Testing the helix model for protein folding on four simple proteins

Pierpaolo Bruscolini
Unità INFM, Politecnico di Torino,
C.so Duca degli Abruzzi 24, 10129 Torino, ITALY

(February 1, 2008)

Abstract

We test a simplified, local version of the helix model on two synthetic and two natural proteins, to study its efficiency in predicting the native secondary structure. The results we obtain are very good for the synthetic sequences, poorer for the two natural ones. This suggests that non-local terms play a fundamental role in determining the secondary structure, even if in some cases local terms alone may be sufficient.

I. INTRODUCTION

It is experimentally known that a protein, under proper solvent and temperature conditions, folds from any random-shaped state to its “native” state, whose three dimensional structure is unambiguously encoded in the amino acid sequence. This state is the only one in which the protein is biologically active, and is strictly related to the chemical function of the protein. Unfortunately, experimental determination of this state is usually a rather difficult task, and it would be highly desirable to know how to predict the structure just from the sequence.

In spite of many years of efforts in the field, a clear understanding of protein folding has not been achieved yet. The best results in structure prediction are obtained by algorithms which compare the protein under study with a database of sequences of already known structure. This approach, even when successful, does not shed any light on the underlying physics.

Because of the wide range of time scales involved ($10^{-13} \div 10^2$ s and to the complexity of the system, ab-initio simulations of the folding process are today (and will most probably be for a long time) out of reach. They could help to understand the fast events involved in the folding process.

Simple simplified physical models have been proposed to capture the most relevant aspects of the problem. In these approaches the protein is usually described as a chain endowed with “charged” beads, representing the residues, which attract each other according to their nature. These model have been studied both on- and off-lattice, resorting to Monte Carlo simulations.

Most of the theoretical understanding of the thermodynamics and dynamics of the folding process comes from these models; yet their relationship with natural proteins is somewhat qualitative, since there is no well defined mapping between the configuration spaces of real
and model proteins. Hence, it is difficult to say which results can be extended to real proteins and which are model dependent, and the debate is still opened\textsuperscript{13–15} on the identification of the relevant features that distinguish a good folder from a poor one.

In a recent paper\textsuperscript{1} we proposed a new model, which gives a coarse-grained description of a protein in terms of helices. This choice stems from the fact that the elements of native secondary structure can be well approximated resorting to one or a few helices. Even loops can be partitioned in smaller parts and approximated in such a way: of course their description will not be as good as that of $\alpha$ helices. However, loops are usually found at the surface of the native globule and are affected by less severe geometrical constraints, so a less precise representation should not be a major problem.

The motivation for a coarse-grained description is related to the fact that a certain degree of redundancy is observed in structure encoding: several different sequences are known to fold to essentially the same structure. This suggests that an appropriate average description of the sequence could be enough to predict most of the features of the native states (even if the details of the three dimensional structure are probably related to close packing of side-chain, and cannot be easily captured in a simplified description).

In this letter we use a simplified version of the helix model, where nonlocal interactions are neglected, to predict the secondary structure of two synthetic\textsuperscript{16} and two natural proteins, which are known to fold into a “four-helix bundle”. We aim on the one hand to test the reliability of our model, and on the other to understand the role of local periodicities of polar and non-polar residues in determining the secondary structure of the protein.

The paper is organized as follows: in Sec. \textsuperscript{II} we recall the main characteristics of the model, in Sec. \textsuperscript{III} we test the efficiency of the local version of the model in predicting the secondary structure of the four proteins; finally, in Sec. \textsuperscript{IV} we briefly summarize and comment our results.

\section{II. THE MODEL}

Considering that their secondary structure is a fairly general feature of native states, and that its elements can be well represented by regular helices, we describe any protein configuration as a continuous curve made of pieces of helices sequentially linked together. This description is particularly suited for $\alpha$ and $3_{10}$ helices, but it can also be applied to $\beta$-strands (which, in the ideal case, are helices with two residues per turn) and, to a lesser extent, to the finite class of tight-turns presently known and to coil regions, once they are divided into smaller parts.

The equation of the curve representing the protein chain is assumed to be:

$$
\mathbf{r}(s) = \sum_{i=1}^{N_h} b_i(s) \mathbf{h}_i(s) \, ,
$$

where the parameter $s$ ranges from 0 to $N$, the total number of residues; $b_i(s) = 1$ if $s \in) s_{i-1}, s_i$ and $b_i(s) = 1/2$ if $s = s_{i-1}$ or $s = s_i$ whereas $b_i(s) = 0$ if $s \notin (s_{i-1}, s_i)$.

The $\mathbf{h}_i$ are the helices expressed in their reference frame ($\mathbf{e}_{1,i}, \mathbf{e}_{2,i}, \mathbf{e}_{3,i}$):

$$
\mathbf{h}_i(s) = a_i \left[ (\cos(u_i(s - s_{i-1})) - 1) \mathbf{e}_{1,i} + \sin(u_i(s - s_{i-1})) \mathbf{e}_{2,i} + u_i\mathbf{h}_i(s - s_{i-1}) \mathbf{e}_{3,i} \right] + \mathbf{h}_{i-1}(s_{i-1}) \, ,
$$

(2)
labelled so that helix $i$ starts at $s_{i-1}$ and ends at $s_i$, with $s_0 = 0$ and $s_{N_h} = N$. $N_h$ is the total number of helices, residues are labeled from 1 to $N$, and the convention holds that a residue sitting at the junction between two helices belongs to the first one. We let $n_i = s_i - s_{i-1}$ denote the length of helix $i$. We define also

\[ u_i = \frac{L}{a_i \sqrt{1 + h_i^2}} , \quad (3) \]

where $L$ is the length of a peptide unit, so that the line element on each helix is $|\dot{\mathbf{h}}_i| \, ds = L \, ds$. We assume the sign $\sigma_i = \pm 1$ of $u_i$ positive for right-handed and negative for left-handed helices, while the product $u_i h_i$ is always positive. We also ask that helices have the same length of the chain they represent, setting $\Delta s = 1$ for a peptide-unit move along the protein chain. This requirement implies that $n_i$ as defined above coincides with the number of residues in the helix.

In order to write down a simple hamiltonian, we further simplify the model, resorting to the following variables:

\[
\begin{align*}
N_h & \quad \text{the total number of helices} \\
n_i & = s_i - s_{i-1} \quad (n_i \in [p_1, p_2]) \\
l_i & = \frac{1}{2} (s_i + s_{i-1} + 1) \\
v_i & = \mathbf{h}_i(s_i) - \mathbf{h}_i(s_{i-1}) \\
\mathbf{B}_i & = \frac{1}{2} (\mathbf{h}_i(s_i) + \mathbf{h}_i(s_{i-1}))
\end{align*}
\]

where $p_2 = N - (N_h - 1)p_1$ and $p_1 = 3$, since a helix cannot be defined with less than three residues. $n_i$ is the length of the $i$-th helix expressed in residues; $i \in [1, N_h]$; $l_i$ represents the position along the sequence of the center of the $i$-th helix; $v_i$ is the vector joining the end-points of helix $\mathbf{h}_i$; $\mathbf{B}_i$ is the the spatial position of the middle point of $v_i$.

Two other variables are necessary to specify the "shape" of a helix: a particularly useful choice is to introduce:

\[
\begin{align*}
z_i & = \frac{L \tau_i}{u_i} , \\
w_i & = u_i - 2\pi \vartheta(-u_i) ,
\end{align*}
\]

where $u_i = L \sigma_i (\kappa^2_i + \tau^2_i)^{\frac{3}{2}}$ ($\kappa_i$, $\tau_i$ are the constant curvature and torsion of the $i$-th helix) and $\vartheta(\bullet)$ is the Heaviside function. The definition of $w_i$, in $w_i \in [0, 2\pi]$, allows us to remove the discontinuity between right and left-handed helices at $u = \pm \pi$, which is model-induced but inevitable in a description of the chain in terms of helices. The sequence enters the model through the variables $q_k$ ($k = 1 \ldots N$) and $p^2(l, w)$. The former are related to the nature of each residue $k$, and measure its coupling to the other residues, due to the fact that the Mijazawa-Jernigan interaction matrix can be written as:

\[
M_{\rho\sigma} = \mu_0 + \mu_1 (q_\rho + q_\sigma) + \mu_2 q_\rho q_\sigma \quad (\rho, \sigma = 1, \ldots, 20) .
\]

Since we deal with entire helices at a time, and not with single residues, we introduce the average $q$ of a helix, centered in $l_i = l$, as
\[ \varpi(l) = \begin{cases} \frac{1}{2m+1} \sum_{j=-m}^{m} q_{l+j}, & \text{if } l = 1, 2, \ldots \\ \frac{1}{2(2m+1)} \sum_{j=-m}^{m} (q_{l-j} + q_{l+j}), & \text{if } l = \frac{1}{2}, \frac{3}{2}, \ldots \end{cases} \] (8)

(integer or half-integer values of \( l \) are the only ones allowed for the central points of the helices, \( l_i \); the variable \( m \) is an arbitrary number, comparable with the mean length of the helices).

The other variables are defined by:

\[ p_2(l, w, n) = \frac{1}{(\sum_{j=-n}^{n} Q_{l+j})^2} \sum_{j,k=-n}^{n} Q_{l+j}Q_{l+k} \cos((j - k)w), \] (9)

where \( p_2(l, w, n) \) is the projection on the plane perpendicular to the helix axis of the "hydrophobic dipole moment", calculated at a point on the axis and normalized with respect to the total hydrophobic charge \( \sum_{j=-n}^{n} Q_{l+j} \), where:

\[ Q_{\rho} = \frac{\mu_0}{2} + \mu_1 q_{\rho} + \left( \frac{\mu_2}{2} \right) q_{\rho}^2. \]

The quantity \( p_2 \) reveals the prevalence of non polar residues on one side of the helix, characterized by the periodicity \( w \).

The following constraints hold among the variables previously defined:

1. the sum of the residues of all the helices must be equal to the total length of the chain:
   \[ \sum_{i=1}^{N_h} n_i - N = 0; \] (10)

2. the length of \( v_i \) is related to the length and shape of the helix:
   \[ v_i^2 - |h_i(s_i) - h_i(s_i-1)|^2 \equiv v_i^2 - n_i^2L^2 \left[ z_i^2 + (1 - z_i^2) \frac{\sin^2(\theta_i)}{\theta_i^2} \right] = 0, \] (11)
   where \( \theta_i = n_i u_i / 2; \)

3. the end of one helix must coincide with the beginning of the following one, both in sequence and in space:
   \[ B_i - B_{i-1} - \frac{(v_i + v_{i-1})}{2} = 0, \] (12)
   \[ l_i - l_{i-1} - \frac{n_i + n_{i-1}}{2} = 0. \] (13)

In these equations, \( i \) ranges from 1 to \( N_h \), and, to be consistent with the definitions of \( l_i \), we set \( l_0 = 1/2, n_0 = 0. \)

With the above defined variables we write a hamiltonian of the form:

\[ H = H_{nn} + \sum_{i=1}^{N_h} (H_i^0 + H_i^1) + \sum_{i<j=2}^{N_h} H_{ij}, \] (14)

where we have defined:
\[ H_{nn} = \gamma_2(N_h - 1) \]
\[ H^0_i = (n_i - 1)\gamma_0 \left[ c_1 \left( (w_i - c_2)^2 - c_3 \right)^2 + c_4 + c_5 \left( z_i - c_6 + c_7(w_i - c_8)^2 \right) \right], \]
\[ H^1_i = -\gamma_1 n_i P(l_i, w_i), \]
\[ H_{ij} = \gamma_4 \vartheta(\rho_1 - \Delta B_{ij}) \gamma(\Delta B_{ij} - \rho_0) \left[ \gamma_3 X(\mu_0 + \mu_1(\varphi(l_i) + \varphi(l_j)) + \mu_2 \varphi(l_i) \varphi(l_j)) \right] + +\gamma_4 \vartheta(\rho_0 - \Delta B_{ij}). \]

Here \( \gamma_i \) are dimensional parameters weighting the various contributions, while \( c_k \) are known adimensional constants and \( \Delta B_{ij} = |\mathbf{B}_i - \mathbf{B}_j| \).

Constraints will be implemented explicitly, by direct substitutions of the variables in the above Hamiltonian, which will eventually be written as a function of the independent variables.

A detailed discussion of the various terms appearing in Eq. (14) has been given elsewhere, here we just recall that \( H^0_i \) recovers in an effective way the experimental Ramachandran plot, thus dictating which kind of helices are more likely to be formed. \( H^1_i \), on the other hand, is sequence dependent and favours the separation of polar and nonpolar residues on the helices: \( P(l_i, w_i) = \mathcal{F}(\mathbf{p}_1^2(l_i, w_i, n)) \) is some simple function of \( \mathbf{p}_1^2(l_i, w_i, n) \).

\( H_{nn} \) represents an extremely simplified way to keep next-neighbours interactions into account: a constant, positive energy is involved in helix breaking, independently on their orientation. \( H_{ij} \) has the simple form of a square-well with an infinite barrier on one side, representing hard core repulsion between helices. The interaction, in the range \( \Delta B_{ij} \in [\rho_0, \rho_1] \) has the form of Eq. (7), calculated with the average “charges” \( \varphi_i \) of the helices.

For the sake of simplicity inter-helical hydrogen bonds are not distinguished from hydrophobic interactions (hence we disregard their dependence on orientation), and both are described by \( H_{ij} \).

### III. ANALYSIS OF THE FOUR PROTEINS

We now consider Eq. (14) in the limit \( \gamma_1 \ll \gamma_0 \), without non-local interactions (\( \gamma_3 = \gamma_4 = 0 \)) and at fixed number of helices \( N_h \), and ask ourselves to what extent the correct native secondary structure can be recovered by local terms only.

The former limit is equivalent to studying the ground state of \( H^1 = \sum_{i=1}^{N_h} H^1_i \) with only two allowed values \( (w_\alpha, w_\beta) \) for each \( w_i \), corresponding respectively to \( \alpha \) and \( \beta \) configuration. We shall look for the values of \( (n_i, w_i) \), at fixed \( N_h \), which best represent the native secondary structure, in the cases of two synthetic sequences and of two natural proteins, identified by PDB codes 2mhr (myohemerythrin) and 2asr (aspartate receptor, ligand binding domain). These proteins are known to fold in the “four-helix bundle” conformation.

We assume that the function \( P(l_i, w_i) \), appearing in the expression of \( H^1_i \), has the form:

\[
P(l_i, w_i) = \begin{cases} p_1^2(l_i, w_i, 3), & \text{if } l_i \text{ is an integer,} \\ \frac{1}{2} \left[p_1^2(l_i - \frac{1}{2}, w_i, 3) + p_1^2(l_i + \frac{1}{2}, w_i, 3)\right], & \text{if } l_i = k + \frac{1}{2}, \text{for integer } k. \end{cases}
\]

We have chosen \( n = 3 \) in expression (9) since this involves calculating the hydrophobic dipole on an helix of seven residues, a reasonable length for \( \alpha \)-helices and for \( \beta \)-strands.
First of all we plot \( P(l, w_\alpha) \), \( P(l, w_\beta) \) for all the proteins: Figures (1, 2, 3, 4) reveal that indeed a clear dominance of \( P(l, w_\alpha) \) seems to be a sufficient condition for \( \alpha \)-helices, though not a necessary one.

Then we study the ground state of the local hamiltonian \( H_1 \): we set \( \gamma_1 = 1 \) and exhaustively search the configuration space with \( N_h = 4 \), recording the best ten configurations we find. The choice of \( N_h \) is suggested by our a-priori knowledge of the native state of these proteins, and by the reasonable assumption that the existence of short turns is related rather to the three-dimensional structure than to sequence periodicity requirements, so that they could not be efficiently recovered by the local hamiltonian.

To test the goodness of the configurations we find, we proceed as follows: first of all we divide each protein into four parts, corresponding to the four “arms” in the native bundle conformation, and look at those which are in a helical configuration (for 2asr, we consider the short 3\(_{10}\)-helices together with \( \alpha \)-helices).

Then we consider our configurations and compare each element in the bundle with the corresponding native one, and count the residues that have been correctly predicted as belonging to an \( \alpha \)-helix. If \( n_\alpha \) is their number, the quantities:

\[
C_{\text{tot}} = \frac{n_\alpha}{N}, \quad C_{\text{rel}} = \frac{n_\alpha}{n_\alpha^{\text{nat}}},
\]

will give the percentage of success in relation respectively to the total number of residues and to the number \( n_\alpha^{\text{nat}} \) of residues belonging to helices in the native state.

We obtain the following results:

| protein | energy | \((n_1, n_2, n_3, n_4)\) | helix | \(C_{\text{tot}}\) | \(C_{\text{rel}}\) |
|--------|--------|------------------------|-------|---------------|---------------|
| seqB   | -20.290| \((25, 3, 27, 29)\)     | \((\alpha, \alpha, \beta, \alpha)\) | 0.42          | 0.55          |
|        | -20.170| \((25, 13, 19, 17)\)    | \((\alpha, \alpha, \alpha)\)     | 0.70          | 0.93          |
| seqF   | -19.203| \((5, 39, 13, 17)\)     | \((\alpha, \alpha, \alpha)\)     | 0.55          | 0.73          |
|        | -19.062| \((11, 27, 19, 17)\)    | \((\alpha, \alpha, \alpha)\)     | 0.69          | 0.91          |
| 2asr   | -36.550| \((3, 3, 129, 7)\)      | \((\beta, \beta, \alpha, \alpha)\)| 0.24          | 0.27          |
|        | -35.830| \((4, 3, 127, 8)\)      | \((\beta, \alpha, \alpha)\)      | 0.25          | 0.28          |
| 2mhr   | -35.297| \((5, 47, 49, 17)\)     | \((\beta, \alpha, \alpha, \beta)\)| 0.24          | 0.34          |
|        | -35.192| \((39, 13, 49, 17)\)    | \((\alpha, \alpha, \beta)\)      | 0.42          | 0.60          |

For each protein the first line refers to the ground state, while the second refers to the configuration with the highest correlation to the native state, among the ten recorded. The most native-like conformations for the four proteins appear at position 3, 8, 9, 2 respectively, in the list of the best ten configurations.

For both the synthetic sequences native \( \alpha \)-helices correspond to residues (3-16; 22-35; 41-54; 60-73); the secondary structure of 2mhr presents \( \alpha \)-helices at positions (12-14, 19-37; 41-64; 70-85; 93-109, 111-114); that of 2asr shows \( \alpha \)-helices at positions (2-38; 49-72; 80-104; 117-141), while residues 44-48, 77-79 are in 3\(_{10}\) conformation.

**IV. COMMENTS AND CONCLUSIONS**

In this letter we addressed three questions: how good is the hydrophobic dipole moment in describing the relationship between sequence and secondary structure? What is the role
of local terms in the hamiltonian? Is it possible to predict the native secondary structure on the grounds only of the hydrophobic dipole?

The results we obtain show that the choice of describing the sequence periodicity by means of $p^2(l, w, n)$ and $P(l, w)$ (Eqs.(9,15)) is substantially correct, both at a descriptive and at a more quantitative level.

Indeed, a qualitative correlation is evident between regions where $P(l, w_\alpha)$ dominates and the position of $\alpha$ helices, in all the proteins considered. It is however not straightforward to describe this correlation quantitatively, since it is not easy to unambiguously express in mathematical language what one should recognize as “dominant”. For this reason, we cannot exclude that better definitions than Eq.(15,9) may be found to characterize local periodicities in the sequence, even if we consider our choice to be a reliable one.

Moreover, we have introduced an objective way to assess how similar is the ground state to the native one, and indeed the minimal energy configurations we find suggest that our variables and hamiltonian are not so bad in describing the system.

It can indeed be noticed that, despite the strong simplifications introduced in considering only $\alpha$ and $\beta$ helices and in taking $N_h = 4$ (that forbids a simultaneous description of both the helices and the turns), we obtain good results for the two synthetic sequences: among the low energy states a configuration is found which shows a high degree of correlation to the native secondary structure, and the fact that this configuration is not the ground state can be considered a minor problem, at this level of simplification.

The results for 2mhr and 2asr, on the other hand, leave us with several open questions about the relative importance of local and nonlocal terms in the hamiltonian. The hydrophobic moment diagrams Fig.(3, 4) are more complex than those for the synthetic proteins, which could signal a minor importance of the local terms with respect to the nonlocal ones. Indeed, it is commonly believed that the secondary structure results from the need to maximize compactness of the protein and protection of the non-polar residues from water. According to these ideas the periodicity of the sequence could be an outcome of evolution, useful to remove a possible source of frustration and prevent misfolding, while increasing the stability of the native state; yet proteins need not be optimized with respect to their periodicity.

On the other hand, the poor results we obtain with these proteins could also be partially due to the approximations introduced, and we cannot exclude that better predictions could be obtained just resorting to a more complete expression of the local terms. A more definite answer to the above questions is left to future efforts.
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Sequence seqB: MG DLNLLEKFEQLIK GPDSG KLNVVQELQELVQ GPSSG KLKNLLNFEDLIN GPRSG NVQQLLKKLQQMIQ R
Sequence seqF: MG EIIDLQLQELME GPDSG KIQKIKEKVNELMQ GPSSG DLHNLINKLDDVMQ GPRSG KMHDIIIDHLHLLN R.

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FIGURES

FIG. 1. Plot of $P(l, w_\alpha)$ (continuous line) and $P(l, w_\beta)$ (dotted line) for the sequence seqB.

FIG. 2. Plot of $P(l, w_\alpha)$ (continuous line) and $P(l, w_\beta)$ (dotted line) for the sequence seqF.

FIG. 3. Plot of $P(l, w_\alpha)$ (continuous line) and $P(l, w_\beta)$ (dotted line) for the protein 2asr.

FIG. 4. Plot of $P(l, w_\alpha)$ (continuous line) and $P(l, w_\beta)$ (dotted line) for the protein 2mhr.
Sequence seqF
Sequence 2mhr

![Graph showing sequence 2mhr](image-url)