Geometry of compact tubes and protein structures

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Proteins form a very important class of polymers. In spite of major advances in the understanding of polymer science, the protein problem has remained largely unsolved. Here, we show that a polymer chain viewed as a tube not only captures the well-known characteristics of polymers and their phases but also provides a natural explanation for many of the key features of protein behavior. There are two natural length scales associated with a tube subject to compaction – the thickness of the tube and the range of the attractive interactions. For short tubes, when these length scales become comparable, one obtains marginally compact structures, which are relatively few in number compared to those in the generic compact phase of polymers. The motifs associated with the structures in this new phase include helices, hairpins and sheets. We suggest that Nature has selected this phase for the structures of proteins because of its many advantages including the few candidate structures, the ability to squeeze the water out from the hydrophobic core and the flexibility and versatility associated with being marginally compact. Our results provide a framework for understanding the common features of all proteins.
A revolution [1] in the understanding of biomolecular structure took place about 50 years ago using the precise geometrical relationships among the atoms and molecules and the rigorous application of the new structural principles enunciated by Linus Pauling [2]. Earlier, in 1939, J. D. Bernal [3] had noted that the symmetry of protein crystals is much higher than would be expected statistically from compounds of such great complexity. This would seem to indicate that each molecule is built of subunits, themselves unsymmetrical but arranged in a symmetrical way. The protein folding problem, the determination of the structure of the folded state of a protein from knowledge of the sequence of amino acids, has remained unsolved despite a large amount of experimental information on protein structures, the availability of powerful computers and detailed knowledge of the building blocks of proteins and their chemistry. The approach pioneered by Pauling is exceedingly effective for understanding small scale structures in great detail but becomes harder to apply at the scale of full protein structure. The complexity arises from the 20 types of naturally occurring amino acids and the solvent and their mutual interactions.

An independent approach to the study of such complex problems consists of stepping back and adopting a coarse-grained view which incorporates just the most essential elements which capture the important emergent features. For example, given a specific chemical compound, one may use the principles of quantum mechanics and chemistry to determine its crystalline structure. Alternatively, in a coarse-grained sense, one may deduce the existence of several types of crystalline structure based on general symmetry and packing considerations. In this case, the specific chemistry of a material would dictate which one of these candidate structures the material would adopt. A common example is the face-centered-cubic (fcc) arrangement adopted both by common salt with strong electrostatic interactions and hard spheres (oranges packed by a grocer) in order to achieve the most efficient packing.

In order to attack a problem of the magnitude of the structure of complex biomolecules, we suggest that it is necessary to consider both points of view. Even assuming that computational power in the future reaches a point which allows a brute force solution incorporating all details, one might be able to mimic Nature but not necessarily understand her. On adapting a statement by Pauling [4] (we have added three words of our own), the problem has been examined, in the main, from one point of view only – not the wrong point of view, but one which, unaided, gives a vista insufficient to reveal the simplicity underlying the true complex nature.

The power of the coarse-grained approach is illustrated by considering some of the familiar states of matter. The gas, liquid and solid phases can be understood in terms of atoms and their interactions modelled as hard spheres (there is no distinction between the gas and liquid phases in the absence of any attractive interaction) or Lennard-Jones systems. On varying the nature of interactions and thermodynamic quantities such as temperature and/or pressure, it is possible to obtain these states of matter, which arise from the collective, emergent behavior of a large number of atoms. Glassy behavior ensues when the crystallization is thwarted by dynamical constraints. Entirely new classes of behavior are found on considering anisotropic molecules as in liquid crystals [5] – the breaking of the symmetry of the building blocks introduces qualitatively new features.
The molecules of life are chain molecules, polymers, which introduce the feature of connectivity along the chain. Indeed, this feature has been exploited by Nature in the DNA molecule to code for genes. Detailed studies of polymers have revealed several phases including a swollen phase (analogous to the gas phase) corresponding to self-avoiding conformations, a highly degenerate compact phase in which different monomers of a chain have an effective attraction to form a dense globule and semi-crystalline phases [6].

Proteins are an important class of chain biomolecules made up of amino acids. These molecules fold into a somewhat compact state with the folded structures controlling their functionality. The folding is driven by hydrophobic interactions or the tendency of certain amino acids to avoid water. The structures of folded proteins do not correspond to the generic compact phase of a polymer. First, the total number of protein folds is only a few thousand [7] instead of the innumerable compact conformations of a generic chain of the same length and second, the building blocks of biomolecular structure are pretty motifs including helices and sheets. Indeed, generic compact conformations are neither suitable for encoding specificity nor are they dynamically accessible in a simple manner. Furthermore, proteins, while stable and able to fold rapidly and reproducibly to their native state structures [8], are sensitive to the right types of perturbations and are consequently able to perform a dizzying array of functions. Here, we shall argue that this phase adopted by molecules embodying life is a new one distinct from the well-studied polymeric phases.

Let us begin with a set of unconstrained hard spheres with an attraction of a given range. When the attraction has a range smaller than the diameter of the hard sphere, it is ineffective and the ground state is a gas. On increasing the range to a value equal to the hard sphere diameter, the ground state changes from a gas to a fcc crystal – each hard sphere is surrounded by the maximum possible number of other hard spheres. Consider now a polymer chain made up of hard spheres tethered together. When the attraction between the hard spheres is sufficiently short range, the analog of the gas phase is a swollen phase corresponding to self-avoiding conformations of the chain. On turning on the attraction by increasing its range, there is a change in the ground state structure. One now gets a fcc lattice but this time with the tethers running through the hard spheres. There are a huge number of ways of doing this and so the ground state is highly degenerate. Of course, a plethora of other compact states would be obtained if this crystallized state is dynamically inaccessible due to the tethering or other constraints. Such compact states are not good candidates for structures of proteins because there are too many of them and accessing a specific structure is next to impossible. Of course, constraints on the local curvature of the chain could lead to other structures, such as lamellar semi-crystalline phases [6], at low temperatures.

The model of spheres tethered together to form a chain does capture the notion of connectivity of a chain but it leaves out a second key factor, the inherent anisotropy associated with the local directionality of the chain. In other words, for a given sphere, the adjacent spheres along the chain define a local direction adopted by the chain. This anisotropy is most easily captured by replacing the sphere by an object with axial symmetry, the simplest
example of which is a coin or a circular disc. Indeed, an object made up of tethered coins of identical size resembles a tube of uniform thickness such as a garden hose. Such a tube like geometry is a coarse-grained representation of the well-known steric effects [9] of the basic constituents of proteins. One may then ask what the analog of the fcc crystal phase is for such a tube.

There are two physical consequences of the tube picture. First, there is a local curvature constraint that does not permit radii of curvature smaller than the tube thickness. This feature may equally well be captured for the case of spheres tethered together by an explicit bond-bending energy term. The second consequence, which is unique to the tube description, is the inherent anisotropy which is reflected in the dependence of the potential energy of interaction between two spatially nearby segments of the tube, not only on their distance from each other but also on their mutual orientation (Figure 1). This change in the symmetry of isotropic interactions between spheres (that is conventionally considered in the well-studied polymer case) to the cylindrical symmetry associated with the tube leads to qualitative changes in the nature of the ground state conformations. At high temperatures, for a tube, one expects a swollen phase in which the local directions defining the tube are distributed isotropically. At sufficiently low temperatures, there is a spontaneous symmetry breaking leading to the selection of a preferred tube direction. This transition is analogous to the isotropic-nematic transition in liquid crystals [5] for tubes. For tube segments, which are thin compared to the range of the attractive interaction and which are near each other, there is a larger flexibility in their relative orientation (see Figure 1). Thus, in this case, a dense globule phase with no significant orientational ordering is expected at intermediate temperatures between the swollen and the orientationally ordered phases.

We now turn to the ground state structures of a simpler situation, one exploited by Nature in proteins, of a short tube when the forces promoting its compaction just set in. Indeed the interaction range and the tube size are matched for proteins because, on the one hand, the effective interactions in the presence of the solvent are short range and the squeezing out of water is facilitated by the outer atoms of nearby side chains coming together and, on the other, it is these same side chains that determine the effective thickness of the tube. Detailed analytic and numerical calculations show that one obtains far fewer conformations than the corresponding generic polymer chain or a thin tube (allowing a protein only relatively few selection choices for its native state conformation) because the different parts of the tube have to position themselves just right relative to each other in order to respect the inherent anisotropy and yet avail of the effective attraction (Figure 1).

Consider the effect of tuning the thickness of the short tube for a given range of attraction. When the tube thickness is bigger than the range of attraction, one obtains a swollen phase because the attraction is ineffective. The other extreme is when the tube thickness is very small compared to this range. In this limit, the greater degrees of freedom for the relative positioning of nearby tube segments due to the longer range of attraction leads to many degenerate conformations.

On varying the tube thickness, near the point when the attractive forces have just set in,
as described below, different segments of the tube have to position themselves just right with respect to each other in order to avail of this attraction. This has two important consequences: first, the tube compaction leads to the formation of a hydrophobic core in the interior of the folded structure and, second, this careful relative positioning of the tube segments combined with the anisotropy associated with the tube weeds out all but a few from the list of possible candidate structures for ground state conformations. These conformations may be thought of as being marginally compact and are thus attractive candidates for versatility and flexibility, because they are able to respond to small changes in an effective manner. Furthermore, on lowering the temperature, starting from the swollen phase, one would expect an almost immediate ordering at a relatively low temperature (with respect to a thinner tube) into one of the ground state conformations without any partially folded intermediates. This “two-state” character is an important feature of small globular proteins and arises in the tube context because the scale of the interaction energy goes down as the tube becomes thicker, entropic effects are less important at the low temperatures of ordering and the orientational effects become stronger (see Figure 1).

We show below that the building blocks of these structures are the familiar helix, hairpins and sheets. Furthermore, elementary considerations predict the geometry of an ideal helix, which is very close to that observed in Nature, and the zig-zag appearance of the strands.

We have carried out computer simulations and analytic calculations of short tubes in the marginally compact phase. The resulting structures are shown in Fig. 2. Helices and hairpins (sheets) are of course the well-known building blocks of protein structures [10,11] (see Fig. 2 (A1) and (D1) for two examples from a protein and (A2), (D2) and (D3) for the tube structures in our simulations). In addition to the prediction of these motifs in our calculations, it is interesting to note that some of the other marginally compact conformations bear a qualitative resemblance to secondary folds in biopolymers. Helices analogous to Fig. 2 (A3) with an irregular contact map occur, e.g., in the HMG protein NHP6a [12] with pdb code 1CG7. Fig. 2 (C1) shows the “kissing hairpins” [13] of RNA (pdb code 1KIS), each of which is a distorted and twisted hairpin structure while Fig. 2 (C2) is the corresponding tube conformation. Fig. 2 (B1) shows a helix of strands found experimentally in Zinc metalloprotease [14] (pdb code: 1KAP), whereas Fig. 2 (B2) is the corresponding marginally compact conformation obtained in our calculations.

It is possible to understand the results shown in Fig. 2 by means of simple arguments. Let us begin by taking a piece of tube of radius $R_0$ and length equal to $2\pi R_0$. Gonzalez and Maddocks [15] have shown that a simple description of a tube is obtained by taking all triplets of points along the axis of the tube and measuring the radii of the circles passing through them with a view of ensuring that none of these radii is smaller than the thickness. In particular, the local radius of curvature of a tube can never be smaller than its thickness. By placing the tube in the form of a donut of radius $R_0$, one can effectively fill all the space in the middle of the donut. When the tube is longer than $2\pi R_0$, the most efficient means of compactifying it is to place it in a helical conformation with local radius of curvature equal to $R_0$ and with the pitch chosen so that the segments of the tube in successive turns lie on top of each other. This is, of course, a valid structure only when the range of attractive
interactions allows contacts to be made in this geometry. This ideal space-filling helix has a special pitch to radius ratio (see Fig. 2 (A2)), which is observed not only in $\alpha$-helices in globular proteins but also in the helices of collagen [16]. An effective squeezing out of the space between the successive turns of the helix is accomplished by the fact that the orientations of the interacting segments of the tube are parallel to each other. Were this not to be the case, the inherent anisotropy of the tube (imagine a tube made up of discrete coins) would lead to a mismatch, a factor of no consequence in a chain made up of tethered hard spheres. One may show analytically that, on increasing the tube thickness, helices are excluded from being the ground states, when the tube thickness exceeds $R_{0}^{\text{max, hel}} \sim \left( \sqrt{1 + R_1^2} \right)/2 \sim 0.943$ ($R_1$ is the range of the attractive interaction and is chosen to be 1.6 units in Fig. 2 – all lengths in the simulation are measured in units of the distance between successive $C_\alpha$ atoms) which is obtained when two parallel straight lines (successive turns of the helix treated as circles with infinite radius) are at a distance of $R_1$ from each other. Indeed this structure is one that corresponds to a hairpin.

The zig-zag hairpin of Fig. 2 (D2) is a distorted version of this idealized case due to the discreteness of the protein chain. One can use elementary geometrical considerations to prove that the zig-zag nature accommodates a tube of larger thickness compared to straight segments. For two zig-zag antiparallel strands facing each other, one can show analytically that the maximum thickness is obtained (leaving aside the edge effect of how the strands are connected together in a hairpin) when one has a space-filling conformation. Indeed, this condition leads to the following relationship between the tube thickness $R_0$ and the interaction range $R_1$

$$R_1^2 + 2 + \frac{R_1}{R_0} - 4R_0^2 = 0,$$

which yields a value of $R_0 \sim 1.2124$, when $R_1 = 1.6$, in perfect accord with our simulations.

For intermediate tube thicknesses between those corresponding to a helix or a hairpin, we find only a few other structures that may be thought of as interpolating between the two limiting cases. In order of decreasing thickness, one obtains first the kissing hairpin structure (Fig. 2 (C2)), which is a hairpin twisted into three dimensions – a feature allowed for by the slightly smaller thickness compared to the planar hairpin; a helix made up of strands (Fig. 2 (B2)); and irregular, somewhat non-ideal helices (Fig. 1 (A3)). In all cases, nearby parts of the tube are oriented parallel to each other.

It is interesting to consider the ground state of many long tubes subject to compaction. Packing considerations suggest that the tubes become essentially straight and parallel to each other and are arranged (when viewed end on) in a triangular lattice, analogous to the Abrikosov flux lattice phase in superconductors [17]. Returning to the case of a single tube, in the very long length limit, a similar phase would be expected with the additional constraint of the bending of the tube segments at the ends. As stated before, for a discrete chain, a planar placement of zig-zag strands is able to accommodate the largest thickness tube that can yet avail of the attraction – however, the thickness for this limiting case is too large to produce the three dimensional ordering alluded to above. It would be interesting to consider how the ground state structure crosses over from the “flux-lattice” type phase.
to the familiar planar phase. Indeed, for thick tubes of moderate length, one may expect
to form a large sheet-like structure analogous to the cross-β-scaffold observed as a building
block of amyloid fibrils [18]. Such fibrils have been implicated in a variety of human disorders
including Alzheimer’s disease and spongiform encephalopathies such as Creutzfeldt-Jakob
disease. Remarkably, recent findings suggest that the ability of proteins to form amyloid is
a generic property of polypeptide chains [18].

Many strategies for attacking the protein folding problem have been put forward which
employ a coarse-grained description [19]. None of the currently used methods has been
successful. Our results suggest that a deficiency of all these methods has been that the
context provided by the local tube orientation is neglected while considering the interaction
between coarse-grained units. The novel phase discussed here arises from the addition of
anisotropy to the well-studied polymer problem just as one obtains rich liquid crystal behav-
ior on studying anisotropic molecules. A mapping of the phase behavior of tubes on varying
the nature of interactions, the thickness of the tube, the length of the tube and temperature
might yield additional surprises.

In 1939, J. D. Bernal [3] wrote: *Any effective picture of protein structure must provide at
the same time for the common character of all proteins as exemplified by their many chemical
and physical similarities, and for the highly specific nature of each protein type.* Our results
provide a simple framework for the common character of all proteins. Our analysis is based
on just three ingredients – all proteins share a backbone, there are effective forces which
promote the folding of a protein and the one and only new idea that a protein can be viewed
as a tube (see Fig. 3). We have not introduced any input into our analysis which pertains
to the highly specific nature of each protein type [3] as encoded by the amino acid sequence.
It would be interesting to extend our calculations to a tube of non-uniform thickness. For
example, the presence of a small amino acid like glycine at backward bends allows for tight
turns to be formed to facilitate good packing and lead to low values of local thickness. Also,
the wide variety of amino acid properties such as hydrophobicity, charge and ability to form
disulfide or hydrogen bonds may be captured in a coarse-grained way by inhomogeneous
attractive amino acid specific interactions, which respect the inherent anisotropy of a tube.

It is important to stress that our results are not at odds with or meant as a substitute for
the detailed and beautiful work involving the laws of quantum mechanics and biochemistry.
The virtue of our approach is that it predicts a novel phase with selected types of structures
and the attendant advantages. It is then necessary to complement this information with the
principles of quantum chemistry to assess whether a given biomolecule would fit one of these
structures. We do not invoke hydrogen bonds as Pauling did in his prediction of protein
secondary motifs [10,11] and indeed not all the structures in the marginally compact phase
are compatible with hydrogen bond placement. What is remarkable, however, is that the
lengths of the covalent and hydrogen bonds and the rules of quantum chemistry conspire to
provide a perfect fit to the basic structures in this novel phase. One cannot but be amazed
at how the evolutionary forces of Nature have shaped the molecules of life [20] ranging from
the DNA molecule, which carries the genetic code and is efficiently copied, to proteins, the
work horses of life, whose functionality follows from their form which, in turn, is a novel
Acknowledgements We are indebted to Flavio Seno and Michele Vendruscolo for useful discussions. This work was supported by INFM, MURST cofin2001, NASA and the Penn State MRSEC under NSF grant DMR-0080019.
REFERENCES

FIGURE CAPTIONS

Figure 1:
Potential energy of interaction of two straight tubes as a function of their mutual distance and relative orientation.

The top panel shows the simplified geometry that we have considered. Two straight tubes, each of length $2l$, are placed a distance $d$ from each other with their axes making an angle $\theta$ with respect to each other. The line joining the centers of the tubes is perpendicular to both the tube axes. We consider a favorable energy of interaction when a pair of infinitesimal segments of the axes of the two tubes are within a distance $R_1$ (chosen to be 1.6 units as in the simulations described in Fig. 2), which is the range of a uniform attractive interaction. The lower panel shows plots of the potential energy both as a function of $d$ and $\theta$ for $l = 1$. The left hand figure shows how the tube geometry leads to an anisotropic interaction, reflected by an energy which depends on $\theta$, for three values of $d$. Note that in each case the energy has been scaled by the energy when the tubes are parallel to each other with the corresponding value of $d$. The anisotropy becomes more pronounced as the tubes become thicker because this restricts the possible range of $d$ to values closer to $R_1$. The weak minimum for the $d = 0$ case away from $\theta = 0$ is due to the short length of the tubes. The lower right hand panel shows a plot of the magnitude of the potential energy when the tubes are oriented parallel to each other as a function of $d$. (we have chosen units such that the scale of the attractive interaction energy of two segments within the range of attraction is simply given by the product of their lengths.) The potential energy is zero when the value of $d$ exceeds that of $R_1$. Note that, for a continuum tube, as the tube thickness, $R_0$, increases towards $R_1/2$, restricting $d$ to values close to but smaller than $R_1$, there are two simultaneous effects. First the scale of the interaction energy becomes very weak and second, the anisotropy becomes pronounced. As described in the text, both these effects play a crucial role in simplifying the behavior of proteins.

Figure 2:
Building blocks of biomolecules and ground state structures associated with the marginally compact phase of a short tube.

In order to mimic a protein, the axis of the tube of non-zero thickness (radius of cross-section) $R_0$ is modelled as a one dimensional discrete chain, whose bonds are of fixed length (set equal to 1 without loss of generality – all other lengths will be measured in these units from now on) and which connect neighboring $C_\alpha$ atoms along the chain. The thickness [15] of the tube is captured by disallowing conformations for which $R_0 > \min_{i\neq j\neq k} R_{i,j,k}$, where $R_{i,j,k}$ is the radius of the circle going through the centers of the atoms $i$, $j$ and $k$:

$$R_{i,j,k} = \frac{r_{i,j} r_{j,k} r_{i,k}}{4A_{i,j,k}}$$

where $A_{i,j,k}$ is the area of the triangle through $i$, $j$ and $k$ and $r_{i,j}$ is the distance between the centers of the $i$-th and the $j$-th atoms. Indeed, one may ascribe a local thickness to the tube by measuring all three body radii associated with a given atom and all other pairs and selecting the smallest radius among these. Figure 3 shows the distributions of the local tube thickness for the native state structures of 30 proteins and underscores the excellent...
approximation of viewing the protein as a tube of uniform thickness.

The interaction between non-consecutive atoms is modeled via a 2-body potential with a hard core and a square well:

\[
V(r_{i,j}) = \begin{cases} 
\infty & \text{if } r_{i,j} < 2R_{h.c.} \\
-1 & \text{if } 2R_{h.c.} < r_{i,j} < R_1 \\
0 & \text{if } R_1 < r_{i,j}
\end{cases}
\]

The three-body interactions capture the inherent anisotropy of a tube, whereas the pairwise potential drives the compaction. For the results shown here, \(R_{h.c.}\) has been set to 0.55, \(R_1\) to 1.6 and \(R_0\) was increased in the vicinity of the transition to the swollen phase until the number of pairwise contacts was reduced to three. While these values have been selected in order to mimic the protein backbone formed by the \(C_\alpha\) atoms, we have verified that our results are robust to variations in these values.

The top row shows some of the building blocks of biomolecules, while the second row depicts the corresponding structures obtained for a tube. (A1) is an \(\alpha\)-helix of a naturally occurring protein, while (A2) and (A3) are the helices obtained in our calculations – (A2) has a regular contact map and is obtained when \(R_0 = 0.80267\) whereas (A3) \((R_0 = 0.833)\) is a distorted helix in which the distance between successive atoms along the helical axis is not constant but has period 2. (B1) is a helix of strands in the alkaline protease of pseudomonas aeruginosa, whereas (B2) shows the corresponding structure \((R_0 = 0.88)\) obtained in our computer simulations. (C1) shows the “kissing” hairpins of RNA and (C2) the corresponding conformation obtained in our simulations with \(R_0 = 0.95\). Finally (D1) and (D2) are two instances of quasi-planar hairpins. The first structure is from the same protein as before (the alkaline protease of pseudomonas aeruginosa) while the second is a typical conformation found in our simulations when \(R_0 > 0.98\). The sheet-like structure (D3) is obtained for a longer tube.

Figure 3:
Distribution of local thicknesses of the native state structures of 30 proteins. The peaked distribution shows that it is a good approximation to think of a protein as a tube of uniform thickness of around 2.7\(\AA\).
triplet radius (local thickness) (A)

Number of triplets