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# Protein folding and wring resonances

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**Abstract**

The polypeptide chain of a protein is shown to obey topological constraints which enable long range excitations in the form of wring modes of the protein backbone. Wring modes of proteins of specific lengths can therefore resonate with molecular modes present in the cell. It is suggested that protein folding takes place when the amplitude of a wring excitation becomes so large that it is energetically favorable to bend the protein backbone. The condition under which such structural transformations can occur is found, and it is shown that both cold and hot denaturation (the unfolding of proteins) are natural consequences of the suggested wring mode model. Native (folded) proteins are found to possess an intrinsic standing wring mode. © 1997 Elsevier Science B.V.

*Keywords:* Protein folding; Linking; Excitations; Resonator; Cold and hot denaturation

## 1. Introduction

It is well known that interaction between residues situated far from each other in the linear sequence of amino acids of a protein, plays a crucial role in the folding of the protein. These long range effects also represent some of the obstacles in current algorithmic approaches to protein folding. Several types of long range regularities in proteins have been investigated, for example, the preference for identical or similar residue partners in beta-sheets [1–3], in close contact pairs [4], and the long and short distance periodicity in packing density [5]. Mutations in the amino acid sequence are significantly correlated over long distances [6–8]. The relation between the amino acid sequence and its three dimensional structure, is a mapping where many sequences assume the same fold [9–12]. Interestingly, order exists at a level more basic than that of the compositional sequence information. The typical length of a domain in a prokaryotic protein is about 150 amino acids, and in eukaryotes about 125 amino acids [13–15]. In some cases the initiation of protein folding has been shown to be a fast seeding process that precedes slow and rate-determining steps [16–18]. In other cases the entire process is intrinsically fast [19]. This rapidness indicates that the initiation of protein folding is not necessarily best understood on the basis of an entropy–energy balance [20–23]. It has been suggested that protein folding is governed by a fast hydrophobic collapse followed by a slower annealing [24]. It has also been suggested that the essential features of protein folding can be derived from spin glass and lattice gas models [25–28]. Such descriptions have

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been very useful in forming an improved understanding of protein folding. By means of statistical physics, the models are able to account for denaturation at high temperature. In order to understand the denaturing at low temperature, cold denaturation, it is necessary to assume that the intermolecular potentials depend on temperature.

The length of the polypeptide chain of globular proteins is much longer than typical diameters of the chain. It is therefore reasonable to speak about a chain and a knot topology. In fact, the chain is never knotted, except for the possible exception where the knot topology is ill-defined [29,30]. Thus, the number of possible different protein structures seems to be limited by a "knot preventing mechanism" that nevertheless can lead to very different protein structures [31–34]. In this paper we suggest that the initiation of protein folding is a resonance phenomenon, and that protein folding takes place when the amplitude of the resonance mode exceeds a certain threshold. This mechanism for initiation of folding of polypeptide chains is justified for a general chain. Folded structures become stabilized by van der Waals forces, hydrogen bonds, disulphide bridges etc., and as we shall see in some cases even by dynamic forces.

## 2. Protein topology

The application of topological arguments in molecular biology was introduced in investigations of supercoiled DNA [35–37], and in investigations of differential geometrical aspects of biological membranes [38–40]. For circular DNA it was possible to utilize the concepts of twist, writhing and linking to establish a conservation law [41], and for membranes [38,42] to obtain a comprehensive differential geometrical analysis of phase separation, vesicle formation, and the associated critical exponents. Topological methods [43,44] have been applied to protein structures for the purpose of energy minimization [45], and it was believed to lead to folding pathways that would minimize the sum of the self-interactions [46,47]. In this paper, we argue that topological constraints lead to a deterministic model of folding, and that it is not primarily the self-interactions in a protein that topologically constrain protein folding. Rather, the topological constraints are enforced by the interaction of the protein with its environment. Such topological constraints of the protein backbone lead to torsional modes, which we will call *wringons*. It is discussed how resonance phenomena may involve rotational excitations of sidechains and/or short peptide strings, and how a coherent resonating state can appear.

The winding of a tube will be defined as $2\pi$ times the number of rotations one end of the tube has made relative to the other end: A straight tube has zero winding. However, in general the winding of a tube cannot be calculated as a continuous measure depending uniquely on the local geometrical progression. This is illustrated in Fig. 1, where nearly identical and approximately flat structures are depicted. While one is not winded, Fig. 1a, another is winded by two turns ($4\pi$), Fig. 1b. The shortened path, Fig. 1c, is a part of the motif of Fig. 1a as well as of Fig. 1b and it is consequently not possible to assign an unambiguous winding to Fig. 1c. In order to assign winding to a polypeptide backbone, it is therefore necessary to know what path the backbone has taken during folding. However, most often the folding pathway is unknown. In the case of closed curves the winding of the backbone can be found as the *linking* of the curve; the linking is topologically conserved [48].

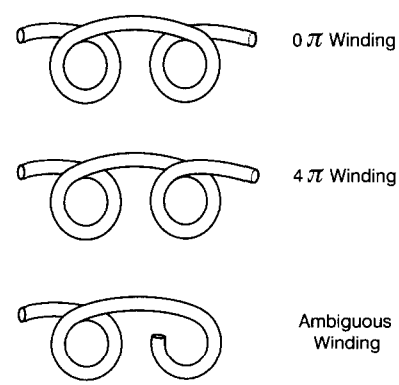


Fig. 1. Examples of winding conformations: (a) an almost flat double loop structure with zero winding; the two loops have opposite chirality, (b) an almost flat double loop structure with $4\pi$ winding; the two loops have identical chirality. For a shortened structure such as the one depicted in (c), it is not possible to say whether it is a part of (a) or (b), and hence it is not possible to assign to it an unambiguous winding.

## 3. Linking, twisting, and writhing

The interactions of proteins with their environment are important for the progression of protein folding. As proteins fold in contact with their environment, large motions of the protein backbone are unlikely on short timescales. At any moment in time changes in torsion that are related to the twist of the backbone must be defined relative to the geometrical path describing the protein backbone. One can define a ribbon by the frame in which the twist is equal to the geometrical torsion, $\tau$, of the curve:

$$\tau = \frac{\vec{r}' \times \vec{r}'' \cdot \vec{r}'''}{|\vec{r}' \times \vec{r}''|^2} \tag{1}$$

where $\vec{r}$ is a vector representation of the curve and the primes denote derivatives.

A linking number of zero for a closed curve means that if one cuts the corresponding ribbon (with scissors) along the central line, one will end up with two non-interwoven ribbons. If the linking number is $\pm 1$ the result will be two ribbons that are linked as links in a chain [49]. The linking number, $L$, is related to the total twist, $T$, and the writhing number, $W$, through the White theorem:

$$L = W + T \tag{2}$$

The total twist can be calculated as

$$T = \frac{1}{2\pi} \int_0^l \vec{v}^{\perp} \cdot \mathrm{d}\vec{v} \tag{3}$$

and the writhing as

$$W = \frac{1}{4\pi} \iint_{c \times c} \frac{\partial \vec{e}}{\partial s_1} \times \frac{\partial \vec{e}}{\partial s_2} \cdot \vec{e}\, \mathrm{d}s_1\, \mathrm{d}s_2 \tag{4}$$

where

$$\vec{e} = \frac{\vec{r}_2 - \vec{r}_1}{|\vec{r}_2 - \vec{r}_1|} \tag{5}$$

and the vectors $\vec{t}, \vec{v}$, and $\vec{v}^{\perp}$ describe a geometrical right-handed frame, $\vec{t}$ being a unit vector parallel to the velocity $\vec{r}'$. The vectors $\vec{v}$, and $\vec{v}^{\perp}$ are the normal and binormal vector, respectively. Writhing can be formed at the expense of twist as has been discussed for the case of circular supercoiled DNA [35–37,48].

## 4. Wringons

Closed chain molecules such as circular DNA must obey the White theorem, and so must collective modes of the DNA molecule. Proteins are not circular but linear. Nevertheless, on a sufficiently short timescale they are also topologically constrained. The reason for this is that the chain molecules will interact with their environment in a viscous manner and may cause dispersion as well. The time it takes for one part of a polypeptide chain to move through distances that are comparable to its separation from other parts of the chain has been measured to be about $10^{-5}$–$10^{-6}$ s using the efficiency of fluorescence energy transfer [29,50]. Below we will show that the typical period of a wringon is a factor of about 1000 shorter as illustrated in Fig. 2. In this case, the wringons are so fast that the path of the protein backbone can be considered to be effectively maintained during a cycle. This is the reason that such torsional modes can be considered separately.

Proteins are single and not double stranded as is typical for DNA. The protein backbone traces a curve in space and consequently has an orientation as any twice differentiable three dimensional curve, where the main normal, $\vec{v}$, and the binormal vector, $\vec{v}^{\perp}$, can be calculated. Notice that this geometrical frame may be different from the actual physical frame imposed by the molecular structure due to the existence of additional twist of the physical backbone. The reason that long range correlations exist is

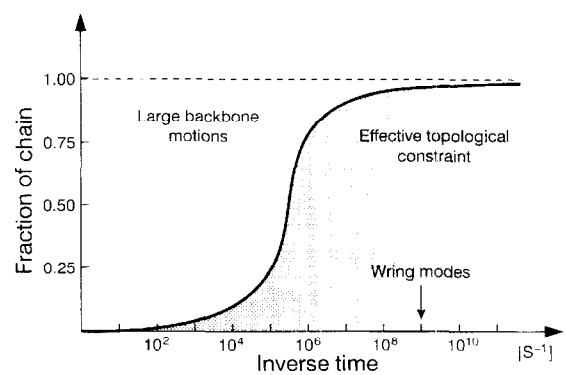


Fig. 2. An illustration (schematic) of the region in inverse time where chain molecules are effectively topologically constrained. Also shown is the typical period of a wring mode which appears within the area of topological constraints.

that the chemical bonds which define the dihedral angles are not coaxial. Pairs of solitons (localized incremental/decremental twist) do not destroy the long range order because they consist of an equal amount of clockwise and counterclockwise twist, see Fig. 3a. The actual wring modes, or wringons, that can be excited depend on the conditions at the two ends of the polypeptide chain. At non-fixated ends, the torsion must be zero while the amplitude can vary, see Fig. 3b. At fixated ends the amplitude must be zero. Here we will separate the modes and consider the ones with no net rotation of the molecule. In some cases, higher order modes as the one shown in Fig. 3c may be less damped because a smaller fraction of the molecule acts as effective ends.

Wringons are long range collective excitations over an entire protein folding domain. In general, they will involve non-zero values of all the different types of dihedral angles, and the backbone will therefore appear to be fairly stiff. The characteristic time involved can consequently be much shorter than the characteristic time associated with an apparent random motion of the unfolded backbone, which mainly involves the dihedral angles $\phi$ and $\psi$. An isolated change in one of the dihedral angles, $\phi$ and $\psi$, will lead to a change in the path of the backbone.

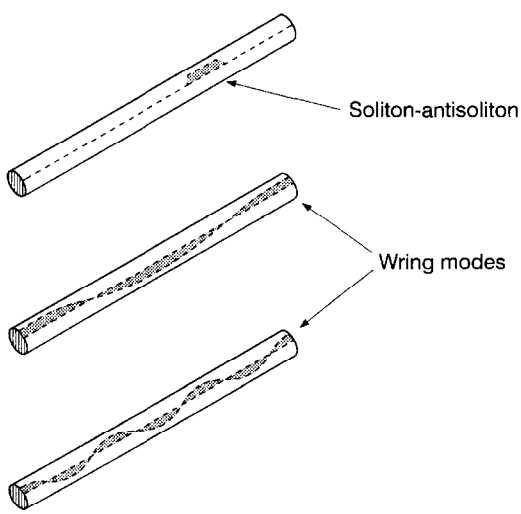


Fig. 3. Three examples of wring modes of a tube. (a) Solitons, local increment (or decrement) in the twist amplitude. Two solitons of opposite twist have no long range implication for the twist amplitude. (b) and (c) show collective twist excitations over the entire tube. We call such excitations wring modes.

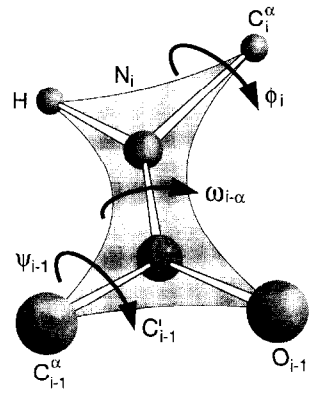


Fig. 4. Part of the polypeptide backbone depicting the dihedral angles $\psi$, $\phi$, and $\omega$. The shaded area is approximately planar.

In contrast, a change in the twist of the backbone maintains its path. A wring mode therefore involves strained chemical bonds. If the dihedral rotations $\psi_{i-1}$ and $\phi_i$ are almost coaxial, see Fig. 4, such a rotation, and counter rotation, does not have any long range implication for the twist of the backbone (see discussion of solitons above in this section), and will therefore not interfere with long wavelength twist modes. The persistence length of proteins as defined for a simple random polymer is short, maybe even only a few residues long. However, topological constraints can be valid over a much wider range. In the case of a circular chain molecule with non-coaxial bonds, the White theorem must be strictly obeyed, no matter how random the molecule appears to be when observed in a local frame. For linear chains the topological constraints are not valid on an infinite timescale. When finite timescales are considered, the range of topological constraints is wider the shorter the timescale.

## 5. Resonator driven transition

A phase transformation in proteins can be initiated by long range collective wring modes of the backbone. We suggest that this is an important part of the underlying mechanism behind the transformation of a protein from the unfolded to the folded structure. We assume that a wring mode is being pumped to levels of higher and higher amplitude, and that eventually this wring mode becomes unsta-

ble in favor of curvature. The nature of such a transition is better characterized as being catastrophic than entropic. By this we mean that the primary reason for the transition is not a change in entropy. Rather, a resonator is responsible for pumping the twist mode to a higher and higher level. The resonator must continuously be re-energized; for example from a thermal bath. One interesting possibility is that the amplitude magnification is due to the formation of a coherent wring excitation involving several resonators.

The condition for the validity of such a scenario for the folding of proteins can be further investigated. Consider the simplified case of a continuous isotropic model with the potential energy:

$$E_{\text{pot}} = \frac{1}{2}\int k_\tau \tau^2(l) + k_\kappa \kappa^2(l)\mathrm{d}l \tag{6}$$

where $\tau$ is the torsion, $\kappa$ the curvature, and $k_\tau$, $k_\kappa$, the torsion and curvature elastic constants, respectively. Curvature is given by the space geometry of the backbone, torsion is given by a combination of space geometry and additional wringing. A wring state is stable when, differentially, a twisted line is stable against the formation of curvature, thereby preventing the formation of a helical line (screw line). The condition for stability depends on the degree of stretching allowed and is obtained after differentiating twice:

$$2k_\tau < k_\kappa \tag{7}$$

Molecular systems are not linear because the effective torsion and curvature constants change with the magnitude of the torsion and curvature variables. It is consequently necessary to consider the following three possible cases:

1. $2k_\tau > k_\kappa$. In this case the chain molecule will not sustain a wring mode as torsion always gives way to bending.
2. $2k_\tau < k_\kappa$, for small amplitudes of the wring modes, and $2k_\tau > k_\kappa$ for larger energy levels. In this case there is an amplitude of the wring mode, where a phase transformation changes the conformation of the chain molecule.
3. $2k_\tau < k_\kappa$, for all amplitudes of the wring state that are biologically interesting. In this case the phase transformation that allows for structure formation in biomolecules, such as for example protein folding, will not take place. On the contrary, excitation of wring modes can enforce a tendency to straighten the chain molecule (for proteins, towards spontaneous denaturation).

Case (2) is relevant for protein folding. It specifies the novel condition for automatic structure formation in biological chain molecules. Accurate numerical values for the torsion and bending constants for proteins are not available at present. It is interesting to compare the two constants for DNA:

$$k_\tau = K_T \cdot [\Delta a]^2 = 1.4 \times 10^{-28}\text{JM} \tag{8}$$

$$k_\kappa = kTL_p = 3.6 \times 10^{-28}\text{JM} \tag{9}$$

where $k_T$ is the Young module per unit length $3.8 \times 10^{-12}$ dyn cm [51], $\Delta a$ is the length of a unit here taken to be 5 Å, $k$ is Boltzmann's constant, $T$ the temperature, and $L_p$ the persistence length. Eq. (9) and the value of the persistence length (861 Å) can be found in ref. [52]. Within the accuracy of the estimates of $k_\tau$ and $k_\kappa$ the requirement that $2k_\tau \approx k_\kappa$ is nearly fulfilled. Thus, it is possible that dynamically induced structure formation also plays a role in some phenomena, which involves a reconfiguration of the DNA molecule, e.g. in connection with transcription, DNA repair, and possible rearrangement of nucleosomes in chromatin.

A number of unique features follow from the scenario of a pumped transition. Firstly, the resonance will be sensitive to the length of the polypeptide chain. This is consistent with protein folding domains all being of a typical size [53] as is the case for the immunoglobulins, and the fact that insulin is synthesized as a single chain which is folded before it is cleaved to its relatively short constituents [54]. Secondly, the part of the protein which first begins to fold will depend on the local amplitude of the global wring mode, and on the local amino acid composition, i.e. on the local values of $k_\tau$ and $k_\kappa$. It is worthwhile to notice that wring modes will divide a protein chain into regions, which are subject to large and small torsional amplitudes, respectively. An amino acid substitution in a region with a high torsional amplitude is more likely to have a modifying (damaging) effect on the folding of a protein than a substitution in a region of low torsional amplitudes. It is possible that this principle is utilized in the variable domains of the antibodies: the hyper-

variable regions may be located to coincide with the regions which are subject to low torsional amplitudes.

## 6. Eigenfrequencies

An upper limit for the energy stored in a twist mode can be estimated by considering the energies of the chemical bonds of the backbone. We take a rotation of $\pi/2$ of a double bond to correspond to about 1 eV/Å, in accordance with typical bond energies, although the energy involved in strained chemical bonds is often smaller. Hence, the torsion constant per inverse unit length, $y$, is limited to about 0.4 eV/Å. The moment of inertia of the backbone (per unit length), $i$, is about 100 a.u. Å, depending on the degree to which the sidechains are involved in the twisting. The eigenfrequency can be estimated to be

$$\nu = \frac{1}{D}\sqrt{\frac{y}{i}} \qquad (10)$$

where $D$ is the length of the backbone. Eq. (10) can be derived as the classical solution to a torsional Lagrange equation, or by modifying the equation describing the Young's modulus. For a typical folding domain, e.g. 125 amino acids, $D$ is about 475 Å, and the frequency $\nu$ becomes about 10 GHz. This frequency corresponds to rotational frequencies of sidechains, and is slightly higher than the rotational frequency of short peptides. The numerical estimate given above assumes a linear torsion term. However, the torsion term is not linear, and the rough estimate of $\nu$ given above is therefore more likely to be too high than too low.

The relaxation time for the rotational motions of the atoms in the backbone has been measured by $^{13}C$ nuclear magnetic resonance. It was found that the relaxation time is much longer than the approximately $10^{-12}$ s that should be expected for individual atoms. Instead, values typically measured are about $5.4 \times 10^{-10}$ s for *Lys* $C^{\gamma}$ in ribonuclease [55]. This more than 100-fold enlargement of the relaxation time is in good agreement with Eq. (10), where it was shown that the relaxation time would scale proportionally to the length of the polypeptide chain, and the corresponding frequency, 185 MHz, is within two orders of magnitude of the estimate. It is worthwhile to note that this is a frequency range (100–600 MHz) where microwave absorption has been observed for proteins [56–58]. The absorption has been interpreted as rotational spectra of *bound water molecules*. It would require that the rotation of bound water molecules is damped to such a degree, when compared to free water, that the frequency is reduced by a factor of about 100. Such a high degree of damping must be almost *critical*, and it therefore seems somewhat peculiar that microwave absorption can be observed over a very broad temperature range of about 100 degrees. It seems more plausible that the damping would become critical and the corresponding frequency therefore go to zero. The reason that the signal was interpreted as coming from bound water is that it is unambiguously linked to the presence of the first monolayer of water on the protein. We suggest a possible alternative explanation, namely, that the microwave absorption is caused by wring modes of the protein. This possibility needs to be addressed in more detail experimentally.

Only in a small window for the values of the constants $k_{\tau}$ and $k_{\kappa}$, corresponding to scenario 2 can wring modes in chain molecules initiate automatic structure formation, see Fig. 5. Specifically, this explains why proteins are unique when compared with many other chain molecules, and may explain why life is carbon based. Chain molecules can loose the ability to form specific structures at low, as well as high, temperatures. Interestingly, for proteins both

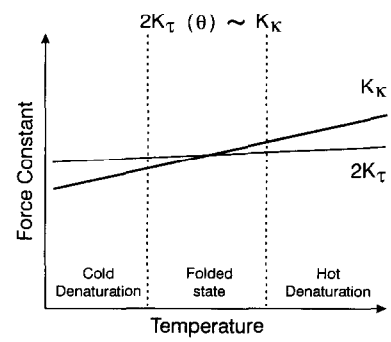


Fig. 5. Schematic drawing of the temperature dependence of the two force constants, the torsion and curvature constants. The three protein phases are indicated below and depend on which line is above which.

cold and hot denaturation are often observed. Proteins are known to release heat when unfolding at low temperature, and to absorb heat when unfolding at high temperature [59]. It is possible that denaturation at low temperature corresponds to a transformation from 2 to 1 and that the released heat is related to the disappearance of wring modes. Likewise, denaturation at high temperature may correspond to a transformation from 2 to 3, where the heat uptake is related to the increased wring mode activity. Interestingly, the two types of denaturation are therefore thermodynamically distinguishable.

In accordance with the above given considerations of which wring modes can be supported by a protein backbone, it can be seen that folded proteins can also sustain wring modes, and that it is the disappearance of these wring modes that upon lowering the temperature can lead to the cold denaturation of some proteins. Some proteins may therefore require dynamic activity in order to be structurally stable. Recently, such dynamic activity has been experimentally verified in a study of the glycosylation of proteins. An analysis of the exchange rates of amide protons indicates that glycosylation is responsible for an overall decrease in the dynamic fluctuations in a protein [60]. The addition of sugar moieties to the polypeptide chain was found to affect the global stabilization of the protein. This is consistent with our proposed wring mode model, as the dynamic fluctuations of the entire protein must be correlated. One can make a crude estimate of the amplitude of the wring modes in a protein by assuming that the only contribution to the change in free energy, when going from an unfolded to a folded, and from a folded to an unfolded protein, is caused by their differences in wring activity. Using the elastic constants above, one can show that the typical amplitude of a wring mode is about 10 degrees of rotation around the backbone. Of course the basis for this estimate is over-simplified as it assumes that all other forces are perfectly compensated by the interaction of the polypeptide chain with the aqueous medium.

Dynamic motion in folded proteins has been studied experimentally by Mössbauer spectroscopy and X-ray diffraction, and theoretically by molecular dynamics simulations. In Mössbauer measurements, it is seen that additional dynamic motions exist in folded proteins at room temperature, which disappear at lower temperature [61]. This low temperature transition has been interpreted as the signature of a glass transition. However, we notice that it could be the signature of the disappearance of wring modes at low temperature. Interestingly, it suggests that what previously has been interpreted as a glass transition, is caused by the same physical phenomenon that is responsible for cold denaturation. In X-ray diffraction studies of protein crystals unexpected dynamic motions have been revealed, when the crystallographic structures are modeled using individual B-factors for the atoms [62]. Although, X-ray studies do not probe the coherence between such motions, they nevertheless reveal a unique pattern along the backbone of the protein which could originate from wring modes. Commonly, it is suggested that the magnitude of the B-factors of specific atoms are correlated with their proximity to the protein surface. We note that there is no contradiction between these two views. In the wring mode model the largest amplitudes are to be found at locations along the backbone where large amounts of curvature were introduced during the folding of the protein. Many of these locations are for structural reasons on the surface of proteins. Molecular dynamics simulations have revealed patterns of excess atomic motions much like the B-factors obtained in X-ray diffraction studies [63].

## 7. Summary

The basic consideration for connecting the winding properties of polypeptides with the protein folding process may be addressed with two conjectures: (a) The path of the segments of the polypeptide chain trace out a "simple motion", (b) the backbone does not rotate unnecessarily. The physical reason for this is that proteins do not fold in a vacuum but in a viscous aqueous medium. Often they also interact with carbohydrates, other proteins and membranes. It is shown that proteins can sustain eigenmodes involving a wringing around the polypeptide backbone, and that the transition from the unfolded state to the folded state of a protein can occur when a wring mode of the protein backbone becomes unstable to curvature.

It is shown that the eigenfrequency of the wring mode may be as high as values typical for excitations of molecular structures. This points to an intriguing possibility for resonances, for example with rotating units, such as sidechains and short peptide strings. As the frequency ($\nu$) scales inversely with the length of the backbone, resonance will not occur before a sufficiently long polypeptide chain has been synthesized in order to facilitate approximate frequency match. This may explain why some shorter polypeptide chains, such as the ones appearing in insulin, are folded while connected to form one longer chain. The fact that the typical number of amino acids in a protein domain is somewhat larger than 100 shows that the length of the protein plays an important role. In this paper we have suggested that protein folding is driven by collective modes in proteins, a hypothesis which is consistent with such observations.

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