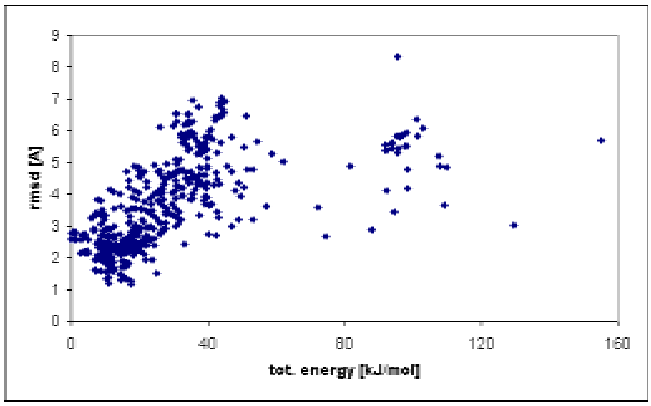
Our test protein 1R69 contains 5 alpha helices (first and last residue in brackets): 1(#2-#12), 2(#17-#24), 3(#28-#35), 4(#45-#51) and 5(#56-#61). We have decided to treat consecutive pairs of them as mobile segments: 1-2, 2-3, 3-4 and 4-5, including loops between these regular fragments, so that a protein scheme R-M-R is in all cases retained. Our mobile segments are: I(#2-#26), II(#15-#37), III(#26-#53), and IV(#43-#62), so they vary in length between 20(IV) and 27(III) residues. For each case, 1000 initial structures were subjected to energy minimization, resulting in 520 energy minimized structures in case I, 647 for II, 532 for III, and 710 for case IV.

Each conformer obtained can be assigned a score (final total energy) and an rmsd (CA atoms only) between it and the experimental 1R69 structure. Plots of rmsd vs. score are presented in Figs. 4a-d. Based on the energy score 10 top scoring structures were pre-selected in each case. These selected structures are presented in Figs. 4e-h and in Table 3.

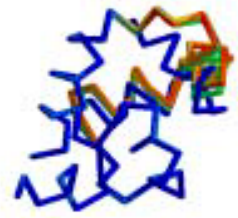

rmsd[A]
tot. energy [kJ/mol]

a

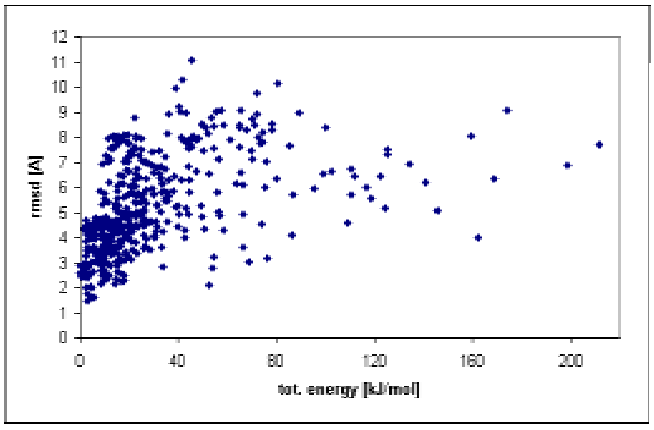
e


rmsd [A]
tot. energy [kJ/mol]

b

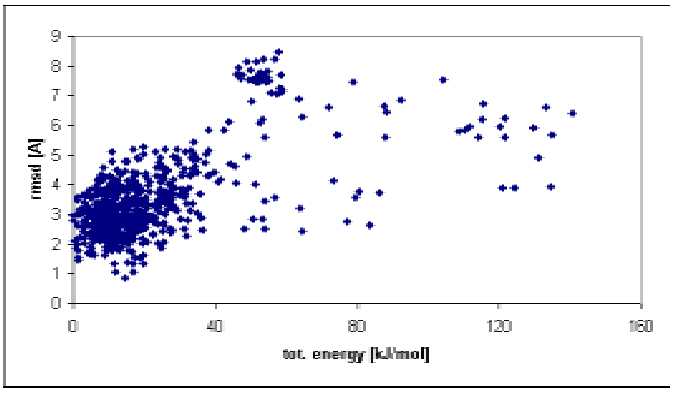


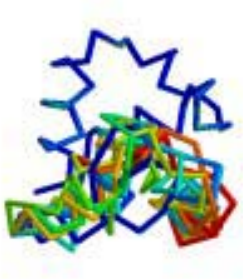
f


rmsd [A]
tot. energy [kJ/mol]

c

g





d h

Fig. 4 Results for mobile segments I (a, e), II(b,f), III(c,g) and IV(d,h). a-d: diagrams rmsd (CA) with respect to experimental structure vs. total energy e-h: ten structures of lowest energy (CA backbones only; restrained blue segments overlap, experimental result in blue, putative mobile fragments in different colours).

The following factors should be taken into account to assess the method: selectivity (i.e. low energy conformations ought to be close to experiment in rmsd, and high energy ones relatively far), unequivocality (procedure should lead to a limited number of acceptable minima), correlation between energy and rmsd (although, e.g., linear regression is not expected, it would be desirable), and finally, it is desirable to obtain low energy structures close to experiment preferably with an rmsd comparable to the experimental resolution (in case of 1R69: 2Å).

Our results on the present test case reveal a quite good selectivity and reasonable correlation between energy and rmsd from the experimental structure in all cases. From the results in Table 4 one can estimate an average accuracy of the segment placement of ~2.5-3.0 Å depending on the selected segment. It should be pointed out, that our test protein is relatively small, and the mobile segment contains approximately 1/3 of the whole structure. It is expected that in case of larger structures with less conformational flexibility for the whole structure the prediction for the mobile segment might further improve.

Mobile segment:											
I			II			III			IV		
E [kJ/mol]	rmsd [Å]	# out of 520	E [kJ/mol]	rmsd [Å]	# out of 647	E [kJ/mol]	rmsd [Å]	# out of 532	E [kJ/mol]	rmsd [Å]	# out of 710
0.000	2.47	1	0.000	2.79	7	0.000	2.58	1	0.000	2.80	1
0.113	2.47	1	0.008	2.77	4	0.141	2.87	1	0.017	2.94	1
0.137	2.49	1	0.041	2.78	1	0.289	2.45	1	0.463	2.10	1
0.190	2.60	1	0.048	2.58	7	0.537	2.57	1	0.674	1.87	2
0.289	2.51	1	0.052	2.78	2	0.546	2.58	1	0.736	1.87	1
0.603	2.55	1	0.056	2.58	2	0.553	2.58	1	0.892	2.65	1
0.622	2.55	1	0.065	2.59	2	0.594	2.59	1	0.958	2.99	1
0.664	2.22	1	0.283	2.80	1	0.698	2.50	1	1.091	1.83	1
0.718	3.42	1	0.986	2.79	2	0.711	2.50	1	1.138	3.49	1
0.801	2.51	1	0.996	2.80	3	0.727	2.50	1	1.146	3.55	1

Table 3 10 structures of lowest energy for each R-M-R case (green/red lowest/highest rmsd).

4 CONCLUSIONS

The concept of a reduced protein modeling approach was introduced to improve homology modeling efforts in regions of low target- template sequence similarity. The initial tests of the model on a mainly alpha-helical structure showed quite reasonable performance. Further testing of the model on more protein structures and protein classes and use of alternative scoring functions is required to make this approach generally applicable.

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