A coarse-grained, “realistic” model for protein folding

Pierpaolo Bruscolini

*Dipartimento di Fisica Teorica, Università di Torino,
v. Giuria 1, 10125 Torino, ITALY

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A phenomenological model hamiltonian to describe the folding of a protein with any given sequence is proposed. The protein is thought of as a collection of pieces of helices; as a consequence its configuration space increases with the number of secondary structure elements rather than with the number of residues. The hamiltonian presents both local (i.e. single helix, accounting for the stiffness of the chain) and non local (interactions between hydrophobically-charged helices) terms, and is expected to provide a first tool for studying the folding of real proteins. The partition function for a simplified, but by no means trivial, version of the model is calculated almost completely in an analytical way. The latter simplified model is also applied to the study of a synthetic protein, and some preliminary results are shown.

I. INTRODUCTION

Protein folding is one of the most challenging problems in molecular biology, and many theoretical models have been proposed, aimed to explain the thermodynamics as well as the kinetics of this process.

It is experimentally known that a protein, under proper solvent and temperature conditions, folds from a denatured random-shaped state to its “native” state, which is characterized only by the amino acid sequence (“the primary structure”), and does not depend on the initial state.

The experimental data for the folding process support the so-called “molten-globule” picture: folding of small single-dominated proteins in proper solvent conditions would start with a rapid collapse from a coil state to a compact one, which is not unique, but is in metastable equilibrium with several other compact states. The protein would appear at this point as a molten globule, not presenting a definite shape. The latter diffuses among the various conformations until it finds its way across the free-energy barrier (probably unique) separating it from the most stable (native) state, and eventually would reach the native state. Because of the cooperativity of the process, folding appears from a thermodynamical point of view as an all-or-none transition, similar to a first-order phase transition in infinite systems.

The understanding of the physics involved in protein folding has greatly profited by ideas and techniques coming from statistical mechanics of disordered systems and random energy models (R.E.M.). As far as thermodynamics is concerned, the number and the complexity of interactions in which residues are involved, as well as the fact that functionally similar proteins may have somewhat different sequences (for instance, lysozymes of different species), have suggested to approach the folding problem by means of an analogy with R.E.M. Analytic results predict a glass-transition when the probability distribution of the couplings is broad enough (that is, when the residues behaves very differently from one another). Several studies have been carried out on short model heteropolymers on a lattice (both with random and with specified interactions), where a preliminary complete enumeration always allows us to find the energy of any configuration. These studies have revealed some important requirements that a sequence should fulfill in order to be a good folder (gap in the spectrum, non-degeneracy of the ground state, particular values of the ratio between collapse and folding temperatures). Debate is still open on the relative importance of these properties for characterizing good sequences. However, what the analysis has assessed is that these requirements are not typical of random sequences, so that a true protein cannot be considered a random heteropolymer, as long as the feature of being able to fold to a stable and unique native state is concerned.

Lattice models have also been employed to study the kinetic aspects of the folding process under the hypothesis that they do not depend on microscopic details of the dynamics. One still gets thus meaningful results when using fictitious Monte Carlo dynamics instead of the true one (which, of course, cannot be easily implemented on a lattice). The above assumption seems reasonable, since the predictions of various diffusive regimes and relaxation times, that come out of these studies, are in good qualitative agreement with phenomenology. Besides, these works have revealed that kinetic accessibility of the native state from a generic initial condition is as important as the already mentioned thermodynamical requirements, for a sequence to be a good folder. As a consequence, it is commonly believed that real proteins present a rough but funnel-shaped free-energy landscape, which provides an overall bias towards the native state.

However, the success of simple lattice models also states their limits.
The results obtained so far constitute an increasing evidence of the fact that proteins are very peculiar heteropolymers; far from being random, they present an "energy-landscape" with correlated minima, and a native state which has probably been selected to be the ground state of the highest number of sequences (to face the risk of mutations) and to be kinetically accessible from all initial configurations of the chain. The role played by local contacts along the chain (the secondary structure), which had been disregarded by REM, is now coming again strongly to attention, as it seems to be crucial for rapid folding and resistance against mutations.

Simple heteropolymer models on a lattice are not well suited to deal with this kind of features, and the only way to improve our knowledge within this scheme would be that of making simulations with longer chains: this is clearly unfeasible, as the only way to find the native state relies on a complete enumerations of all compact configurations of the polymer, whose number grows exponentially with the number of residues.

The need for an exhaustive enumeration comes from the fact that chain connectedness is responsible for a strong frustration of a generic polymer, so that the energy landscape is rugged, and states of the same energy (but very different in shape) may be found anywhere, asking for a complete searching of the configuration space.

Real proteins circumvent this problem when folding to the native state, since they are provided with a sequence which, given the geometrical constraints of connectedness and microscopic steric hindrance to be fulfilled, encodes the smallest frustration, and the smoothest energy landscape (essentially, this is the statement of the "principle of minimal frustration").

The problem with lattice models comes from the fact that we do not know a-priori, given the constraints of lattice geometry (intrinsically different from the natural ones), which sequences of what hydrophobic charges correspond to the smoothest landscapes, and the only way to find it out consists in an exhaustive numerical analysis of the entire configuration space, since no definite hints can be provided by real proteins.

The situation would greatly improve with a model directly related to the real systems, such that a mapping would exist between protein and model configurations. In this case, a direct comparison of the ground state with true native one would be possible, and one could check the goodness of the model by direct inspection.

In this paper, we present a model which, in a coarse-grained way, allows us to deal with any chosen sequence of any length. Such model is based on a description of the protein chain in terms of pieces of helices, implying that the building blocks are indeed the elements of the protein secondary structure, which have an "internal energy" related to Ramachandran’s maps and mutually interact according to the mean "charge" they contain. The Hamiltonian we obtain is realistic, yet quite complicated, just because of its generality. However, simplified models can be extracted from it and studied independently, and the results can be compared to real native states.

The paper is organized as follows: in Sec. II we define the model, discussing the various terms in the Hamiltonian, in Sec. III we derive a simplified model and calculate its ground state and partial partition function; in Sec. IV, we briefly summarize and comment our results.

II. THE MODEL

A. Preliminary remarks

We start from the observation that accurate studies of the phenomenology reveal a number of common features of the native states of the majority of simple, single–domain proteins:

- the native state is organized hierarchically in secondary, supersecondary, and tertiary structures. Typical elements of the secondary structure are α–helices, β–strands and tight–turns; supersecondary rules tell us how these elements pack together locally (prescribing, for instance, the right-handedness of β–X–β units), while the tertiary structure refers to the way the above mentioned elements are arranged in space. As a general remark, one can say that “pieces of secondary structure that are adjacent in the sequence are also often in contact in three dimensions”; knots in the chain seem also to be generally forbidden;

- the native state is highly compact, with the non–polar residues buried on the inside, in order to minimize their contact with the solvent. The urge to protect the hydrophobic residues from water is believed to be the leading factor in the folding process: the secondary and supersecondary structures would emerge in order to accomodate in the best way the hydrophobic core, with the minimal frustration of local interactions;

- the periodicity of the helices tends to mimick that of the sequence, when there is one. It is known, for instance, that α–helices on the surface of the protein’s native state usually present an external side, exposed to the solvent, with polar residues, while the other one is hydrophobic;
the partial success of structure identification methods, based on the analysis of the homologies between sequences, suggests that, even though the folding process is dominated by hydrophobic interactions, local properties pose serious constraints on the final structure, and somewhat limit the number of possible choices.

For the above reasons, we aim to construct a model able to handle both the local and the global aspects of the main chain geometry (disregarding side-chain configurations).

One way to cope with the two contrasting requirements of a coarse-grained “effective” modelling of the interactions, and of a good control of the local constraints imposed by steric hindrance and chain connectedness, is to think of the protein as made up by pieces of different helices, linked together one after the other. This picture is general enough to describe probably all the relevant conformations of a protein: it is built having in mind the above-mentioned features of native states, but it may also represent chains in coil conformations, when many small helices are present, with random orientation. For the consistency of this approach, we shall approximate with a helix a part of the protein at least three peptide units long; shorter helices will not be allowed. Since a perfect helix involves repetition of a fixed dihedral angle \((\phi, \psi)\) at each peptide unit, this approximation may appear to be somewhat crude when there is a strong local variability of the above variables, as it happens in loops and turns. However, this is not a major problem, because a tight turn can be fairly well represented by a short piece of regular helix, and the shortness implies that only a small error in the energy is introduced. The same holds true for any “coil” region of the chain, which may be partitioned and treated in the same way.

The helices are described by their radius and pitch, their length, and the orientation of a reference frame attached to each of them, which specifies the direction of their axis. We shall see later that these are not the most useful variables to introduce in the Hamiltonian, but we start with them for the sake of simplicity.

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

\[
r(s) = \sum_{i=1}^{N_h} b_i(s) h_i(s)
\]

where \(s\) is a continuous variable parametrizing the curve points, and ranging from 0 to \(N\), the total number of residues; \(b_i(s)\) is the limit for \(\lambda \to 0\) of the function

\[
b_i(s, \lambda) = -g_i(s, \lambda) + g_{i-1}(s, \lambda)
\]

which represents a “barrier”: \(g_i(s, \lambda)\) is a function of the variable \(s\) designed in such a way that in the limit \(\lambda \to 0\) it becomes a step function. For instance one could choose

\[
g_i(s, \lambda) = \frac{1}{2} \tanh\left(\frac{s - s_i}{\lambda}\right)
\]

whereby \(b_i(s_{i-1}, 0) = b_i(s_i, 0) = 1/2\), and

\[
g_i(s, 0) \equiv \frac{dg_i}{ds}(s, 0) = \delta(s - s_i)
\]

The \(h_i\) are the helices expressed in their reference frame \((e_{1,i}, e_{2,i}, e_{3,i})\):

\[
h_i(s) = a_i \left[ (\cos(u_i(s - s_{i-1})) - 1) e_{1,i} + \sin(u_i(s - s_{i-1})) e_{2,i} + u_i h_i(s - s_{i-1}) e_{3,i} \right] + h_{i-1}(s_{i-1})
\]

labeled 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 of them. We will name from now on \(n_i = s_i - s_{i-1}\) the length of helix \(i\). We choose

\[
u_i = \sigma_i \frac{L}{a_i \sqrt{1 + h_i^2}}
\]

where \(L\) is the length of a peptide unit (the distance between two neighboring \(\alpha\)-carbon atoms), so that the line element on each helix is \(ds = Lds\). 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 piece of chain they represent: this may be done by requiring that \(\Delta s = 1\) when we move along the protein chain of one peptide unit: in this way the above defined \(n_i\) coincides with the number of residues in the secondary structure element that the helix describes.
We see that six scalar variables are needed to specify a helix: they are \( n_i, a_i, h_i \), and three Euler rotation angles relating the helix reference frame to the fixed "laboratory" one. For an infinite helix, the radius \( a_i \), the pitch \( h_i \) and the angular parameter \( u_i \) are related to curvature \( \kappa_i \) and torsion \( \tau_i \) in a straightforward way:

\[
a_i = \frac{\kappa_i}{\kappa_i^2 + \tau_i^2}, \quad h_i = \frac{\tau_i}{\kappa_i}, \quad u_i = L\sigma_i(\kappa_i^2 + \tau_i^2)^{\frac{3}{2}}.
\]

Obviously curvature and torsion are the natural candidates to appear in the local part of the Hamiltonian, which will take into account the stiffness of the chain and the steric hindrance of the residues. One is therefore led to study the algebraic operations on curvature and torsion.

For instance we obtain for the curvature (see Appendix [A]):

\[
\kappa = \sum_{i=1}^{N_h} \left( \kappa_i \delta(s - s_{i-1}) \delta(s_i - s) + \delta(s - s_i) \left( \frac{1 - (h_i \cdot h_{i+1})^2}{1 + h_i \cdot h_{i+1}} \right)^{\frac{1}{2}} \right)
\]

where dots indicate derivatives with respect to \( s \), and \( \delta(s - s_i), \delta(s - s_i) \) are respectively Dirac delta and Heaviside theta functions.

We see that the relevant quantity at the interface is the scalar product \( h_i \cdot h_{i+1} \) between the right and the left limit in \( s = s_i \) of the tangent vectors. Similar results hold for the torsion, as well as for any other quantity obtained by algebraic operations on curvature and torsion.

The fact that it is possible to reduce the expressions of curvature and torsion of the whole chain to sums of the corresponding quantities for each helix, plus "interface terms" depending only on nearby elements, suggests that also the Hamiltonian may be built as a sum of "local" single-helix terms with next-neighbour interactions, accounting for the stiffness of the chain. In addition to these, a third, non-local term, will describe the interactions between non-neighbouring helices. Therefore we write:

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

The protein sequence will come into play in the last term, because the interaction between helices obviously depends on the residues they are made of, but will also have a role in the first one, as helices are preferred if they present the same local periodicity as the sequence.

The explicit form of the Hamiltonian will be discussed in section \[\text{II C}\]; in the next one, we introduce a formalism allowing us to treat conveniently the non-local term, which is awkward to handle in the variables that appear in Eqs. [1] and [2].

\[\text{B. Dynamical variables}\]

In order to specify the position and the kind of each helix, we introduce the following variables:

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

where \( p_2 = N - (N_h - 1)p_1 \) and \( p_1 = 3 \), since a helix cannot be defined with less than three residues; \( q_{1,i} = \frac{1}{2}[1 + p_1(2i - 1)], q_{2,i} = N + \frac{1}{2}[1 - p_1(2(N_h - i) + 1)] \). In the above equations \( n_i \) 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 \( h_i \); it is the geometrical analogue of \( n_i \); \( B_i \) is the spatial position of the middle point of \( v_i \).

Two other variables, related to curvature and torsion, are needed to completely specify the characteristics of a helix: a useful choice, which will allow us to write a realistic potential in a simple form (see Section \[\text{II C}\] below), is to introduce:
where \( u_i \) has been defined in Eq. (4). Note that \( z_i \) ranges between 0 and 1, while \( w_i \in [0, 2\pi] \). The definition of \( w_i \) 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 term of helices. A chain in such a conformation, where there are exactly two residues per turn, may be regarded as both left-handed and right-handed, and a little deformation can bring to an arbitrary number, comparable with the mean length of the helices).

The above variables specify how the chain is partitioned into helices and is embedded in three-dimensional space, providing a sequence-independent formalism. The sequence enters the model through new variables \( q_k \) \((k = 1 \ldots N)\) and \( p^2_\perp(l, w) \). The former are related to the nature of each residue \( k \), and measure its coupling to the other residues. In the following we shall refer to Li and coworkers\(^{21}\) who write the Mijazawa-Jernigan interaction matrix\(^{21}\) as:

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

(a slight change of notation is performed here with respect to the original paper). This equation can be recast as:

\[
M_{\rho\sigma} = Q_\rho + Q_\sigma + \frac{\mu_2}{2}(q_\rho - q_\sigma)^2
\]

with

\[
Q_\rho = \mu_0/2 + \mu_1 q_\rho + (\mu_2/2)q_\rho^2.
\]

The authors show that \( Q_\rho \) correlates well with the hydrophobicity of the residues; for this reason we can call it "the hydrophobic charge" of aminoacid \( \rho \).

Since we deal with entire helices at a time, and not with single residues, we shall introduce the average \( q \) of a helix, centered in \( l_i = l \), as

\[
\bar{q}(l) = \begin{cases} 
\frac{1}{2m+1} \sum_{j=-m}^{m} q_{l+j}, & \text{if } l = 1, 2, \ldots, 2n+1 \\
\frac{2}{2m+1} \sum_{j=-m}^{m} (q_{l-\frac{1}{2}+j} + q_{l+\frac{1}{2}+j}), & \text{if } l = \frac{1}{2}, \frac{3}{2}, \ldots
\end{cases}
\]

(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 corresponding values for the hydrophobic charge \( \bar{Q}(l) \) are obtained from Eq. (12) with \( q \) replaced by \( \bar{q} \). Notice that this is the correct way of evaluating \( \bar{Q}(l) \), since it is easy to show that the average interaction between \( n_1 \) residues on a helix and \( n_2 \) on another is given by:

\[
\mathcal{M} = \frac{1}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} M_{ij} = \frac{1}{n_1 n_2} \left[ n_1 n_2 \mu_0 + \mu_1 \left( n_2 \sum_{i=1}^{n_1} q_i + n_1 \sum_{j=1}^{n_2} q_j \right) + \mu_2 \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} q_i q_j \right] = \\
= \mu_0 + \mu_1 (\bar{q}_1 + \bar{q}_2) + \mu_2 \bar{q}_1 \bar{q}_2,
\]

and hence is naturally written as a function of the average \( \bar{q}_1 \) and \( \bar{q}_2 \) on the helices.

The other variables are related to the local periodicity of the sequence, and are defined in the following way. Considering a generic helix of length \( 2n+1 \), centered at the point \( l \) along the sequence, the quantity:

\[
P_{\perp}(l, w, n) = \frac{1}{\sum_{k=-n}^{n} Q_{l+k}} \sum_{k=-n}^{n} Q_{l+k} (\cos[w(l+k)]e_1 + \sin[w(l+k)]e_2)
\]

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 charge \( \sum_{j=-n}^{n} Q_{l+j} \) (for the sake of simplicity, we take \( l \) to be an integer in these equations).

We observe that \( p^2_\perp \) reveals the prevalence of non polar residues on one side of the helix, characterized by the periodicity \( w \). Therefore, in Sec. [II C 1] we shall write a local hamiltonian depending explicitly on
and favouring configurations which maximize $p_\perp^2$.

The dynamical variables previously defined are not completely independent from each other, and the following constraints hold:

1. the sum of the residues of all the helices must be equal to the total length of the chain:

$$ \sum_{i=1}^{N_h} n_i - N = 0 $$

2. the length of the end-to-end vector $v_i$ is related to the length and shape of the helix:

$$ v_i^2 - |h(s_i) - h(s_{i-1})|^2 \equiv v_i^2 - n_i^2 L^2 \left[ z_i^2 + (1 - z_i^2) \frac{\sin^2(\theta_i)}{\theta_i^2} \right] = 0 $$

3. the end of one helix must coincide with the beginning of the following one, both in sequence and in space:

$$ B_i - B_{i-1} - \left( \frac{v_i + v_{i-1}}{2} \right) = 0 $$
$$ l_i - l_{i-1} - \frac{n_i + n_{i-1}}{2} = 0 $$

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

From the above discussion the following picture emerges: we describe the geometric shape of the protein by the variables $B_i, v_i, w_i, z_i, n_i, l_i$; besides, we give the sequence of “charges” $q_k$ which represent its residues.

Notice that the above variables do not specify completely the position of the helices in space, because, when the constrains are satisfied, there is still a complete degeneracy for rotations of each helix $h_i$ around the vector $v_i$.

Actually, given a chain conformation, only one of these degenerate configurations, which are indistinguishable in our scheme, corresponds to it. Therefore, provided we choose a good criterion to identify helices out of real chain conformations, we have that any protein configuration can be mapped in exactly one model configuration.

We may ask if the converse is also true: considering a given a model helix, specified by the variables $n, w, z, v$, with its end points pinned at $P_1 = B - v/2$, $P_2 = B + v/2$, we see that:

a. many chain conformations, slightly different from one another, correspond to it, since real helices are not made up by an exact repetition of dihedral angles; anyway, we assume we can consider these to be represented by the same geometrical helix;

b. it’s quite unlikely that two (or more) real helices, with shape and length well described by the above variables, but corresponding to different degenerate positions around vector $v$, may exist;

c. it could be possible, on the other hand, that no real helix with the above parameters could fit between $P_1$ and $P_2$, due to the stiffness of the chain at those points.

If case ‘c’ holds, troubles arise, since forbidden chain configurations could appear as allowed model ones.

In the following, we shall make the assumption that, given a model helix (of length $n \geq 3$), with its end-points at $P_1$ and $P_2$, it is always possible to replace it with the corresponding real chain helix, in such a way that no relevant perturbation is introduced in the total energy of the protein.

This is reasonable, since small adjustments of the chain, out of a perfectly regular configuration (yet not affecting its overall helical shape), can intervene and prevent forbidden joint conformations, thus reducing the energetic penalty to a small fraction of the total energy. We shall come back to the discussion of the energy contributions from the helix junctions in the next section, where we discuss the explicit form of the hamiltonian.
C. The Hamiltonian

1. The local hamiltonians $H_i$

Two terms will contribute to the internal energy of a helix: the first one, $H^0_i$, is purely geometric, and takes into account the experimental Ramachandran plot to dictate which kind of helices are more likely to be formed. The second one, $H^1_i$, on which we commented above, is sequence dependent, and acts as an external field, biasing the helices towards a certain periodicity.

It is important to notice that, if proteins were made by a sequence of exact repetitions of the same $(\phi, \psi)$ angles, the association of a geometrical helix to each part of the chain would be straightforward and the geometrical quantities $w, z$ of the helix could be written as functions of $(\phi, \psi)$:

\[
\begin{align*}
\frac{\cos\left(\frac{\pi}{2} \right)}{z} &= \sigma(s_2) c(\phi, \psi), \\
\frac{\sin\left(\frac{\pi}{2} \right)}{z} &= \frac{\sigma(s_2) |s_2|}{\sqrt{1 - c^2(\phi, \psi)}},
\end{align*}
\]

(18)

where we have defined:

\[
\sigma(x) = \begin{cases} +1, & \text{if } x > 0 \\ -1, & \text{if } x \leq 0 \end{cases}
\]

and use the explicit expressions

\[
c(\phi, \psi) = a \sin\left(\frac{\psi + \phi}{2}\right) + b \sin\left(\frac{\psi - \phi}{2}\right),
\]

\[
s_2 = c \cos\left(\frac{\psi + \phi}{2}\right) + d \cos\left(\frac{\psi - \phi}{2}\right),
\]

(see Appendix[3] for details). The mapping $(\phi, \psi) \rightarrow (w, z)$ in the above equations is two-to-one, as can be seen from the study of the solutions of the fourth degree polynomial equations involved in the inversion of Eqs.(18). This reflects the fact that $(\phi, \psi)$ provide a complete description of the geometry, specifying not only the position of the $C_\alpha$ atoms, but also the orientation of the peptide planes, which have been disregarded in our approach.

Dealing with real proteins implies that each helix is associated to a portion of chain where a certain amount of irregularity in $(\phi, \psi)$ is inevitable. This has no practical consequences when the irregularities are small and the dihedral angles are clustered around a particular position (which happens for long elements of secondary structure). However, for short coil regions with a great variability in the dihedrals, it would be quite artificial to relate the best fitting values of $w, z$ to an hypothetical $(\phi, \psi)$ repeating couple. It is clear that in the latter case the relationship between $(w, z)$ and $(\phi, \psi)$ is weakened. For this reason we shall make the simplifying assumption that $w, z$ can be assumed as fundamental variables, and that the helix-model configurations are in a one-to-one relationship with them. Since $(w, z)$-couples that cannot correspond to any real protein configuration are introduced by this ansatz, we shall write the hamiltonian $H^0_i$ in such a way that these values have a vanishing weight in the evaluation of the partition function.

The choice of an explicit expression for the hamiltonian requires a careful analysis, because no reliable potential function based on first principles is known. This is not surprising, if one thinks that each residue is itself a many-body system, usually in interaction with the solvent molecules. On the other hand, the fact that even a crude hard-sphere model for a dipeptide reproduces the experimental Ramachandran’s maps in an essentially correct way (compare for instance Fig. (12A, 13A) in Ramachandran’s article[22] with Fig. (5) in Morris et al.[23]), implies that a simplified description of the potential function should be possible, and suggests its main characteristics.

We already mentioned the fact that, even in the case a perfect helical-shaped chain, the variables $w, z$ give a description of the chain geometry which is less detailed than that provided by $(\phi, \psi)$ angles, and even more so in comparison with an all-atom description. Despite this, it is possible to write a potential function in the variables $w, z$ which correctly reproduces the main features of the Ramachandran’s plots, in the form

\[
H^0_i = (n_i - 1)\gamma_0 \left[ c_1 \left( (w_i - c_2)^2 - c_3 \right)^2 + c_4 + c_5 \left( z_i - c_6 + c_7 (w_i - c_8)^2 \right) \right].
\]

(19)

Here the $c_k$’s are fixed adimensional constants; the factor $(n_i - 1)$ takes into account the feature that each residue in the helix feels the same potential, except the last one, which corresponds to the junction with the following helix, and must be treated in a different way. In Figs. (12) we plotted the contour lines of this potential, with $n_i - 1 = 1$, respectively as a function of $w, z$, and of their images on the $(\phi, \psi)$ plane.
Note that, since $b/a \approx 1/20$, there is nearly symmetry under the exchange of $\phi$ and $\psi$, so that there is an intrinsic difficulty in distinguishing the region above from that below the diagonal $\phi = \psi$ by means of the quantities at the right member of Eq. (13). This can be considered a minor problem, since the physically allowed region roughly coincides with that above the diagonal $\phi = \psi$ and we aim to study, as a first approach, only the behaviour of a system which presents two energy minima roughly corresponding to the $\alpha$- and $\beta$-regions of Ramachandran’s map (as a consequence, we disregard left-handed $\alpha$-helices, since a residue in that position usually belongs to a turn, and not to an actual left-handed helix).

The above ad hoc hamiltonian provides a correct qualitative description of the phenomenological results, without introducing expressions more complicated than a fourth degree polynomial. In the following we shall consider it as an unperturbed hamiltonian, to which the non local-interactions add as perturbations, which remove its degeneracy without affecting its overall shape in plane $(\phi, \psi)$.

Coming to the sequence dependent part of the local hamiltonian, a very natural choice is that of taking

$$H^1_i = -\gamma_1 n_i P(l_i, w_i), \quad \text{(20)}$$

where $\gamma_1$ is an appropriate dimensional constant, and $P(l_i, w_i) = F(p^2_i(l_i, w_i, n))$ is some simple function of $p^2_i(l_i, w_i, n)$. For instance $F$ could be either the average of $p^2_i(l_i, w_i, n)$ calculated for different accessible lengths of the helices (the values of $n$ in Eq. (13), or $p^2_i$ itself, evaluated with a particular phenomenological mean value of $n$.

A more detailed study on the best expression for $F$ is left to future work on the subject: in the following, we shall choose a particular form for $F$ only when, in section II B, we shall study the ground state of hamiltonian Eq. (20) for a small synthetic protein, showing that $P(l_i, w_i)$ can indeed provide partial information about the native state.

2. The interaction between neighbouring helices $H_{i,i+1}$

So far we have disregarded the contributions to the energy coming from the aminoacids at the junctions between helices: they have been kept out from Eq. (20) thanks to the factor $(n_i - 1)$. When we try to keep them into account, we immediately face many difficulties, since there is apparently no natural way to relate exactly the microscopic potential determining the possible $(\phi, \psi)$ values to the description of the chain in terms of helices.

We saw in Eq. (5) that, at the junctions between helices, the curvature depends on the scalar product of the right and left limits of tangent vectors in those points. This would suggest to write an interaction penalizing discontinuities in the tangent vector; yet, this is quite awkward for the following reasons:

- while it is possible to relate curvature and torsion of a helix to the dihedral angles specifying the chain, it is extremely complex to do the same for the scalar product $\mathbf{h}_i \cdot \mathbf{h}_{i+1}$: the knowledge of $(\phi, \psi)$ at the junction is not sufficient to specify the direction of the tangent vectors (remember that the $C_\alpha$ atoms of the chain do not even lie on the helices, due to the requirement that lengths be the same when measured along the (continuous) helices or the (discrete) chain);

- even if it were possible to find a mapping, relating the dihedral angles at the junction to the helix geometrical description, in terms of tangent vectors, uncontrollable mistakes would be done in evaluating the energy. In fact, real helix junctions are different from ideal ones, since structural adjustments are allowed in real chains to minimize the energetic cost of the junction which cannot be described by ideal chain geometry, where helices are stiff. Hence, if we are interested in a reasonable estimate of the energy, a detailed description of the tangent vectors’ dependence on the $(\phi, \psi)$ angles at the junction is essentially useless.

- Finally, tangent vectors are very difficult to write down within the adopted formalism (because of the degeneracy under rotations, discussed in Sec. IV B) even if we don’t relate them to real chain quantities.

Therefore, we have to write down an expression for the interaction energy between neighbouring helices without relating it to the real chain geometry, and without resorting to tangent vectors. The natural candidates to appear in such an expression are, of course, the vectors $\mathbf{v}_i, \mathbf{v}_{i+1}$, but the functional form to be chosen is by no means obvious.

In principle one could study first the total energy of two successive secondary structure elements (for instance, by looking at dihedral angles and applying microscopic potentials) as it comes out from phenomenology, then represent such elements with model helices, and finally get the interaction energy as the difference between the total and the sum of the single helices’. It would be possible, in this way, to relate the interaction energy to the relative positions of $\mathbf{v}_i$ and $\mathbf{v}_{i+1}$.
Yet, as a first approach, we assume this energy to be a constant, at each junction, regardless of the values of the dynamical variables: for instance, we can take the mean energy of the residues calculated with the Ramachandran’s map distribution. Since \( N_h - 1 \) is the number of junctions, we set:

\[
H_{nn} = \gamma_2 (N_h - 1) .
\]

This simple hypothesis entails an important effect: if one neglects the sequence-dependent hamiltonian \( H^1_1 \) and the non-local interactions, splitting up a helix in two pieces with the same \((w, z)\) as the former one involves the substitution of a residue of energy \( H^0(w, z) \) with a residue of mean energy \( \gamma_2 \). Hence helix breaking will be penalized for helices with ”good” values of \((w, z)\) (\(\alpha\)-helices and \(\beta\)-strands, for instance), and favoured in the opposite case.

This is very important, because both entropic effects and non-local interactions, as we shall see below, would favour configurations with many short helices, regardless of \((w, z)\) values: \(H_{nn}\) competes with the above effects, allowing, in principle, the existence of equilibrium states of the model presenting long elements of secondary structure.

3. The non-local interactions \(H_{ij}\)

The modeling of non-local interactions requires a careful analysis of their nature and characteristics. Two different contributions are to be dealt with: hydrogen bonds and hydrophobic interactions. The former are responsible of orientational preferences of the couplings between elements of the secondary structure, but are believed to play an insignificant role as a driving force for folding, since hydrogen bonds to solvent are of the same energy than orientational preferences of the couplings between elements of the secondary structure, but are believed to play to model, since they come mostly from entropic effects involving the solvent, and not from a true coupling between residues. For these reasons, we abandon the idea of writing non-local interactions on the grounds of microscopic considerations, and once more resort to phenomenology.

First of all we notice that typical distances between the axes of interacting secondary structure elements in the native state range from 0.46 nm (two hydrogen-bonded \(\beta\)-strands) to about 1 nm (two \(\alpha\)-helices or two \(\beta\)-sheets). Therefore we simply write down an attractive square-well potential in the variable \(\Delta B_{ij} = |B_i - B_j|\), taking \(B_i\) as representative of the \(i\)-th helix, with a hard core repulsion preventing overlap between helices.

Then, we look at the phenomenology of non-local interactions in the native state, considering at first only the hydrophobic effect and disregarding hydrogen bonds in \(\beta\)-sheets. We see that usually two elements of secondary structure tend to pack as closely as possible, just due to the hydrophobic effect. For geometrical reasons this usually means that they cannot be parallel; thus the number of residues which are actually into contact is independent of the length of the helices, and also, roughly, of their characteristics. If, following Li and coworkers\(^2\) we take as the ”microscopic” contact interaction between two residues that given in Eq. (8), we can write for the interaction between helices:

\[
H_{ij} = \vartheta (\rho_1 - \Delta B_{ij}) \vartheta (\Delta B_{ij} - \rho_0) \left[ \gamma_3 \chi (\mu_0 + \mu_1 (\varphi(l_i) + \varphi(l_j)) + \mu_2 \varphi(l_i) \varphi(l_j)) + \right. \\
\left. + \gamma_4 (\rho_0 - \Delta B_{ij}) \right],
\]

where \(\varphi(l_i)\) are the quantities defined in Eq. (12); \(\chi\) is the average number of contacts between residues in two close-packed elements of the secondary structure, \(\gamma_4 \gg 0\) provides an hard-core repulsion when the distance is less than \(\rho_0\); \(\rho_1\) is the range of the attractive interaction \(\gamma_3 > 0\) ”normalizes” the interaction with respect to the other terms in the hamiltonian: again, it should be small compared to \(\gamma_0\).

Coming to the hydrogen bonds in a \(\beta\)-sheet, we see that they tend to align the two interacting strands, independently of their charge. Yet, it is known that \(\beta\)-sheets show very little stability when exposed to the solvent, because residues easily form hydrogen bonds with water. In our approach, where the solvent is taken into account implicitly in the coupling strenght, one should relate hydrogen-mediated interactions to the geometry of the helix and to the surrounding environment (in order to distinguish between exposed and buried sheets). Hence, hydrogen bonds between \(\beta\)-strands should depend on the overall hydrophobic charge of the environment they are embedded in: this is far too complex to be described exactly.

For the sake of simplicity, we shall use Eq. (23) also to describe interaction between \(\beta\)-strands, neglecting the tendency towards alignment. A more detailed representation would involve the introduction of terms involving \(v_i \cdot v_j\), and also, perhaps, of \(v_{i-1} \wedge v_{i+1} \cdot v_i\), to account for right-handedness of super-secondary structures like \(\beta\)-X-\(\beta\).

As a result of the above discussion, the complete hamiltonian of our model, also including the constraints, reads:

\[
H = H_{nn} + \sum_{i=1}^{N_h} (H_i^0 + H_i^1) + \sum_{i<j=2}^{N_h} H_{ij} + \lambda_0 + \sum_{i=1}^{N_h} (\lambda_i^1 + \lambda_i^2 + \lambda_i^3),
\]
where, recalling here all the results for the sake of clearness:

\[
H_{nn} = \gamma_2(N_h - 1) ,
\]

\[
H^0_i = (n_i - 1)\gamma_0 \left[ c_1 \left( (w_i - c_2)^2 - c_3 \right)^2 + c_4 + c_5 \left( z_i - c_6 + c_7(w_i - c_8)^2 \right)^2 \right] ,
\]

\[
H^1_i = -\gamma_1 n_i P(l_i, w_i) ,
\]

\[
H_{ij} = \vartheta(\rho_i - \Delta B_{ij})\vartheta(\Delta B_{ij} - \rho_0) \left[ \gamma_3 \chi \left( \mu_0 + \mu_1 (\vartheta(l_i) + \vartheta(l_j)) \right) + \mu_2 \vartheta(l_i)\vartheta(l_j) \right] + + \gamma_4 \vartheta(\rho_0 - \Delta B_{ij}) ,
\]

\[
\mathcal{V}^0 = \lambda_0 \left( \sum_{i=1}^{N_h} n_i - N \right) ,
\]

\[
\mathcal{V}^1_{ij} = \lambda_{1,i} \left( \mathcal{V}_{ii} - n_i^2 L^2 z_i^2 \right) ,
\]

\[
\mathcal{V}^2_{i-1,i} = \lambda_{2,i} (\mathbf{B}_i - \mathbf{B}_{i-1} - \frac{(\mathbf{v}_i + \mathbf{v}_{i-1})}{2}) ,
\]

\[
\mathcal{V}^3_{i-1,i} = \lambda_{3,i} \left( l_i - l_{i-1} - \frac{n_i + n_{i-1}}{2} \right) ,
\]

\[
\lambda_0 = \lambda_0' - \gamma_0 c_4 , \quad \gamma_2 = \gamma_2' + \gamma_0 c_4 ,
\]

The \( \lambda_{a,i} \)'s are Lagrange multipliers that allow us to insert the appropriate constraints in the hamiltonian.

Notice that we have introduced an approximated expression of \( \mathcal{V}^1_i \) (compare it with Eq.(14)): this is possible because, for the most significative regions of \((w, z)-\)plane, with any allowed value of \( n_i \) (remember that \( n_i \geq 3 \)), the \( u \)-dependent term in Eq.(14) is negligible.

Without loss of generality, we can moreover take \( c_4 = 0 \): in fact, \( c_4 \) can always be eliminated by the transformation:

\[
\lambda_0 = \lambda_0' - \gamma_0 c_4 , \quad \gamma_2 = \gamma_2' + \gamma_0 c_4 ,
\]

whereby the hamiltonian changes of the constant term \((N - 1)\gamma_0 c_4 \). The way we have chosen to implement the constraints is particularly suitable to carry on some analytic calculations on the model. Obviously, it is not the only possible one, and different choices may be useful in different approaches.

An important remark concerns \( H_{nn} \): the choice of an expression independent of \( \mathbf{v}_i \) introduces possible symmetries in the model that could be exploited to some extent. If, in fact, we disregard the sequence, taking \( q_k = q \) as a constant and neglecting \( H^1 \), we see that, given a set of \( B_i \), and a choice of \( n_i, w_i, z_i \), providing a total internal energy \( E^0 = \sum_{i=1}^{N_h} H^0_i \), we can certainly change the values of \( n_i, w_i, z_i \), together with the vectors \( \mathbf{v}_i \), in such a way that \( E^0 \) is unchanged and the \( \mathbf{B}_i \) fixed, so that also the non-local interaction remains the same. This symmetry is reduced, or removed, by the introduction of the real charges \( q_k \), but this has to be studied independently in each case.

The picture that emerges from the above hamiltonian is that of a complicated interplay among dynamical variables: a helix of a certain shape and length, specified by \( w, z, n \), will be attributed an energy based on \( H^0 \) (which is related to dihedral angles configuration), plus a sequence-dependent contribution \( H^1 \) depending on its position \( l \) along the chain. The values of \( z \) and \( n \) then determine, through constraint \( \mathcal{V}^1 \), the length of the vector \( \mathbf{v} \), and through \( \mathcal{V}^2 \), the spatial coordinates \( \mathbf{B} \) of the helix. The latter, in turn, determine whether the helix considered interacts with other helices by the term \( H_{ij} \), where the charges \( \vartheta(l) \) again depend on the internal coordinate \( l \).

For the above reasons, the hamiltonian Eq.(23) is inevitably complicated. It should be remembered, though, that it describes a generic protein, with any sequence, and within a very realistic framework, which deals directly with secondary structure elements. Moreover, the model involves a reduction of the intervening number of independent dynamical variables \( 5N_h \) against \( N \) couples \((\phi, \psi) \), if \( N \) is the number of residues, with a rough estimate for the ratio as \( 5N_h/2N \approx 1/3 \), so the shortest proteins could lie within the reach of numerical studies.

In this case predictions of the model can be compared directly with experimental findings, which could remove the need of an exhaustive search for the ground state, that is the starting point of many lattice models currently studied. Quantities like:

\[
\delta^2 = \frac{1}{N_h} \sum_{i=1}^{N_h} \left( \left( \mathbf{B}_i - \mathbf{B}_i^{nat} \right) \right)^2 ,
\]

measuring the distance of equilibrium structure from the experimental native state (the average is taken e.g. in a canonical ensemble), as well as its analogue referring to line coordinates:
can give information on the most appropriate choice of the parameters in the hamiltonian, and on the folding transition.

Another important topic to be investigated is that of the identification of order parameters, which could characterize the folding transition in an intrinsic way, with no reference to a native state known a priori. The study of the temperature dependence of correlation functions could possibly distinguish a true folding transition of a “good” sequence from the freezing of a “bad” one into any minima of a rugged landscape.

In order to get information about such thermodynamical quantities, we must be able to evaluate the partition function associated to the protein hamiltonian $H$. This is of course a very complicated task, and deserves a complete and specific analysis which is beyond the scopes of the present paper. In the next section we shall show, anyway, that one can resort to the study of simplified models and get indeed useful information both on the protein under investigation, and on the best way to extend the analysis to the more general case of the complete model.

III. A SIMPLIFIED MODEL

In this section, we shall mainly deal with a simplified version of the model, where only the local terms $H^0_i + H^1_i$ are kept into account and the number of helices is fixed. Eventually we shall add non-local interactions to it as a small perturbation, in order to retrieve information about the spatial conformation of the protein.

When dealing with the local model, in fact, we loose the description of the spatial structure, but we are left with a highly non-trivial model, with the sequence coming into play through $H^1_i$, which provides interesting information about both the native state and the relative importance of the interactions stabilizing it. Indeed, the requirement of maximizing the separation of hydrophobic charges on the helices generates a scenario in which the most amphiphilic helices tend to compete with each other in order to grow as long as they can. The equilibrium configuration one finds in this way specifies how the protein should be partitioned in secondary structure elements to obtain the highest amphiphilicity. Therefore, it provides some important insight on the secondary-structure composition of the native state, so that it is natural to ask oneself if, at least in some cases, the three-dimensional structure could be superimposed to the resulting secondary one, introducing non-local interactions as a small perturbation driving the helices to the correct configuration.

In the first part of the present section we indeed show that, for the simple synthetic protein (already studied by Kolinski and coworkers$^{[a]}$ and Raleigh and DeGrado$^{[b]}$) specified by the sequence GEVEELLKKFKELWKG PRR GEVEELLKKFKELWKG PRR GEIEELFKKFKELIKG, the above scheme may be successfully applied. We shall in fact find the set of $n_i$, $w_i$ corresponding to the minimum of the local hamiltonian; then, we shall switch on the non-local interaction as a small perturbation and eventually find that native-like configurations are indeed the ground state. Encouraged by this result, we shall pursue the study of the local model and, resorting to some general assumptions on $P(l_i, w_i)$ and to suitable approximations, we shall find out – in an almost completely analytical way – an expression for the partition function of a generic protein, which can represent a good starting point to study the thermodynamics of the complete model.

Both these investigations are intended as preliminary tests, aiming to demonstrate on the one hand that the variables we have chosen accurately describe the sequence, and on the other that the model we propose, despite its complexity, is indeed analytically manageable, at least in some simplified case.

We start with the hamiltonian:

$$H\{w_i, n_i, l_i\} = \sum_{i=1}^{N_h} (H^0_i(w_i, n_i) + H^1_i(w_i, l_i, n_i)) .$$

(24)

where the constraints are exactly implemented, through the equations:

$$l_i = \frac{1}{2} (n_i + 1) + \sum_{k=1}^{i-1} n_k$$

$$n_{N_h} = N - \sum_{i=1}^{N_h} n_i$$

(25)

We assume that the function $P(l_i, w_i)$, appearing in the expression of $H^1_i$ (Eq. (24)), has the form:

$$P(l_i, w_i) = \left\{ \begin{array}{ll}
\frac{1}{2} p_1^2 (l_i, w_i, 3), & \text{if } l_i \text{ is an integer} \\
\frac{1}{2} p_1^2 (l_i - \frac{1}{2}, w_i, 3) + p_1^2 (l_i + \frac{1}{2}, w_i, 3), & \text{if } l_i = k + \frac{1}{2}, \text{ for integer } k
\end{array} \right.$$
We choose $n = 3$ in expression (13) since this involves calculating the hydrophobic dipole on an helix of seven residues, a reasonable length both for $\alpha$-helices and for $\beta$-strands. We assume that $\gamma_1/\gamma_0 \ll 1$, so that we can approximate the minima of $H$ with those of $H^0_i$, as far as $w_i$ is concerned. In this way we are left with only two values for the $w_i$, namely $w_{00}$, $w_{03}$, which in turn entails that we can forget about $H^0_i$, whose minima are symmetric, and only deal with $H^0$.

Since the $n_i$ are integers and the $l_i$ are integers or half-integers, we are left with a discrete configuration space, whose size depends on the number of helices we are considering.

Obviously, we have $2^{N_h}$ configurations for the set of $w_i$, but, once given the set of positions $l_i$, one “a-priori” knows which has the lowest energy, by a direct comparison of $P(l_i, w_{00})$ and $P(l_i, w_{03})$ (see Fig. 3). Hence, we have to find the energy minimum in a space that contains as many points as the number of possible partitions of $N$ residues in $N_h$ helices which have a minimum length of $p_1$ residues. One can easily convince oneself that this number is given by:

$$\pi(N_h) = \sum_{j=0}^{N_h-1} \left( \frac{N - N_h \rho_1 - 1}{j} \right) \left( \frac{N_h}{j + 1} \right).$$

(27)

For the protein considered we a priori know that its native state is made up of four $\alpha$-helices (indeed the sequence has been designed to produce a 4-helix-bundle), so we try $N_h = 4$ and ask ourselves if our model will be able to find out the correct position, length and kind of helices.

We set $\gamma_1 = 1$, $c_4 = 0$ and exhaustively searched the configuration space with $N = 73$ and $N_h = 4$, which contains $\pi(N_h) = 41664$ points. We found that $(n_1, n_2, n_3, n_4) = (17, 19, 20, 17)$, with all the helices being $\alpha$-helices, is the ground state of our simplified model. This result is in excellent agreement with the experiment, and suggests that, at least for some proteins, the only requirement of maximal local amphipilicity may be enough to get the correct composition of the secondary structure.

Encouraged by this result, we will not the non-local interactions Eq. (22) as a small perturbation ($\gamma_3 \ll \gamma_1$) to the simplified model (24), and try to predict the tertiary structure. We proceed as follows: relying on the fact that the gap between the ground-state and the first “excited state” of our simplified model is necessarily finite (the $n_i$ may only assume integer values), we freeze the secondary structure $(n_1, n_2, n_3, n_4)$ we have obtained and look for the minimum of $H_{int} = \sum_{i=1}^{N_h-1} \sum_{j=i+1}^{N_h} H_{i,j}$, where $H_{i,j}$ is given by Eq. (22). We choose in that equation the values $\rho_0 = 5$ Å, $\rho_1 = 9$ Å, and show that the bundle-like configurations are indeed the ground state (even if degenerate) of the model. First of all we notice that, once fixed the length in residues $n_i$ of each helix and its $z_i$ according to the fact they are all $\alpha$-helices, we have a unique set of $v_i$, coming from Eq. (15). Then, we see that, upon defining $d_i = B_{i+1} - B_i$, the set of twelve cartesian components of the four vectors $v_i$ are subjected to the thirteen equations (see Eq. (17)):

$$d_j = \frac{1}{2} (v_j + v_{j+1}) \quad (j = 1, 2, 3)$$  

(28)

$$v_i^2 = v_i^2 \quad (i = 1, 2, 3, 4)$$  

(29)

One of the above equations represents a constraint on the allowed $d_j$: it is easy to see that Eqs. (29) lead to the explicit expression:

$$v_3^2 - v_4^2 + 4d_3^2 - 8d_2d_3 + \frac{d_3}{(d_1 \wedge d_2)^2} (d_1 \wedge d_2) \times$$

$$\left[ (d_3 \wedge d_1) A_2 - (d_2 \wedge d_3) A_1 + \sigma_0 \hat{d}_3 \sqrt{16v_2^2 (d_1 \wedge d_2)^2 - (d_1A_2 + d_2A_1)^2} \right] = 0,$$

(30)

where we have written $d_i = d_i \hat{d}_i$ ($d_i$, $i = 1, 2, 3$ are unit vectors) and we have defined $A_1 = (v_3^2 - v_4^2 + 4d_3^2)/d_1$, $A_2 = (v_3^2 - v_2^2 - 4d_2^2)/d_2$ and $\sigma_0 = sgn(d_1 \wedge d_2 \cdot v_1)$.

The above constraint, together with the requirement that all the distances among the helices range between $\rho_0$ and $\rho_1$, defines the ground state configuration of our protein.

It is straightforward to see that Eq. (30) has solutions on the plane: for instance upon choosing $d_3 = -d_1$, we see that any configuration satisfying:

$$\rho_0^2 + \frac{1}{4} |v_3^2 - v_4^2| \leq d_1^2 + d_2^2 \leq \rho_1^2 - \frac{1}{4} |v_3^2 - v_2^2|$$

(31)
is a solution, and indeed a bundle-shaped one, because of the geometrical conditions we have imposed. These solutions share the same topology and present no barrier in between, since the corresponding domain of \((d_1, d_2)\) plane is simply connected (Eq. (31)). For these reasons they can be considered as small deformations of the same "native state". Of course solutions exist which are not planar, but they are difficult to single out analytically. Hence, we resorted to numerical calculations to find some of the energy minima, which, due to the oversimplified, square well interaction potential Eq. (22), are highly degenerate. First, we characterized the conformations of the protein by spherical coordinates \(v_i = (v_i, \theta_i, \phi_i)\) calculated with respect to vector \(v_1\). Since the lengths are known, and we can set \(\phi_2 = 0\), we were left with the five angular coordinates \(\theta_2, \theta_3, \phi_3, \phi_4\), to describe any configuration. We also calculated, for each configuration, the “lack of planarity”, as given by the volume we were left with the five angular coordinates \(\nu\). We found that configurations with the highest degree of parallelism among the \(\nu\)’s are also those with the most planar set of vectors \(d_i\). The best and the worst structure are presented in Fig. (4) and Fig. (5), respectively: they correspond to the values \(\beta_{bun} = 0.031, V = 0.072\) and \(\beta_{bun} = 0.32, V = 0.64\) respectively. Notice that, even though our model does not single out a unique native state for the proposed protein, it is nevertheless very encouraging that its results can be easily pruned, according to some simple considerations of excluded volume, leading to essentially correct configurations. This, in spite of the many simplifying assumptions made.

Having investigated the characteristics of the ground state of the simplified model, we now come to the study of its thermodynamic properties, and write, under certain simplifying assumptions, its partition function.

We consider again the hamiltonian:

\[
H = V^0 + V^3 + \sum_{i=1}^{N_h} (H^0_i + H^1_i) ,
\]

and perform some further modeling on the explicit expression of \(H^1_i\), under the assumption that Eq.(20) contains more more details than needed. To this purpose we keep into account only the most relevant maxima in \(P(l_i, w_i)\), whose positions we specify by \((l_{0,\nu}, W_{\nu})\).

The general requirements that \(H^1_i\) has to fulfil are then the following:

- along the w-axis, it must present minima which are symmetrically disposed around \(\pi\), since \(p^2_w\) is invariant under the exchange \(w \rightarrow 2\pi - w\) (corresponding to the inversion of handedness of the helix);
- in the physically interesting domain, \(l_i \in [q_{1,i}, q_{2,i}]\) and \(w_i \in [-\pi/3, 5\pi/3]\) (which is the image of Ramachandran’s plane \((\phi, \psi)\)). \(H^1_i\) must amount to a perturbation of \(H^0_i\), hence it must be bounded within a range small compared to \(\gamma_0 c_{1i}^2\), namely to the difference between the maximum and the two (symmetric) minima of \(H^0_i\);
- the width, shape and depth of the minima of \(H^1_i\) must resemble those of \(P\).

In the following, we shall assume that, near positions \(l_{0,\nu}\) along the sequence, only two minima in \(w\) are present, and they are placed at \(\Omega_{1,\nu} = W_{\nu}\) and \(\Omega_{2,\nu} = 2\pi - W_{\nu}\). We choose for \(H^1_i\) the expression:

\[
H^1_i = -n_i \sum_{\nu=1}^{M} \sum_{a=1}^{2} \gamma_{1i} \delta_{\nu} G_{i,\nu,a} ,
\]

where we have defined:
\[ G_{i,v,a} = \exp\left[ -\frac{(l_i - l_{0,v})^2}{\eta_v^2} + \frac{(w_i - \Omega_{a,v})^2}{\tau_v^2} \right] \]  

(35)

\(H_i^1\) amounts to a collection of 2\(\mathcal{M}\) gaussian wells, whose overlap will be considered negligible for all practical purposes, which have a depth \(\gamma_1\delta_i\) (with \(\gamma_1 \ll \gamma_0 c_1 c_2^2\)), are centered at positions \(l_{0,v}\) along the \(l\)-axis and positions \(\Omega_{a,v}\) along the \(w\)-axis, and have widths \(\eta_v/\sqrt{2}, \tau_v/\sqrt{2}\). We also assume that, at the end points of the chain, all the gaussians Eq. (35) are negligible, which will allow us to extend the integrations to the range \(l_i \in [\pm \infty, \infty]\).

Notice that, while \(\gamma_1\) is a tunable parameter, \(\delta_i\) are given (sequence dependent) positive constants, corresponding to the ratio between the depth of the \(\nu\)-th minimum and that of the deepest one. Without loss of generality we can take \(\delta_{\nu} \leq 1\).

Expression (34), (35) for the hamiltonian relies on the implicit assumption that the height, position and width of the maxima of \(P(l, w)\) are more important than the details of its shape, so that a coarse grained description is sufficient. Of course, the use of gaussians is arbitrary, and is dictated by the fact that they allow us to model accurately the shape of \(P\) around its maxima, and decrease rapidly to zero, preventing or reducing spurious overlaps.

In order to perform the calculations, it is useful to write the constraint \(\mathcal{V}_3\) appearing in Eq. (34) as:

\[ \mathcal{V}^3 = \lambda_3 \sum_{i=1}^{N_t} (l_i - L_i)^2 \quad , \]  

(36)

where we have introduced, remembering the definition of \(l_i\):

\[ L_i = \frac{1}{2} (s_i + s_{i+1} + 1) = \frac{n_i + 1}{2} + \sum_{k=1}^{i-1} n_k \quad . \]  

(37)

Our goal is to evaluate the partition function, so we write

\[ e^{-\beta H} = e^{\beta (\mathcal{V}^0 + \mathcal{V}^3)} \prod_{i=1}^{N_t} e^{-\beta (H_0^0 + H_i^1)} \quad , \]  

(38)

and introduce two approximations, in order to make an analytic integration possible. Namely we replace:

\[ \exp \left\{ -\beta(n_i - 1)\gamma_0 c_1 [(w_i - c_2)^2 - c_3]^2 \right\} \cong \]  

\[ \cong t_i^0 \equiv \exp \left\{ -\beta(n_i - 1) \frac{(w_i - \mu_1)^2}{\sigma^2} \right\} + \exp \left\{ -\beta(n_i - 1) \frac{(w_i - \mu_2)^2}{\sigma^2} \right\} \quad , \]  

(39)

where

\[ \mu_1 = c_2 - \sqrt{c_3} \quad , \quad \mu_2 = c_2 + \sqrt{c_3} \quad , \quad \sigma^2 = \frac{1}{4 \gamma_0 c_1 c_3} \quad . \]  

(40)

We also write:

\[ e^{-\beta H_i^1} \cong f_i^1 \equiv 1 + \sum_{\nu=1}^{M} \sum_{n=1}^{2} \exp\left\{ -\beta e_{\nu}(n_i) \left( \frac{(l_i - l_{0,v})^2}{\eta_v^2} + \frac{(w_i - \Omega_{a,v})^2}{\tau_v^2} \right) \right\} \quad , \]  

(41)

where

\[ e_{\nu}(n_i) = n_i \gamma_1 \delta_{\nu} e^{\beta n_i \gamma_1 \delta_{\nu}} - 1 \quad . \]  

(42)

Notice that in this case both \( e^{-\beta H_i^1} \) and \( f_i^1 \) tend to one as we move far from the maxima: nevertheless, this fact does not bring any further difficulty in the integrations involved in the partition function, since the dependence of \(H\) on \(w_i\) is dominated by \(H_0^0\), and that on \(l_i\) (which anyway ranges between the two finite values \(q_{1,1}\) and \(q_{2,N_h}\), see Eq. (34)) by \(\mathcal{V}^3\). With the above approximations, which are thoroughly discussed in Appendix C, we are finally able to calculate the partition function. First of all we integrate on \(w_i\) and \(z_i\), performing the change of variable \(\zeta_i = z_i - c_6 + c_7 (w_i - c_8)^2\), which does not affect the jacobian:
\[ Z(\{n\}, \{l\}) = e^{-\beta(y^0 + y^2)} \prod_{i=1}^{N_h} \int_{-\infty}^{\infty} dw_i d\zeta_i^f \exp[-\beta g_{05}(n_i - 1)\zeta_i^2] . \]

We insert Eqs. (33, 34) in the above expression and perform the integrations on \( \zeta_i, w_i \) and then the summations on \( l_i \), which we extend to the range \([-\infty, \infty]\). Eventually, after some lengthy calculations, and resorting to the definition of elliptic theta functions \([3]\) we can write:

\[ Z(\{n\}) = e^{-\beta y^0} \prod_{i=1}^{N_h} \left\{ \frac{2\sigma \pi}{\beta(n_i - 1)\sqrt{\gamma_0 c_5}} \theta_3(0, e^{-\frac{1}{2} \beta \lambda_3}) + \sum_{\nu=1}^{M} \sum_{a,b=1}^{2} \left[ d_{\nu}(n_i) e^{(A_{a,b,\nu}(n_i) - C_{\nu}(\{n_k\}, \lambda_3))} \right] \right\} \times \frac{\sqrt{\pi}}{x_{\nu}(n_i, \lambda_3)} \theta_3 \left( \frac{y_{\nu}(\{n_k\}, \lambda_3)}{x_{\nu}(n_i, \lambda_3)}, e^{-\pi^2 \zeta_i^2(n_i, \lambda_3)} \right) , \]

where \( \theta_3(z, q) \) is the elliptic \( \theta_3 \) function of argument \( z \) and nome \( q \), and we have defined:

\[
\begin{align*}
    d_{\nu}(n_i) &= \frac{\pi}{\beta} \left( e^{\beta n_i \gamma_1 \delta_\nu} - 1 \right) \left[ (n_i - 1)\gamma_0 c_5 \left( \frac{n_i - 1}{\sigma^2} + \frac{e_{\nu}(n_i)}{\tau_{\nu}^2} \right) \right]^{-\frac{1}{2}}, \\
    A_{a,b,\nu}(n_i) &= \frac{\beta}{\sigma^2 \tau_{\nu}^2} \left\{ \left[ (n_i - 1)\mu_a \tau_{\nu}^2 + \frac{e_{\nu}(n_i)\Omega_{b,\nu}\sigma^2}{\tau_{\nu}^2} \right]^2 - \left[ (n_i - 1)\frac{\mu_a^2 \sigma^2}{\tau_{\nu}^2} + \frac{e_{\nu}(n_i)\Omega_{b,\nu}}{\tau_{\nu}^2} \right] \right\} \\
    x_{\nu}(n_i, \lambda_3) &= \frac{1}{2\eta_{\nu}} \sqrt{\beta(\epsilon_{\nu}(n_i) + \lambda_3 \eta_{b,\nu}^2)} , \\
    y_{\nu}(\{n_k\}, \lambda_3) &= \sqrt{\frac{\beta \eta_{b,\nu}^2}{\epsilon_{\nu}(n_i) + \lambda_3 \eta_{b,\nu}^2}} (0, \nu \frac{e_{\nu}(n_i)}{\eta_{b,\nu}^2} + \lambda_3 L_i(\{n_k\})) , \\
    C_{\nu}(\{n_k\}, \lambda_3) &= \beta \frac{e_{\nu}(n_i)\lambda_3}{e_{\nu}(n_i) + \lambda_3 \eta_{b,\nu}^2} (0, \nu - L_i(\{n_k\})) ,
\end{align*}
\]

At this point we should perform the sums over \( n_i \). They are clearly unfeasible in analytical way, yet, they are very simple to perform numerically. To this end it may be useful to implement directly the constraint \( S_0 \) by setting

\[ n_{N_h} = N - \sum_{k=1}^{N_h-1} n_k \ , \]

and paying the necessary attention to discard the collection of \( n_k \)'s leading to negative values for \( n_{N_h} \).

In this way, for each given sequence one can obtain an expression for the partition function which depends only on \( \beta, \lambda_3 \), and the ratio \( \gamma_1 / \gamma_0 \). The Lagrange multiplier must be evaluated by minimizing the free energy. This involves the condition

\[ \frac{\partial Z}{\partial \lambda_3} = 0 \ , \]

that should be solved to give \( \lambda_3 = \lambda_3(\beta, \gamma_1 / \gamma_0) \).

We shall then study the thermodynamic behaviour of the system at different temperatures and values of \( \gamma_1 / \gamma_0 \). We leave this detailed analysis to future studies, since our goal here was only two show that, despite the complexity of the model, interesting calculations, providing new insight in the folding process, can be performed without resorting to heavy numerical work.

**IV. COMMENTS AND CONCLUSIONS**

This paper is mainly devoted to the presentation and analysis of a new model hamiltonian to be used in thermodynamical and dynamical studies of the folding process. The various contributions to the hamiltonian (23), and their relation to phenomenology, have been thoroughly discussed, together with the approximations introduced.

Then we concentrated on the local part of the hamiltonian, and applied it to the study of a small (73-residues long) synthetic protein [24], designed to produce a four-helix bundle.
Even if this part of the model does not contain information on the spatial structure, it takes into account the sequence, so we expect that it can provide relevant information on the secondary structure composition of the native state. To prove that this is indeed the case, we studied the local Hamiltonian minima for the protein considered, and then switched on the non-local interactions as a small perturbation, thus superimposing the tertiary structure to the existing secondary one.

We found that the four-helices ground-state is correctly made up of α-helices, placed in the same positions along the chain as they are found in the experimental native state. Encouraged by this result, we studied the best spatial configuration that the four helices we obtained would assume, due to the non-local mutual interactions. Again we obtained a positive result: bundle-like configurations are indeed the ground-state of the model, even if the latter configuration that the four helices we obtained would assume, due to the non-local mutual interactions. Again we leave to future efforts the refinements regarding, for instance, the explicit expressions for the charges \( q_i \); in this paper they have been fixed in an arbitrary, though reasonable, way just to perform some tests on the model. The same holds true for assumption (34), whose validity could depend on the sequence considered and requires further analysis.

Coming to the partition function for the complete model, a reasonable goal is to perform exact, analytic integration on some of the variables (for instance, \( w_i, z_i, \lambda_i \)), thus providing an effective interaction potential among the others; then, one could resort to numerical simulations. The latter could be approached, for instance, putting the lattice: in this way one could have a true mapping of real proteins on lattice models, and the approximations induced by the lattice could be better controlled in their relationship to protein geometry.

The fact that the known native state of a real protein can be mapped onto a model configuration entails also that the study of the inverse folding problem can greatly benefit from this new approach.

Finally, it is easy to provide a coarse-grained dynamics for the protein through the variables used in the model, and our future efforts will be dedicated also to this line of research.

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APPENDIX A:

Curvature and torsion of the curve \( r(s) \) are defined:

\[
\kappa = \frac{\vec{r} \wedge \vec{r}'}{|\vec{r}|^3}, \quad \tau = \frac{\vec{r} \wedge \vec{r}'}{|\vec{r} \wedge \vec{r}'|^2}.
\]

Performing the calculations for the curve in Eq. (B), and keeping only the leading terms in the limit \( \lambda \to 0 \), we find:

\[
|\vec{r}'| \sim 0 \quad \sum_{i=1}^{N_h} \left[ (b_i \hat{\mathbf{h}}_i + b_{i+1} \hat{\mathbf{h}}_{i+1})^2 - b_{i+1}^2 \hat{\mathbf{h}}_{i+1}^2 \right]^{\frac{1}{2}},
\]

\[
|\vec{r} \wedge \vec{r}'| \sim 0 \quad \sum_{i=1}^{N_h} \left[ \left( \hat{g}_i (b_i + b_{i+1})(\hat{\mathbf{h}}_i \wedge \hat{\mathbf{h}}_{i+1}) + (b_i \hat{\mathbf{h}}_i + b_{i+1} \hat{\mathbf{h}}_{i+1}) \wedge (b_i \hat{\mathbf{h}}_i + b_{i+1} \hat{\mathbf{h}}_{i+1}) \right)^2 - b_{i+1}^2 (\hat{\mathbf{h}}_{i+1} \wedge \hat{\mathbf{h}}_{i+1})^2 \right]^{\frac{1}{2}},
\]

\[
\vec{r} \wedge \vec{r} \cdot \vec{r}' \sim 0 \quad \sum_{i=1}^{N_h} \left[ b_i^2 \hat{\mathbf{h}}_i \cdot \hat{\mathbf{h}}_i + (b_i - b_{i+1}) \left[ 6g_i^2 (\hat{\mathbf{h}}_i \wedge \hat{\mathbf{h}}_{i+1}) (-\hat{\mathbf{h}}_i + \hat{\mathbf{h}}_{i+1}) + \cdots \right] \right].
\]
2\ddot{g}_i(b_i\ddot{h}_i + b_{i+1}\ddot{h}_{i+1}) \wedge \dot{h}_i \cdot \dot{h}_{i+1} + 3\dddot{g}_i(b_i\dddot{h}_i + b_{i+1}\dddot{h}_{i+1}) \wedge \dot{h}_i \cdot \dddot{h}_{i+1} + \frac{1}{2} \sum_{\mu, \nu, \rho = 1}^3 \varepsilon_{\mu \nu \rho} b_i b_{i+1} (b_i \dot{h}_i^{(\mu)} + b_{i+1} \dot{h}_{i+1}^{(\mu)} \wedge \dddot{h}_i^{(\nu)} \cdot \dddot{h}_{i+1}^{(\rho)})

In the above equations, dots as well as greek apices indicate derivative with respect to \(s\), and we have introduced \(\mathbf{h}_0 = \mathbf{h}_{N_{h}+1} = \mathbf{0}\).

Now we observe that the above quantities, in the limit considered, are made up by terms proportional to powers of \(b_i\), that are different from zero in the region \(s_{i-1} < s < s_i\), and terms that live in the interfaces \(s = s_i\) between helices, namely those containing the product \(b_i b_{i+1}\), or the delta function \(\dot{g}_i\). These terms are mutually “orthogonal”, in the sense their product is zero. This fact allows strong simplifications, because one can perform algebraic manipulations at fixed \(s\) (this is allowed, since we are not differentiating), and consider only those terms which are different from zero for that particular value of \(s\). For instance, the curvature becomes:

\[
\kappa = \sum_{i=1}^{N_h} \left( \kappa_i \vartheta(s - s_{i-1})\vartheta(s_i - s) + 2\dot{g}_i(s) \frac{4\delta(s - s_i) (\dot{h}_i \wedge \ddot{h}_{i+1}) + (\dddot{h}_i + \dddot{h}_{i+1}) \wedge (\dot{h}_i + \dddot{h}_{i+1})}{|\dot{h}_i + \dddot{h}_{i+1}|^3} \right)
\]

where \(\vartheta\) is Heaviside’s function, \(\kappa_i\) is the curvature of the \(i\)-th helix:

\[
\kappa_i = \frac{|\dot{h}_i \wedge \ddot{h}_i|}{|\dot{h}_i|} ,
\]

and the delta-like functions

\[
q_i(s) = \begin{cases} 1, & \text{if } s = s_i \\ 0, & \text{otherwise} \end{cases}
\]

have been introduced to single out those terms that live at the interfaces \(s_i\). Obviously, the term containing \(\delta(s - s_i)\) is the only one to take into account, which leads to the result in Eq.(B).

**APPENDIX B:**

In order to study the relationship between geometrical quantities and \((\phi, \psi)\) angles, we observe that a protein may be built up by performing a sequence of rotations and translations of the peptide plane \(C_i^0 C_i^0 NC_i^{\alpha}C_{i+1}^{\alpha}\).

If \(\mathbf{L}\) is the segment joining to successive \(C_i^0\), and if we label \(j\) the peptide plane between \(C_j^0\) and \(C_{j+1}^0\), we have that the position of \(C_N^0\) is given, in the reference frame of peptide plane number 0, by:

\[
\mathbf{r}_0(C_N^0) = \left[ \prod_{i=1}^{N-1} (\mathbf{L} + R(\phi_i, \psi_i)) \right] \mathbf{L}
\]

\[
= \mathbf{L} + R(\phi_1, \psi_1) \mathbf{L} + \cdots + R(\phi_{N-1}, \psi_{N-1}) \mathbf{L} . \tag{B1}
\]

Following Ramachandran\[2\], we associate to each peptide plane \(j\) a reference frame \((\mathbf{x}_j, \mathbf{y}_j, \mathbf{z}_j)\), such that the origin sits on \(C_j^0\), \(\mathbf{y}_j = \mathbf{L}_j\) and \(\mathbf{x}_j\) lies on the plane, with \(\mathbf{x}_j \cdot C^0 \mathbf{O} > 0\).

Now we consider two successive planes, labelled 0 and 1, and take \(\phi, \psi\) to be the unit vectors, expressed in reference frame 0, of the rotation axes \(NC_1^0\) and \(C_1^0 C_2^0\), when \(\phi = \psi = 0\) (according to the standard conventions, this corresponds to having \(C^0 N\), \(NC_1^0\), \(C_1^0 C_2^0\) in “cis” configuration, lying on the same plane with \(C^0 \mathbf{N} \cdot C_1^0 C_2^0 < 0\)).

Given frame 0, the sequence of rotations to perform in order to obtain frame 1 is the following: first one rotates by an angle \(\pi\) about the \(\mathbf{y}\) axis, then of an angle \(\theta = -\pi + C_0^0 C_1^0 C_2^0 = -\pi - |\alpha| + |\zeta| + |\beta|\) about the \(\mathbf{z}\) axis, to recover the standard \(\phi = \psi = 0\) configuration. Here \(\alpha = NC^0 N\), \(\beta = C^0 C_1^0 C_2^0\), and \(\zeta = C_0^0 C_1^0 N\). Then we can perform the \(\psi\)-rotation, and successively the \(\phi\)-one, obtaining:

\[
\mathbf{X}_0^1 = \mathbf{X}_0^0 [R(\phi)R(\psi)R(\theta \mathbf{z})]_{ba} \tag{B2}
\]

where all the rotation axes are expressed in reference frame 0, \((\mathbf{X}_0^1, \mathbf{X}_1^0, \mathbf{X}_0^0) = (\mathbf{x}_j, \mathbf{y}_j, \mathbf{z}_j)\), and

\[
[R(\eta)]_{pq} = \cos(\eta)\delta_{pq} + (1 - \cos(\eta))\eta^p \eta^q - \sin(\eta)\eta^r \varepsilon_{rps} \tag{B3}
\]
Let us take three successive \( \sigma \) and, to distinguish between left- and right-handed helices, we assume that:

\[
\begin{align*}
\hat{c} & \equiv c(\omega) = \hat{\kappa} (s(\psi)c(\phi) + s(\phi)c(\psi)) , \\
\hat{s} & \equiv s(\omega) = \hat{\kappa} [s(\psi)s(\phi) - c(\phi)c(\psi)] - \hat{\kappa} \wedge (s(\psi) \wedge s(\phi)) + \hat{\kappa} \wedge (s(\psi)c(\phi) + s(\phi)c(\psi)) ,
\end{align*}
\]

where, for any argument \( \eta \), \( c(\eta) = \cos(\eta/2) \), \( s(\eta) = \hat{\eta}\sin(\eta/2) \); while

\[
\begin{align*}
\hat{\kappa} &= (-\sin(\frac{\theta}{2}), \cos(\frac{\theta}{2}), 0) , \\
\hat{\phi} &= (\sin(|\zeta|), \cos(|\zeta|), 0) , \\
\hat{\psi} &= (\sin(|\alpha| - |\zeta|), -\cos(|\alpha| - |\zeta|), 0) ,
\end{align*}
\]

The above equations specify completely the rotation matrices appearing in Eq. (B2).

Now we have to relate the parameters identifying a helix, to the repeating value of the angle of rotation \( \omega(\phi, \psi) \).

Let us take three successive \( C^\alpha - C^\alpha \) segments, namely \( L, L', L'' \), and put ourselves in reference frame of segment \( L' \). We have \( L = R^{-1}(\phi, \psi)L' \) and \( L'' = R(\phi, \psi)L' \), whence

\[
\begin{align*}
L &= L\{2(s_1s_2 + c_3s_3), c^2 - s^2 + 2s_2^2, 2(s_2s_3 - c_3)\} , \\
L' &= L\{0, 1, 0\} , \\
L'' &= L\{2(s_1s_2 - c_3s_3), c^2 - s^2 + 2s_2^2, 2(s_2s_3 + c_3)\} ,
\end{align*}
\]

where \( c = c(\omega), s = \sin(\omega/2), s_i = \omega_i \sin(\omega/2) \) (\( s_i \) are the components of the vector \( s(\omega) \)).

Now we ask that the helix axis \( e_3 \) satisfies the following requirements:

\[
L \cdot e_3 = L' \cdot e_3 , \quad L'' \cdot e_3 = L' \cdot e_3 , \tag{B6}
\]

The axis orientation is fixed by:

\[
L \cdot e_3 > 0 , \tag{B7}
\]

and, to distinguish between left- and right-handed helices, we assume that:

\[
\text{sgn}(L \wedge L' \cdot e_3) = \begin{cases} +1, & \text{if right-handed} \\ -1, & \text{if left-handed.} \end{cases} \tag{B8}
\]

We need also to relate the length of the helix arc, corresponding to one peptide segment \( L \) of the chain, to the angle of rotation at each site. From the definition of \( u \) (Eq. (3)), we have that \( \Delta s = 1 \) corresponds to an arc of length \( L \); hence \( u \) is the rotation about the helix axis corresponding to a displacement of one peptide unit along the chain. This angle must be equal to the projection of \( \omega \) on the plane perpendicular to \( e_3 \), namely

\[
\cos(u) = \frac{e_3 \wedge (L \wedge e_3) \cdot e_3 \wedge (L' \wedge e_3)}{|L \wedge e_3| \cdot |L' \wedge e_3|} . \tag{B9}
\]

Equations (B6), (B8) yield:

\[
e_3 = \frac{\sigma s}{|s|} , \tag{B10}
\]

where \( \sigma = \pm 1 \). Observing that \( \omega \in [0, 2\pi] \) \( (\omega \in [-\pi, \pi] \) would not be correct, as \( c(\omega) \) in Eq. (B4) can also be negative), we always have \( s > 0 \), hence

\[
e_3 = \sigma \hat{\omega} . \tag{B11}
\]

From Eq. (B9) we finally get:

\[
\cos(u) = \cos(\omega) .
\]

To establish the sign \( \sigma \) and the explicit expression of \( u \), we use Eqs. (B7), (B8), and find that the product \( s_2c \) determines the handedness of the helix (right-handed if positive), while \( \sigma \) coincides with \( \text{sgn}(s_2) \) whenever \( s_2 \) is different from zero. The analysis of the case \( s_2 = 0 \) lead us to the following general definition:
\[ u = \sigma(s_2) \left( \omega - 2\pi \left\lceil \frac{\omega}{\pi} \right\rceil \right), \quad \text{(B12)} \]

where \( \left\lceil x \right\rceil \) denotes the maximum integer \( \leq x \), and \( \sigma(x) = 1 \) if \( x > 0 \), \( \sigma(x) = -1 \) otherwise. With the above position we have \( u \in [-\pi, \pi] \), with positive values corresponding to right-handedness. Recalling definition (8) we can also write:

\[ w = \pi + \sigma(s_2) (\omega - \pi). \quad \text{(B13)} \]

Now we can derive \( a \) and \( h \), from Eq.(4) and the condition

\[ ahu = L \cdot e_3 = L \frac{s_2}{s}, \quad \text{(B14)} \]

and hence, using once more Eq.(4), curvature and torsion. The former can be expressed as

\[ \kappa = \frac{|u|}{L} \sqrt{1 - \frac{\tau^2 L^2}{u^2}}, \]

while torsion is given by

\[ \tau = \frac{|s_2|}{L} \frac{u}{L}. \quad \text{(B15)} \]

Now we recall that \( \omega \) depends on \((\phi, \psi)\) through Eqs.(B4) and (B5), where \( \cos(\frac{\omega}{2}) \) and \( \hat{\omega} \sin(\frac{\omega}{2}) \) appear. From Eq.(B15) and the relation \( \cos \frac{\omega}{2} = |\cos \frac{\omega}{2}| \), using the explicit expressions of the vectors appearing in Eq.(B5), we find two formulas relating \( u \) and \( \tau \) to the dihedral angles:

\[ \cos \frac{u}{2} = |c(\omega)|, \quad z = \frac{|s_2|}{\sqrt{1 - c^2(\omega)}}, \]

where the quantities at the right hand side of the above equations can be explicitly written as

\[ c(\omega) = \sin \frac{\alpha}{2} \cos(\frac{\zeta}{2} - |\beta|) \sin(\frac{\phi + \psi}{2}) + \cos \frac{\alpha}{2} \sin(\frac{\zeta}{2} + |\beta|) \sin(\frac{\psi - \phi}{2}), \]
\[ s_2 = \sin \frac{\alpha}{2} \cos(\frac{\zeta}{2} + |\beta|) \cos(\frac{\phi + \psi}{2}) + \cos \frac{\alpha}{2} \sin(\frac{\zeta}{2} + |\beta|) \cos(\frac{\psi - \phi}{2}). \]

In analogous way we find for \( w, z \):

\[ \cos \frac{w}{2} = \sigma(s_2) c(\omega), \]
\[ z = \frac{|s_2|}{\sqrt{1 - c^2(\omega)}}, \]

which is identical to Eq.(18), where an obvious definitions of coefficients \( a, b, c, d \) has been performed.

**APPENDIX C:**

In this appendix we discuss the validity of the two approximations Eqs.(39,41) we introduced in section III. The first approximation is the most relevant one, since it involves the leading hamiltonian \( H_0^0 \). The parameters \( \mu_1, \mu_2, \sigma \) have been chosen so that the exact and approximate functions have maxima at the same positions, and the leading order in the series expansion at those points are the same, provided that the overlap between the two gaussians is vanishing:

\[ \exp[-\beta(n_i - 1)\frac{(\mu_2 - \mu_1)^2}{\sigma^2}] \approx 0. \]

We check that the approximation is globally good by a comparison of the two integrals:
\[ I_1 = \int_{-\infty}^{\infty} dw_i e^{-\beta(n_i - 1)\gamma_0 c_1[(w_i - c_2)^2 - c_3]^2} , \]  
\[ I_2 = \int_{-\infty}^{\infty} dw_i \left\{ e^{-\beta(n_i - 1)\frac{(w_i - \mu_1)^2}{\sigma_1^2}} + e^{-\beta(n_i - 1)\frac{(w_i - \mu_2)^2}{\sigma_2^2}} \right\} \]  
\[ = \sqrt{\frac{\pi c_3}{2}} \frac{1}{\sqrt{y}} , \]  
where \( y = \beta(n_i - 1)\gamma_0 c_1 c_2^2/2. \)

The first one can be evaluated explicitly:
\[ I_1 = \frac{\pi}{2} \sqrt{c_3} e^{-y} \left[ I_{-\frac{1}{2}}(y) + I_{\frac{1}{2}}(y) \right] , \]
where \( I_{\pm\frac{1}{2}}(y) \) are modified Bessel function of the first kind.

A numerical evaluation of \( \epsilon = \left| 1 - \frac{I_2}{I_1} \right| \) reveals that, for \( y \gtrsim 0.24 \) it always holds \( \epsilon \lesssim 0.12 \), with \( \epsilon \) decreasing to zero as \( y \) increases.

For the above reasons we consider expression Eq. (3.38) as a good approximation, with a word of caution: the value of its right and left member differ significantly at \( w_i = c_2 = (\mu_1 + \mu_2)/2 \), the maximum of the quartic potential appearing in \( H_i^0 \). We have in fact that the former goes as \( \exp[-2y] \), while the latter gives \( 2\exp[-8y] \). This difference may however be considered negligible when both of the above quantities are very small, namely, for long enough helices and/or low enough temperature \( 1/\beta \).

Coming to the second approximation, we see that also in this case Taylor expansions near the maxima \( (l_{0,\nu},\Omega_{a,\nu}) \) coincide. To check the global behaviour, we proceed as before, evaluating:
\[ I_3 = \int_{-\infty}^{\infty} dw_i dl_i (e^{-\beta H_i^1} - 1) , \]
moving first to polar coordinates:
\[ \frac{l_i - l_{0,\nu}}{\eta_\nu} = r \cos(\theta) , \quad \frac{w_i - \Omega_{a,\nu}}{\tau_\nu} = r \sin(\theta) , \]
then introducing \( z = \exp(-r^2) \), and finally resorting to formula Eq. 5.1.40 in the Abramowitz-Stegun handbook, whereby:
\[ I_3 = \eta_\nu \tau_\nu \pi [Ei(b) - \ln(b) - \gamma] , \]
where \( \gamma \) is the Euler constant, \( Ei \) indicates the exponential integral function, and \( b = \beta n_i \gamma_1 \delta_\nu \).

This is to be compared to:
\[ I_4 = \int_{-\infty}^{\infty} dw_i dl_i (f_1^i - 1) = 4 \frac{\eta_\nu \tau_\nu \pi}{b} \sinh^2\left(\frac{b}{2}\right) , \]
Again, an estimate of \( \epsilon' = |1 - I_4/I_3| \) reveals that \( \epsilon' \to 0 \) both for \( b \to 0 \) and for \( b \to \infty \), with a maximum of \( \epsilon' = 0.27 \) at \( b = 2.97 \).

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FIG. 1. Contour lines of $H_i^0 (n_i - 1)\gamma_0$, showing the two minima in $(w, z)$ plane; the variable $w$, on the horizontal axis ranges from $\pi/3$ to $5\pi/3$, while $z$, on the vertical axis, goes from 0 to 1. Lines are drawn at intervals of 1, in arbitrary units, in the range $[0,5]$ (deeper regions are darker). The saddle point corresponds to an height of 2.7. The figure is obtained with the values $c_1 = 2.73$, $c_2 = 2.77$, $c_3 = 0.99$, $c_4 = 0$, $c_5 = 150$, $c_6 = 0.86$, $c_7 = 0.16$, $c_8 = 3.5$.

FIG. 2. The same as in Fig.(1), but with $w$, $z$ expressed as functions of $\phi$ (horizontal axis) and $\psi$ (vertical axis) through Eq.(14). Both $\phi$ and $\psi$ range from $-\pi$ to $\pi$. The values of the parameters are the same as in Fig.(1). Notice the essentially correct position and shape of the $\alpha$ and $\beta$ minima; the unphysical third minimum is an effect of the approximate symmetry of Eq.(14) under the transformation $\phi \leftrightarrow \psi$.

FIG. 3. Plot of $P(l, w_{\alpha})$ (continuous line), $P(l, w_{\beta})$ (dotted line) and $0.17l$ (dashed line, below) for the considered protein, calculate for a 7-residues-long helix centered at position l (on the horizontal axis); $\bar{q}$ and $P$ have been defined in Eqs. (12, 26). Notice the big maxima of the dipole moment characterising the $\alpha$ helices.

FIG. 4. Ground-state configuration most similar to a bundle, among those obtained by numerical calculations: $\beta_{\text{bun}} = 0.031$, $V = 0.072$ (see text).

FIG. 5. Ground-state configuration with the worst value of $\beta_{\text{bun}}$ and $V$, among those obtained by numerical calculations: $\beta_{\text{bun}} = 0.32$, $V = 0.64$. 
