A Novel Method for Sampling Alpha-Helical Protein Backbones

Boris Fain and Michael Levitt

Department of Structural Biology, Stanford University School of Medicine,

Stanford, CA 94305

Abstract

We present a novel technique of sampling the configurations of helical proteins. Assuming knowledge of native secondary structure, we employ assembly rules gathered from a database of existing structures to enumerate the geometrically possible three-dimensional arrangements of the constituent helices. We produce a library of possible folds for twenty-five helical protein cores. In each case, our method finds significant numbers of conformations close to the native structure. In addition we assign coordinates to all atoms for four of the twenty-five proteins and show that this has a small effect on the number of near-native conformations. In the context of database driven exhaustive enumeration our method performs extremely well, yielding significant percentages of structures (between 0.02% and 82%) within 6Å of the native structure. The method’s speed and efficiency make it a valuable tool for predicting protein structure.

keywords: proteins; helices; decoys; core.

I. INTRODUCTION
A. Outline of current prediction methodology

Prediction of protein structure from sequence is one of the most enticing goals of scientific inquiry today. Currently the most reliable method of determining a protein’s shape is to search for a close homologue in the database of solved protein structures. Although the number of solved structures increases daily, it is estimated (Brenner et al., 1997) that in the near future at least 40% of proteins of interest bear no discernible sequence resemblance to a known macromolecule. Therefore *ab initio* prediction of structure from sequence remains an important challenge.

When sequence homology cannot be used to construct a 3-D model, the current *modus operandi* for predicting structure is composed of three separate (yet interdependent) steps: sampling, searching, and ranking. First one picks a formalism to index and sample the possible structures, then searches through the conformation space and constructs the shapes, and finally uses some ranking criterion to pick out a structure as close to native as possible. Let us provide a few examples of each step. Some of the possible choices in the initial phase, picking a representation, are all-atom, reduced ‘pseudo’ amino-acid interaction centers (Levitt & Warshel, 1975; Levitt, 1976), lattice (Hinds & Levitt, 1992; Covell, 1994; Lau & Dill, 1989; Skolnick & Kolinski, 1990), and many others. Once the representation is chosen, one then picks the degrees of freedom to use. Researchers may vary Cartesian coordinates of all or a subset of the atoms, dihedral angles, relative distances, etc (Eyurich et al., 1999). The decisions of which, how many, discretized or continuous, and so on, are motivated by the amount of detail one wants to capture, as well as by computational complexity of the forthcoming search.

Having picked a representation, one then selects an appropriate search technique, or a mixture of search techniques to march through the enormous space of possible 3-dimensional structures. As we have mentioned above, the three complementary steps of prediction are highly interdependent. The most important factors in picking a search method are computational complexity and the natures of both the energy function and the representation.
Lattice models, for instance, lend themselves easily to exhaustive enumeration. All atom Cartesian coordinate representations usually employ a physical potential, and search for a minimum with either an adaptive minimization routine, or a copy of Nature’s algorithm, molecular dynamics.

The remaining tool employed in protein structure prediction is a potential function constructed to pick out native or near-native conformations from a vast number of alternatives. If the search method employed is a guided one (for example, minimization) then the potential function also provides a landscape that will allow the procedure to converge to the correct answer. Just like sampling and search techniques, potential functions are many and varied: physical potentials with/without water representation, knowledge based potentials of various detail, hydrophobic contact potentials, and many others (Sippl, 1990; Samudrala, 1997; Park & Levitt, 1996). Frequently more than one potential is used to evaluate candidate structures.

II. DESCRIPTION OF THE SAMPLING METHOD

A. Graph-Theoretical representation of secondary structure

We represent alpha-helical proteins as connected graphs. Each helix is represented by a vertex and an edge is drawn between two vertices if the corresponding helices are in contact. To form a protein core one builds a connected subgraph, adding one helix at a time, until the protein is assembled. Figure 1 shows the graph-theoretical representation and the construction of Myoglobin.

We are, in essence, building an off-lattice model for secondary structure segments, but our degrees of freedom are not in the frequently chosen (Park & Levitt, 1996; Eyrich et al., 1999) \((\phi, \psi)\) loop residues (or their subsets), but in the relative geometric position of the helices themselves. The disadvantage of this approach is that a significant fraction of visited structures violate loop constraints - simply put, the ends of helices are too far apart to be
joined together by an intervening loop. There are, however, several advantages. First, the
structures we generate have a much higher tendency to be compact. Second, we are able to
exploit the correlation between the sequence patterns of helix-helix contact to significantly
enrich our sampling with native-like structures. (A simple example of this is two helices with
small residues in the contact area are more likely to be close to each other than those with
large residues between them.) Third, we are able to sample possible packing much better
than when using loop torsion angles, as is usually done in off-lattice models.

B. Exhaustive enumeration of helix-helix contacts

In this work our principal aim is to achieve coarse-grained sampling of the helical protein
core. Our hypothesis is that the contact patches on each helix influence the way the helices
pack. We choose to derive the preferences for helix-helix packing from the distribution of
such orientations in known proteins. If we are incorrect, then our packing strategy will
position helices randomly and no harm will be done. Therefore, the way we choose to travel
through our space is by exhaustive enumeration of a discrete version of our representation.
We can choose to sample relative helix-helix orientation in several ways. (We call a particular
realization of an edge a ‘link’. Figure 2 shows the various attributes of a link). It’s useful,
once again, to draw an analogy to dihedral sampling: there one can discretize a particular
dihedral angle into a (not necessarily) uniform spectrum of values suggested by geometric
considerations; e.g. sampling a dihedral angle in, say, 30 degree intervals. Alternatively, one
can extract the local moves from a database of existing structures, thus sampling the space
more efficiently. One could, for example, assign three possible values - helix, loop, sheet - to a
dihedral angle. Another possibility would be to bias the assignment with sequence matching.
(Simons et al. 1997) or secondary structure prediction. In this work we choose exhaustive
enumeration of possible orientations derived from known structures. This is motivated by
the observed influence sequence has on the packing of proteins (Reddy & Blundell, 1993;
Popov, 1980; Matheson & Sheraga, 1978; Richmond & Richards, 1978).
C. Definition of helix to helix contact ('link') and the contacts’ database

We extract the possible relative orientations of two helices from a database of touching helix-helix pairs obtained from a subset of the SCOP (Murzin et al., 1995) database. The 1305 folds in our library have a sequence identity to each other no greater than 35%. We then parse our database to get touching helix-helix pairs. We define two helices \( X \) and \( Y \) to be in contact if a) the shortest distance between any two CB’s, \( CB_x \) and \( CB_y \), located respectively on helix \( X \) and helix \( Y \), is less than 7.3 Å; and b) if each helix has at least 3 CB atoms within \( d = d_{\min} + 2.5 \) Å. The specific values of \( d_{\min} = 7.3 \) Å and 2.5 Å worked best in our tests. Lowering the \( d_{\min} \) parameter to below 5 Å caused some of the proteins in our database to be represented by disconnected graphs, thus making it impossible to ever reproduce them with our technique. Conversely, making the \( d_{\min} \) cutoff much larger than 7.3 Å decreases the influence the contact sequence has on the relative orientation of the helices. In addition to the minimal three CB's on each helix, all the CB's on both helices that are within 2.5 Å of \( d_{\min} = 7.3 \) are defined to be in a contact ‘patch’. Our definition picks out the residues in the contact region by assigning a ‘patch’ of contact residues between helices. Residues on the far side of each helix, which have very little influence on the relative orientation, are ignored. Figure 2 shows the patches and the structure of each link in the database.

D. The Enumeration Procedure

Figure 1 illustrates the enumeration procedure. The build-up of a helical protein proceeds as follows: given a protein sequence and native secondary structure assignment, we construct idealized helices on the chain. (In a predictive scenario the native secondary structure assignment will be replaced by a prediction, or possibly several alternative predictions.) We

\(^1\)The three CB on each helix are necessary to define a relative rotation from one helix to another.
then go to the library of links and perform a sequence alignment between the patches on each link and all possible pairs of helices on our target sequence. The residues between key ‘patch’ residues serve as spacers to fix the position of the influential patch residues. If the sequence match score is high then this particular link will be used to bring the pair of helices together. The actual threshold for matching depends on the number of structures we want to sample. The sequence match is scored using the Blosum62 (Henikoff & Henikoff, 1992) matrix. The comparison threshold typically varies from 0.1 to 0.6 and is adjusted to give anywhere from 10 to 1000 possible links for a given pair of helices in the target protein. The number chosen depends on how many final structures we wish to generate in the available amount of computer time. The relative orientations of the helices are then loaded into memory and the buildup of structures begins.

We use each topologically distinct pathway to build the target - (refer to figure 1b) - each specific pathway corresponds to a minimal subgraph spanning the protein graph. (Dashed and solid lines, respectively, in figure 1). For a structure consisting of 4 helices this results in $4^{(4-2)} = 16$ possible topologies (see Figure 3). Note that the graph corresponding to each final structure might (indeed should) possess other edges not used in construction; however it’s sufficient to follow a minimally connected subgraph to construct it.

For each topology we construct each possible combination of links (gathered from the sequence-matching procedure above) that can realize a given edge. To improve performance we used branch and cut filters for loop and clash constraints. The clash filter eliminates a conformation if more than 3 residues on one helix are closer than 2 Å to residues on another helix. The loop filter eliminates conformations for which the distance for the loops necessary to connect the helices is longer than the maximum available loop length. The reason for the branch-cut approach is simple: if in a given six-helix enumeration helix one clashes with helix three there is no need to cycle through and build helices four, five, and six. Finally, each geometrically viable structure is tested for compactness. All the tests and filters are extremely fast because whenever possible we use the coarse segment representation of the structure and thus escape having to visit each amino acid’s coordinates. We have
also incorporated other filtering information, most notably disulfide bond locations, into the build-up procedure. In its current incarnation the method is able to generate roughly $10^3$ conformations per second on a 400MHz Pentium workstation. The ultimate speed of the procedure will, in the future, be limited by scoring function evaluations.

III. RESULTS

To test the performance of our technique we have used the coordinates of helical cores for 25 proteins. The molecular sizes range from 31 to 172 amino acids, and the number of helical residues to which coordinates are assigned ranged from 23 to 130.

The position of disulfide bonds can sometimes be easily obtained through chemical methods, and one can then rely on knowing their location prior to prediction of structure. To this end, for 4 of the 25 proteins in our set that have disulfide links, we have also evaluated the performance of our method with and without a priori knowledge of these bonds.

The fact that we assign residues only to the helical backbone leaves open the possibility that our results will be degraded by subsequent assignment of coordinates to all remaining atoms. We investigated this effect by building all atomic coordinates for 5 of our proteins using the program SegMod (Levitt, 1992). Prior to reconstructing full coordinates we pruned the helical cores with a slightly modified Sippl-like function (Sippl, 1990), leaving 500 to 4500 structures for each protein. (We left more decoys for larger proteins).

A. Sampling of helical cores

We used the method to generate conformations for 25 helical proteins, ranging in size from 31 to 172 residues and containing anywhere from two to six helices. The program STRIDE (Frishman & Argos, 1995) was used to determine the secondary structure of the native protein. To check how much the quality of our sampling will be degraded when all the coordinates are reconstructed from the helical core, we have included several proteins with
significant loop content. The assignments of helices to the structure were made identical to the native structure.

The results are summarized in table I. Overall the results are very promising. For nearly every protein a sizeable proportion of the sampled structures is within 3Å of the native. For 9 proteins the best structure produced is closer than 1Å CA RMSD to the actual structure, and is virtually identical to the protein itself. Our procedure clearly samples well enough for a suitable potential to make a successful prediction.

Our method is not only very fast, it is also very efficient, in the sense of being able to generate a large percentage of native-like conformations. We can access the efficiency by estimating how many random protein-like compact structures are needed to obtain the RMSD of our best structure, and compare that number to the number of conformations we have visited. We use the estimate from (Reva et al., 1998)

$$\frac{1}{(\sigma\sqrt{2\pi})} \int_{-\infty}^{R} \exp \left[ -\frac{(x-<R>)^2}{2\sigma^2} \right] dx$$

(3.1)

Where, following Reva et al., 1998, we set $\sigma = 2.0$ and $<R> = 3.333N^{1/3}$, where $N$ is the number of residues in the protein core that we have assigned coordinates to. The proximity of our best structures to native exposes the weakness in estimating probability of low RMSDs, namely that the Gaussian distribution cannot be used to effectively describe sets of conformations which are very close to native. Having said that, we still feel that equation (3.1) gives a good estimate of efficiency. The last column in table I shows the ratio of the number of structures we sample to the number of random structures given by formula (3.1).

The results from this column in table I show that our method is efficient - the sampling is strongly biased towards the native conformation. The worst performance is shown in protein 1res, where we would have done slightly better if we chose relative helical conformations at random. However for most cases we are besting the random sampling by factors of $10^4$ to $10^6$, with efficiency actually improving for large proteins - which is where it matters most.
B. Features of the sampled conformations

Figure 4 shows a typical distribution of the sampled conformations. One particularly interesting feature is the non-Gaussian tail extending towards the native structure. This is most likely the result of using sequence matching to select the possible orientations of the helix-helix pairs. The use of sequence information enriches the set with native-like structures and makes the curve decidedly non-Gaussian.

C. Using disulfide bond information

To test how well the sampling is aided by supplementary information, we took proteins with disulfide bonds and subjected the sampling for those structures to an additional filter. We did not screen 1cc5 because although it does have disulfide bonds, they occur in the loop regions and do not help filter the helical cores. Our filters rejected conformations in which the CYS CB-CB distance was greater than 8 Å. This is not a very strict filter; it simply weeds out grossly incorrect topologies. The results both with and without (The results without disulfide bond information are the same as in table I) disulfide bond information are displayed in table II. The number of structures was cut by approximately a factor of 8. While the filtering did not significantly improve the average RMSD of the ensemble, the proportion of ‘good’ structures was raised approximately fivefold.

D. Completing the structure

The structures described in the previous two sections possess only backbone coordinates for residues in the helical core. It is quite possible that the successes of the sampling can be washed out when coordinates are assigned to the remaining residues and side-chain atoms. To check how the quality of the sampling changes during completion we ran our sets of conformations through the program SegMod (Levitt, 1992). We did not construct full coordinates for all twenty five proteins because we simply wanted to show that reconstruction
does not significantly degrade the quality of the sampling procedure. To make the test credible, we selected proteins of varying size, and with long unassigned loops. The results are shown in table [III].

Once again, the resulting ensembles of decoys contain a sizeable proportion of near-native decoys. Even though we did not produce any decoys closer than 6Å for 2fha, we feel that the sampling of such a large (according to ab initio prediction standards) protein - 172 residues - was successful. The RMSD of the best decoy, 7.3Å has log-odds of 23234 (computed from last column of table [III]. The total ensemble for 2fha has 0.25 results are good for decoy ensembles. Whether they are good enough for prediction remains to be seen.

IV. DISCUSSION

A. Desirable features of search methods

In this paper we presented a novel sampling technique for helical proteins. To provide a context in which one can judge the merits of our procedure, we shall review the features that make a sampling method effective.

Our assertion is that for a search method to be useful in ab-initio structure prediction, the proportion of sampled conformations that are native-like should be statistically significant. Admittedly, the definitions of both “statistically significant” and “native-like” are imprecise - but clearly, the more and the closer, the better.

The need to get close to the native fold is motivated by several considerations. First, we wish to furnish good, i.e. close to native, predictions, Since we cannot select that which we do not have, having at least one native-like conformation is an obvious necessity. Second, an energy functions can only be effective in a slice of conformational space close to the actual protein. Structures can be ‘wrong’ in a vast number of ways, many of which are low scoring alternatives to the native fold in a very large space. Only a small subspace close to the actual structure can be expected to exhibit the properties of an energy funnel; i.e. a region
where the energy decreases uniformly as one approaches the native, and where a majority of conformations are more favorable than all the others in the available space. We can demand that an energy function correctly discriminates a 1 Å fold from all 10 Å folds, but if our best candidate model is 8 Å cRMS from native, we cannot expect an energy function to pick this over one that is 10 Å away. The need to have more than one 'good' structure; indeed to enrich the library as much as possible with near-native choices, is brought about by the imperfections of the available selection methods. A perfect energy function should be able to discriminate a native structure from all other alternatives - this, in fact, is what nature is able to do with striking consistency.

The current generation of potentials (Samudrala & Moult, 1997; Sippl, 1990, Simons *et al.*, 1999) often misidentifies incorrect folds, giving them energies lower than that of the native fold. By increasing the number of 'correct' answers we increase the statistical likelihood of making a successful prediction. In addition, some current techniques use consensus information - selecting a subset and building a consensus model from it (Huang *et al.*, 1999). These methods clearly need a sizeable native-like population to work. To give today’s energy functions all the help they can certainly use, it’s advantageous to have as many native-like folds as possible in one’s decoy set.

To make the above discussion more concrete, we should quantify the definition of being 'near-native. How close is close enough? One possible answer comes from comparing one’s technique to random sampling. Reva *et. al.* (1998) suggest a 'native-like' target value of about 6 Å cRMS deviation relative to the actual structure. Other authors (Park & Levitt, 1996; Simons *et al.*, 1997). also propose a similar distance of 4-6 Å. 

\[^2\] This number depends on the size of the protein.
B. Comparison with other search methods

Our sampling method has both merits and drawbacks when compared with other search techniques. Many classical approaches of assembling alpha-helices into a core have been proposed (Soloviev & Kolchanov, 1981; Cohen et al., 1979, Cohen & Sternberg, 1980). One alternate approach to sampling is to assemble helices using distance geometry by either embedding distance space in three dimensions (Aszodi et al., 1995; Havel, 1991), or by minimizing against restraints (Cohen & Sternberg, 1980; Kuntz et al., 1982; Richmond & Richards, 1978; Huang et al., 1999; Chelvanayagam et al., 1998; Lund et al., 1996; Mutenthaler & Braun, 1995; Ortiz et al., 1998; Smith-Brown et al., 1998). In a recent work (Huang et al., 1999) this method produced decoy libraries with the log-odds of producing the lowest structure in the -4 to -6 range. Our results, which range from log-odds of -4 to -9, are somewhat better. However we must emphasize that the comparison is unfair, since we used actual structure, and Huang et. al. used predicted (albeit well predicted) secondary structure. We are currently examining how well our method will perform with predicted secondary structure. Preliminary indications are that both distance geometry and our method both get sufficiently close to the native fold, with our method having a slight edge due to its speed.

Our method begins to pull away from distance geometry methods in the scaling of CPU time needed when one moves to larger and larger proteins. Current distance geometry methods cannot be extended to proteins of length over 100 residues (Huang et al., 1999). In contrast, because our sampling technique scales with the number of secondary structure segments and not the number of residues, it can easily handle chains of 100 - 200 residues, comparable to single domains of larger proteins. The library for the largest protein in our

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3 Log-odds are 1/N_{best}, where N_{best} is the number of random structures needed to find at least one structure with RMSD equal to our best structure. N_{best} is the first number in the last column of table I.
work - 2fha, 172 residues long - took approximately 15 hours of CPU time on a 400 MHz Pentium II machine. The sampling procedure produces approximately 500 structures per second for two-helical proteins, 100 per second for proteins containing three helices, 10 per second with four, and roughly one structure/second for proteins with five and six helices.

Another sampling technique is to fix the helical segments and then to vary the dihedral angles of the loop regions. This method has been used to produce fold libraries (Park & Levitt, 1996), and, combined with a branch and bound algorithm, in folding studies (Eyrich, et al., 1999). This method carries an advantage over ours because every structure generated automatically satisfies loop constraints, where as many of our potential structures do not. Many of our structures have to be rejected because the physical distance between ends of helices violates chain connectivity. Furthermore, we do not have loops at the end of construction; therefore we are currently working on an algorithm to sample loop regions of our protein cores that would be a good (i.e. fast) match to our helical core sampling.

Our method out-paces the loop dihedral angle sampling in two categories: scaling of computational demands with time, and efficient sampling of structures with plausible contacts. Let us illustrate the first difference with a four-helical protein. Assuming an average loop length of seven residues varying loop conformation demands $7(\text{residues}) \times 2(\phi, \psi) \times 3 = 42$ degrees of freedom. Our procedure needs to position three rigid bodies (the position of the initial helix is arbitrary), requiring $6(\text{rigid body}) \times 3 = 18$ degrees of freedom. The second difference stems from the fact each helix in our construction method is guaranteed to have at least one, and possibly two plausible contacts with other helices. When one varies dihedral angles, most of the non-clashing structures have large voids between helices.

Yet another method for generating possible reduced-model conformations for protein structure are lattice and off-lattice models, such as Hinds & Levitt, 1992, 1994; Covell, 1994. These methods are better ours in their generality because they require no knowledge of either actual or predicted secondary structure. On the other hand it’s difficult to see secondary structure at the resolution of these lattice models. In addition, often secondary structure is fitted onto the conformations at the end of construction. In computational
performance, for small proteins these approaches compare very favorably with ours - a simplified representation which assigns a lattice point to every second residue can exhaustively sample shapes of proteins of up to 100 residues (Hinds & Levitt, 1992). However, because of an exponential increase in the number of shapes of a self-avoiding walk on a tetrahedral lattice, it’s difficult at this point to see a generalization of the lattice methods that would apply to larger molecules without a significant sacrifice in resolution.

One final method we want to mention is that of assembling structures from fragments of existing folds used by Simons et al., 1997, 1999. We find this method very appealing. It uses the information in the sequence to bias the assembly much the same way we use the ‘patch’ information to bias helix-helix orientation. It is reported to produce best structures as good as the lowest RMSDs in our set. In addition, this method - as do the lattice models and the loop angle search - works on beta and mixed alpha/beta proteins: something we cannot yet do. Because we have not had the opportunity to test this method ourselves, we cannot comment on how its efficiency compares with ours. Our feeling is that sequence information in ‘contact patches’ has more correlation on the global geometry of the structure than the local sequence/structure combination employed in these methods. Also our method is much faster than any of the other currently available sampling methods.

C. Advantages and disadvantages

In the beginning of his section we introduced two desirable qualities of a sampling method: speed, and the ability to produce structures close to the native fold. Our technique fills both of these requirements.

The speed of the method depends on the size of the protein and, more specifically, on the number of helices that we are trying to arrange. Each entry in table takes from a fraction of a second to a few hours to produce. A naive count would estimate a factorial growth in the number of graphs, and an additional geometric growth in the number of structures sampled in each graph; however the actual increase is much less. For most structures of 4 helices or
more, cutting the branches that violate self-avoidance results in a significant reduction of the number of conformations we need to sample. In our tests each additional helix increased the time of the runs by approximately a factor of 30. The set of conformations for our largest structure, 1fha, took approximately 4 hours to generate.

In addition to being fast, our method scales well - the use of branch-cutting helps because larger proteins are very constrained by self-intersection. Because of this our technique is able to sample molecules comparable in size to small domains, which are the largest single-chain structures one wishes to predict.

We chose to re-arrange helices using a ‘patch’ contact database derived from existing structures. This approach significantly enriches the sampled ensemble with native-like structures. In addition, the graph-theoretical enumeration of relative orientations ensures that we sample all plausible regions of conformation space.

In its current incarnation our method also possesses some drawbacks. The main disadvantage of our technique is the requirement of a specified secondary structure. Ideally a search technique should also sample alternate secondary structure assignments. Currently we can only do this by specifying different assignments at the beginning of the procedure. Another significant drawback is the absence of a complimentary loop-building method. We need a method with the speed of a loop-library lookup methods, yet able to get a 3 Å or better approximation of the native loop (so that our near-native cores remain near-native). A final challenge to our approach is the lack of an obvious generalization to beta and alpha/beta proteins. The definitions of a sub-segment and the contact patch have to be significantly revised to adapt to beta sheets.

V. FUTURE DIRECTIONS

The deficiencies of our technique, outlined in the previous paragraph, point the way to future developments. We are currently working on a fast loop building procedure for short (3-10 residues) loops. Our next project is to enhance sampling of alpha proteins to include
variations of the boundaries of the helices.

A slightly more distant goal is the extension of the presented technique to construct \( \beta \)-sheet and mixed \( \alpha/\beta \) proteins. In addition we are working on a fast preliminary discrimination function which would be used prior to the reconstruction of all atoms for each conformation.

VI. ACKNOWLEDGEMENTS

Many thanks to the present and former members of the Levitt group for help and discussions. We wish to thank Patrice Koehl for providing excellent routines for superimposing structures. Thanks to Prof. Joseph Rudnick for hints on graph theory. B.F. wishes to thank the A.P. Sloan Foundation, and the U.S. Department of Energy for financial support. This work was supported in part by grant DE-FG03-95ER62135 to M.L. from the U.S. Department of Energy.
### TABLE I. Summary of helical core sampling.

| Name   | Helices | Assigned / Total residues | CA RMSD range | % below 3Å best - worst : ave | % below 6Å | Random / Generated |
|--------|---------|---------------------------|---------------|-------------------------------|------------|-------------------|
| T0065  | 2       | 23/31                     | 0.483 - 7.503 : 4.988 | 3.1%                          | 81.4%      | 291299/2604        |
| 1fc2 C | 2       | 23/43                     | 0.759 - 7.937 : 4.813 | 10.3%                         | 77.4%      | 153634/2144        |
| 1res   | 3       | 24/43                     | 2.265 - 9.646 : 6.546 | 0.055%                        | 29.3%      | 8392/9028          |
| 1erp   | 3       | 26/38                     | 0.986 - 9.052 : 6.191 | 0.26%                         | 41.8%      | 226495/11188       |
| 1mbh   | 3       | 30/52                     | 0.954 - 10.412 : 7.123 | 0.3%                          | 23.3%      | 773292/11724       |
| 1uxd   | 3       | 30/59                     | 0.880 - 10.828 : 6.773 | 0.58%                         | 28.2%      | 927522/16281       |
| 3hdd   | 3       | 40/56                     | 0.240 - 12.022 : 8.001 | 4.0%                          | 11.8%      | 8.3E7/13380        |
| 1trl   | 3       | 42/62                     | 0.617 - 12.920 : 8.002 | 0.24%                         | 12.4%      | 4.0E7/24151        |
| T0073  | 2       | 43/48                     | 0.662 - 8.348 : 4.840 | 11.9%                         | 78.9%      | 5.5E7/522          |
| 1cc5   | 4       | 43/83                     | 3.484 - 13.237 : 9.241 | -                             | 0.84%      | 47664/46725        |
| 1r69   | 5       | 44/63                     | 3.127 - 13.143 : 9.140 | -                             | 1.1%       | 128078/56520       |
| 1lfb   | 3       | 44/77                     | 1.584 - 13.668 : 8.973 | 0.09%                         | 5.4%       | 5.68E6/41505       |
| 2ezh   | 4       | 48/65                     | 3.514 - 15.363 : 9.670 | -                             | 1.4%       | 5E6/61981          |
| 1c5a   | 4       | 49/65                     | 3.774 - 13.731 : 9.064 | -                             | 1.7%       | 78745/98842        |
| 1hssn  | 4       | 50/79                     | 3.321 - 17.380 : 11.211 | -                             | 0.28%      | 266620/32734       |
| 1ropa  | 2       | 51/56                     | 2.432 - 13.751 : 6.765 | 1.3%                          | 40.2%      | 2.9E6/620          |
| 1pou   | 4       | 52/71                     | 4.546 - 14.318 : 10.187 | -                             | 0.95%      | 25296/29283        |
| 1nre   | 3       | 57/81                     | 2.240 - 13.020 : 8.215 | 0.21%                         | 11.3%      | 1.6E7/21716        |
| 1ail   | 3       | 60/70                     | 3.028 - 15.481 : 9.582 | -                             | 2.5%       | 3.6E6/15980        |
| 1nkl   | 4       | 60/78                     | 3.879 - 14.122 : 9.931 | -                             | 1.15%      | 439759/15486       |
| 1aca   | 4       | 60/86                     | 0.752 - 15.059 : 10.442 | 0.13%                         | 1.9%       | 2.5E9/6827         |
| 1flx   | 4       | 67/79                     | 3.306 - 14.786 : 10.869 | -                             | 0.635%     | 6.5E6/37141        |
| 1aj3   | 3       | 88/98                     | 2.594 - 16.309 : 10.125 | 0.05%                         | 3.1%       | 1.6E9/8177         |
Table I summarizes the sampling of helical cores for 25 proteins. Column 3 shows the number of residues that are assigned coordinates as well as the total length of the protein. Column 4 lists the range of CA RMSD of assigned coordinates from native coordinates. Columns 5 and 6 show the percentage of total structures that are closer than, respectively, 3Å and 6Å to the native structure. The last column displays the efficiency of our decoy generation method. It shows the ratio of the number of structures one needs to generate randomly in order to produce the best CA RMSD (computed using Eqn. 3.1) to the number of conformations in our ensemble.

| Protein | ave without | ave with | %below 3Å | %below 6Å | num/numact |
|---------|-------------|----------|-----------|-----------|------------|
| 1lis    | 5           | 91/131   | 4.345 - 18.696 : 12.430 | - | 0.07% | 2.0E7/32805 |
| 2fha    | 5           | 130/172  | 4.855 - 21.671 : 15.912 | - | 0.023% | 1.0E10/4279 |

**Table II. Disulfide bond information included**

| Protein | ave without → with | %below 3Å | %below 6Å | num/numact |
|---------|---------------------|-----------|-----------|------------|
| 1c5a    | 9.064 → 8.047       | -         | 1.7% → 7.4% | 98842 → 6325 |
| 1erp    | 6.191 → 6.017       | 0.26% → 1.3% | 41.8% → 46.9% | 11188 → 761 |
| 1nkl    | 9.931 → 9.254       | -         | 1.15% → 5.8% | 15486 → 889 |
Table II displays the results of pruning the decoy ensembles with disulfide bond information. The columns show shifts in the average Ca RMSD, proportions with RMSD below 3Å and 6Å, and the reduction in the number of structures in each set.

| Name | Size, Helices | CA RMSD range | % below 4Å | % below 6Å | Random/Generated |
|------|---------------|---------------|------------|------------|-----------------|
| T0073 | 48, 2         | 1.435 - 8.320 : 4.840 | 24.5% | 73.7% | 2.14E7/522 |
| 1ropA | 56, 2         | 2.446 - 15.041 : 7.074 | 6.6% | 34.0% | 7.79E6/712 |
| 1ail  | 70, 3         | 3.200 - 15.624 : 9.730 | 0.26 | 3.50% | 1.45E7/1917 |
| 1flx  | 79, 4         | 3.282 - 14.886 : 11.329 | 0.06 | 0.9% | 5.56E7/3342 |
| 1aj3  | 98, 3         | 2.67 - 16.02 : 10.396 | 1.0% | 4.0% | 9.17E9/1880 |
| 2fha  | 172, 5        | 6.644 - 26.323 : 18.668 | - | 0.25 % ≤ 10Å | 7.27E8/4279 |

Table III summarizes the results for decoy sets with all atoms’ coordinates assigned. The columns are similar to table II. The number of decoys in each ensemble is smaller than in table II because of pruning with a statistical pairwise potential. 2fha had no conformations below 6Å, however 0.25 % of structures were closer than 10Å to the native.
FIG. 1. This figure shows the graph representation of the protein Myoglobin, as well as its construction by our method. a) 1mba. This well known globin consists of 6 major and two minor helices. We sample the 6 major ones. b) The vertices of the graph are helices 1 through 6 of 1mba. (helix1: residues 4-19; h2: 21-35; h3: 59-77; h4: 81-98; h5: 102-119; h6: 126-144.) The vertices represent the 6 major helices; the solid lines are drawn whenever two helices are, according to our definition, in contact. The minimal spanning subgraph, outlined in dashed lines, represents one possible way to reassemble the helices. The dashed lines are numbered in the order the protein is assembled in one of our conformations. c) The order of assembly of the helical core. Helices are assembled in the order 1, 6, 4, 5, 2, 3 using the relative orientation from the dashed lines of b). Figures were made with the aid of MOLSCRIPT (Kraulis, 1991). In the process of construction other 'links' may form, however they are not neccesary to reproduce the helical core.
FIG. 2.  A diagram of two typical link configurations. The amino-acids in the shaded areas are involved in sequence matching against the target. The configuration on the left produces shorter patches than the configuration on the right. The figure on the bottom shows the sequence content of the link. This is an actual link taken from Myoglobin 1mba.

FIG. 3.  This figure shows the 16 topologically distinct ways to construct a 4-helix protein. In general there are $N^{(N-2)}$ spanning trees for a N vertex graph.
FIG. 4. RMSD from the native for sampled conformations for 1aca.pdb. The distribution appears gaussian with an enriched tail of native-like structures.
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