

Chirality of coiled-coils: elasticity matters

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Coiled-coils are important protein-protein interaction motifs with high specificity that are used to assemble macromolecular complexes. Their simple geometric organization, consisting of α -helices wrapped around each other, confers remarkable mechanical properties. A geometrical and mechanical continuous model taking into account sequence effects and based on the super-helical winding of the constituent helices is introduced and a continuous family of solutions in which the oligomerization interactions are satisfied is derived. From these solutions, geometric and structural properties, such as the chirality and pitch of the coiled-coil and the location of residues, are obtained. The theoretical predictions are compared to X-ray data from the leucine zipper motif.

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Coiled-coils consist of α -helices wound together to form a rope-like structure stabilized by hydrophobic interactions. The coiled-coil motif is found in about 10% of the proteins in the human genome [1]. Particular examples of coiled-coils are keratin and the muscle protein tropomyosin. The widespread appearance of the coiled-coil motif is due in part to the simplicity, versatility, and economy by which coiled-coil forming sequences can achieve high specificity to select particular binding partners from a large choice of similar sequences, and in part to their mechanical properties. A coiled-coil can extend and twist to store elastic energy and, accordingly, produce mechanical work. These mechanical properties are conferred by an interplay between specific sequences and intrinsic geometrical properties of this structural motif. Whereas α -helices are right-handed, the overall chirality of most coiled-coils built out of α -helices, such as keratin, is left-handed. This difference was first observed by Crick [2] who gave a geometric construction to reproduce both the handedness of keratin fibers and its global structure. Crick's construction was later generalized by Fraser and MacRae [3] who provided a formula to describe the pitch and radius of a general coiled-coil based on the periodicity of hydrophobic residues in the sequence of identical α -helices. Absent from these analyses is a justification of this formula, a convincing explanation of the origin of the coiled-coil chirality, and the response of coiled-coils under mechanical loads.

The purpose of this paper is to model the relation between structure and mechanical properties of coiled-coils. We introduce a continuum representation that takes into account sequence effects and model the long-range elasticity of the structure. We assume that the coiled-coils are in a canonical shape where the central axis of each

α -helix is itself a helix. We show that within the coiled-coil structure, all residues lie on helices. Further, we show that there exists a continuum of possible structures with both chiralities satisfying the geometric requirements that the hydrophobic sides of the helices face each other, thereby disqualifying previous explanations [2, 3] on chirality. Eventually, the selection of a specific structure within this continuum and therefore its chirality is achieved only by taking into account mechanical aspects of the interactions and the energy associated with each structure.

Geometrically, coiled-coils are made out of *primitive helices* wound around each other, *e.g.* in a keratin dimer there are two primitive helices, which are α -helices. In the canonical form of a coiled-coil, the central axes of these primitive helices take a helical form. We parameterize these structures in terms of curves on which residues lie at discrete points. Consider a curve $\mathbf{r}(s) = (x, y, z)$ parameterized by its arc-length s in the three dimensional space equipped with fixed reference frame $\{\mathbf{e}_x, \mathbf{e}_y, \mathbf{e}_z\}$. We make use of the Cosserat moving frame $\{\mathbf{d}_1, \mathbf{d}_2, \mathbf{d}_3\}$, which is a right-handed orthonormal director basis, built from the tangent vector $\mathbf{d}_3(s) \equiv \mathbf{t}(s) = \mathbf{r}' = d\mathbf{r}/ds$. The two remaining vectors $\mathbf{d}_1(s)$ and $\mathbf{d}_2(s)$ lie in the plane spanned by the usual normal $\mathbf{n}(s)$ and binormal $\mathbf{b}(s)$ vectors. The director basis evolves according to $\mathbf{d}'_i = \boldsymbol{\kappa} \times \mathbf{d}_i$, $i = 1, 2, 3$ where $\boldsymbol{\kappa} = (\kappa_1, \kappa_2, \kappa_3)$ is the vector of material curvatures, related to the classical curvature κ and torsion τ by

$$\kappa_1 = -\kappa \cos \phi, \quad \kappa_2 = \kappa \sin \phi, \quad \kappa_3 = \tau + \phi', \quad (1)$$

where ϕ' is the excess twist [6], describing the material twist of a filament superimposed on its torsion. That is, the angle between the normal and the vector \mathbf{d}_1 : $\mathbf{d}_1 =$

$\sin \phi(s) \mathbf{n} - \cos \phi(s) \mathbf{b}$, $\mathbf{d}_2 = \cos \phi(s) \mathbf{n} + \sin \phi(s) \mathbf{b}$.

Polypeptide sequences which specify α -helical coiled-coils can be recognized by the presence of characteristic sequence elements [4] with periodicity p . One prominent class is recognized by a heptad repeat motif [5] where $p = 7$. Positions of the residues within the heptad are labeled A through G , with positions A and D typically occupied by hydrophobic residues. These hydrophobic sites lie along a twisted hydrophobic strip which drives its association with another similar helix. To maximize the burial of these residues the primitive helix is distorted, introducing a bending of the helical axis and a twisting about this axis which affects the disposition of the side chains. We first consider the positions of the residues on a

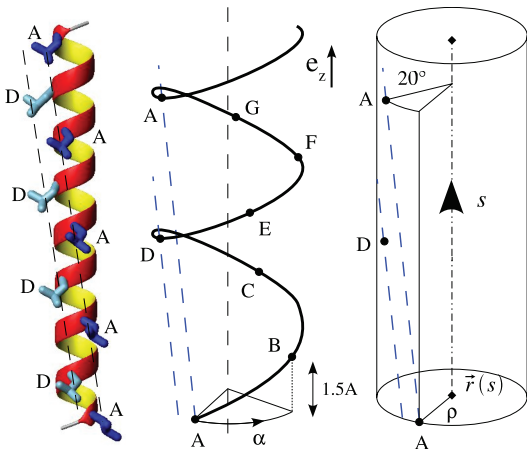


FIG. 1: Left: Idealized alpha helix with hydrophobic leucine sidechains at position A (dark blue) and position D (light blue) in the heptad repeat. Middle: The primitive α -helix. Right: The imaginary cylinder carrying the residues lying at position A, B, \dots, G . The hydrophobic strip is represented as a dotted double line.

canonical α -helix which is modeled as an elastic filament. The axis of the α -helix is identified with the *center line* of the filament and the residues at positions A, B, \dots, G all lie on a cylinder of radius ρ , see Fig. 1. When the filament is straight and unstressed, corresponding to the idealized form of an α -helix, the center line of the filament is the z -axis and the director frame for the filament is $\{\mathbf{d}_1, \mathbf{d}_2, \mathbf{d}_3 \equiv \mathbf{t}\} = \{\mathbf{e}_x, \mathbf{e}_y, \mathbf{e}_z\}$. The residues A and D provide the interaction strip defined by two curves, one joining the A residues and another one joining the D residues (see Fig. 1). The *interface curve* is the curve at the center of the strip. On the cylinder representing the idealized α -helix, the interface curve for a heptad repeat is a left-handed helix. With respect to the center line $\mathbf{r}(s)$ of the filament, this helix is defined by the material line:

$$\mathbf{d}_{\text{hp}}(s) = \cos(\hat{\tau}s) \mathbf{d}_1(s) + \sin(\hat{\tau}s) \mathbf{d}_2(s). \quad (2)$$

The twist $\hat{\tau}$ is an intrinsic property of the filament defined by the periodicity of hydrophobic residues. As the center

line $\mathbf{r}(s)$ deforms, the position of the interaction interface with respect to the director frame remains unchanged. To compute the value of $\hat{\tau}$ we observe that there are 3.6 residues in every turn of the α -helix, that is an angle $\alpha = 2\pi/3.6 = 5\pi/9 = 100^\circ$ between each residue. Each A residue has an angular offset of $-\pi/9$ radians with respect to the previous A residue. Since the rise per residue is $h = 1.5 \text{ \AA}$, we have $\hat{\tau} = -\pi/9/(7 \times 1.5) \simeq -0.033 \text{ rad/\AA}$. In general, let α be the angle between each residue (taken to be positive if the primitive helix is right-handed, and negative otherwise), p the periodicity, then

$$\hat{\tau} = \frac{-\pi + (\alpha p + \pi) \bmod 2\pi}{p h}. \quad (3)$$

The sign of $\hat{\tau}$ gives the chirality of the interface curve.

We now consider a canonical *super-helix*, that is a configuration where the center line $\mathbf{r}(s)$ of the filament is itself a helix, of radius R , axis \mathbf{e}_z , and pitch $2\pi R/\tan \theta$ (the *helical angle* θ is the angle between the tangent $\mathbf{t}(s)$ and the super-helical axis \mathbf{e}_z , see Fig. 2). The center line of the filament in the super-helical configuration is:

$$\mathbf{r}(s) = \begin{pmatrix} +R \sin \psi(s) \\ -R \cos \psi(s) \\ s \cos \theta + z_0 \end{pmatrix}, \quad \psi(s) = \frac{\sin \theta}{R} s + \psi_0, \quad (4)$$

where $\psi(s)$ is the equatorial angle, in the (x, y) plane, perpendicular to the axis of the super-helix. The (constant) curvature and torsion of the super-helix are $\kappa = \sin^2 \theta/R$ and $\tau = \sin \theta \cos \theta/R$. The normal $\mathbf{n}(s)$ has no vertical component and is always facing toward the super-helical axis. The force that holds two (a dimer) or more α -helices to coil around each other is the hydrophobic interaction. In these super-helical structures hydrophobic residues are sequestered from the solvent by facing toward each other. In the case of dimers, symmetry implies that the hydrophobic residues face toward the super-helical axis. This fact can be expressed geometrically by requiring that in the super-helical configuration $\mathbf{d}_{\text{hp}}(s) = -\mathbf{n}(s), \forall s$. Using Eq.(2), we have

$$\cos(\hat{\tau}s + \phi) = 0, \forall s \quad \text{hence} \quad \phi(s) = \pi/2 - \hat{\tau}s. \quad (5)$$

We conclude that the excess twist $\phi'(s)$ in the super-helical configuration is the opposite of the intrinsic twist $\hat{\tau}$ of the hydrophobic strip on the undistorted α -helix structure. In the general case of n primitive filaments the hydrophobic residues do not necessarily face the central axis. Nevertheless they have to face another direction and this also implies $\phi'(s) = -\hat{\tau}$. This last condition has another unforeseen consequence. All A residues lie on a helix of radius R_A with $R_A^2 = (R - \rho \cos \pi/7)^2 + \rho^2 \cos^2 \theta \sin^2 \pi/7$, and helical angle θ_A given by $\tan \theta_A = (R_A/R) \tan \theta$. Similarly, all other residues (B through G) lie on different helices. Remarkably, there is no condition on either the super-helical angle θ nor on

the super-helical radius R . Hence, despite the conventional wisdom based on [2, 3], *the chirality of a coiled-coil (given by the sign of θ) cannot be solely determined from the geometric properties of the hydrophobic strip of the primitive helices*. Moreover, for a given value of R , there exists a one-parameter continuous family of coiled-coils parameterized by the super-helical angle θ which all satisfy the hydrophobic constraint, as seen in Fig.2. Clearly, not all configurations are mechanically acceptable and a selection mechanism is now discussed.

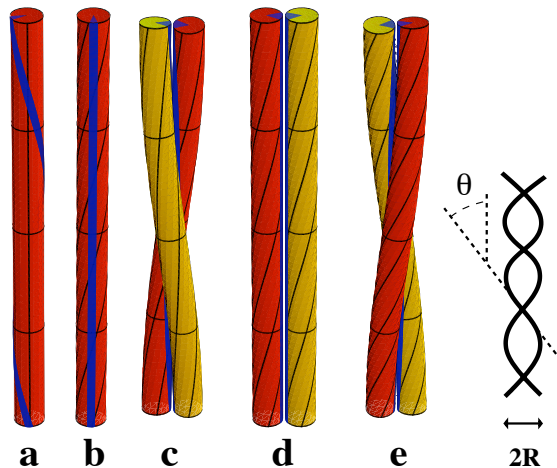


FIG. 2: From left to right: a. Undistorted filament with twisted hydrophobic strip, b. filament twisted in such a way that the hydrophobic strip is straight, c. Left-handed coiled-coil with two facing hydrophobic strips ($\theta = -0.1$ rad.), d. Two parallel twisted filaments ($\theta = 0$ rad.), e. Right-handed coiled-coil with two facing hydrophobic strips ($\theta = +0.1$ rad.). For the last three configurations, the interface curve faces inward. Note that the twist (shown as black lines on the filaments) is lower in the left-handed configuration.

Based on the previous geometric construction, we consider the mechanical aspects of the structures. We first analyze the predicted conformations of the structure in the relaxed (unstressed) state and then when under external axial loads. To begin, we consider n identical primitive α -helices as elastic filaments of length L that are naturally straight and untwisted. To form a coiled-coil, an α -helix will have to bend and twist, but for simplicity, in the following treatment, we assume the primitive filaments to be inextensible [8]. We further assume that when the n primitive filaments coil around one another, the energy associated with the hydrophobic interactions is much larger than the one associated with the elastic deformations (an estimate based on [7] gives $E_{hydro} = 5.1$ kT per residue, to be compared to $E_{elas} = 0.16$ kT per residue (computed with values from Table I)). Thus, the hydrophobic interface is rigidly maintained and the geometric constraint (5) specifies the function $\phi(s)$. There is an energy cost associated with the elastic deformations of

each filament which, in the lowest order theory, is written as the sum of the square of the three material curvatures

$$E_{el} = \frac{1}{2} n \int_0^L (B_1 \kappa_1^2 + B_2 \kappa_2^2 + B_3 \kappa_3^2) ds. \quad (6)$$

The quantities $B_1(s)$, $B_2(s)$ are the (local) bending rigidities along \mathbf{d}_1 and \mathbf{d}_2 and $B_3(s)$ is the twist rigidity. For large-scale deformations taking place over many residues, the bending and twisting rigidities are averaged and replaced by effective constant rigidities $B_1^{\text{eff}} = B_2^{\text{eff}} = B$ and $B_3^{\text{eff}} = C$ [10]. Equation (6) is applicable to general conformations of an elastic rod, which can be specified by any values of the curvatures $\kappa_1(s)$, $\kappa_2(s)$, and $\kappa_3(s)$. Here we consider the case where the center lines of deformed filaments are helices and the super-helix has a fixed (known) radius R and a constant helical angle θ . Curvature and torsion are then $\kappa = \sin^2 \theta / R$ and $\tau = \sin \theta \cos \theta / R$.

To the energy of elastic deformations E_{el} we add the work done by an external force F and torque M which we consider acting along the super-helical axis \mathbf{e}_z . We can now write the total energy in terms of θ and R , that is $E = n \int_0^L V ds$ with

$$V = \frac{B \sin^4 \theta}{2 R^2} + \frac{C}{2} \left(\frac{\sin 2\theta}{2R} - \hat{\tau} \right)^2 - \frac{F}{n} \cos \theta - \frac{M}{nR} \sin \theta. \quad (7)$$

Since R is constant, V only depends on θ and a minimum in the energy is obtained when $dV/d\theta = 0$, that is

$$2B \sin^3 \theta \cos \theta + C \cos(2\theta) (\sin \theta \cos \theta - \hat{\tau} R) + R^2 \frac{F}{n} \sin \theta - R \frac{M}{n} \cos \theta = 0. \quad (8)$$

The solution of this equation gives the super-helical angle θ as a function of the intrinsic parameters of the coiled structure (the rigidities B and C , and the twist $\hat{\tau}$ of the hydrophobic strip) when the structure undergoes tensile ($F \neq 0$) and torsional ($M \neq 0$) deformation. We first focus on the case with no external load ($F = 0 = M$), where the rest state is characterized by a super-helical angle $\theta = \theta_0$ given by the solution of

$$-\frac{2B \sin^3 \theta_0 \cos \theta_0}{R C \cos 2\theta_0} = \frac{\sin \theta_0 \cos \theta_0}{R} - \hat{\tau}. \quad (9)$$

For small angles θ_0 , the l.h.s. provides a correction of order $O(\theta_0^3)$, hence

$$\theta_0 \simeq \hat{\tau} R \text{ if } \theta_0 \ll 1. \quad (10)$$

A few comments are in order. First, an important consequence of Eq.(10) is that elastic equilibrium requires that the chirality of the coiled structure (i.e. the sign of θ_0) is given by the chirality of the hydrophobic strip (i.e. the sign of $\hat{\tau}$) and not by the chirality of the primitive α -helix. For example, in the case of an undecad repeat

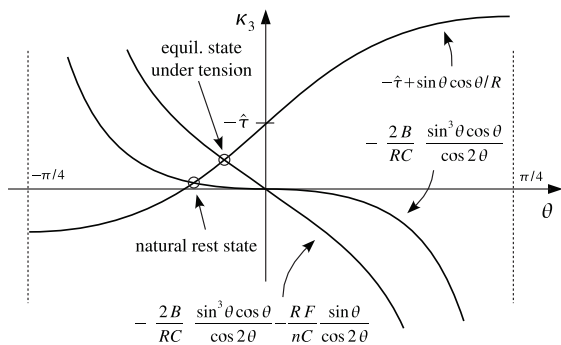


FIG. 3: The mechanical state of the super-helical configuration is at the intersection of the two curves describing the twist in the proto-filaments. In the tensile regime, the mechanical state is shifted toward smaller θ (in absolute values) and higher twist κ_3 .

($p = 11$), the hydrophobic strip on the primitive helix is right-handed and the coiled-coil formed is right-handed as well [9]. Second, we remark that in the limit of small angles, (given by Eq. (10)) we recover the classic formula used in the biochemistry literature and usually attributed to Fraser & MacRae (See Eq. (15.6) of page 458 in [3] where it is given without derivation). Eqs. (9) and (8) are a correction and a generalization of the empirical observation of Fraser and MacRae. A different formula for the pitch of a coiled-coil as a function of the twist $\hat{\tau}$ based also on elastic rod theory has been proposed in [8]. However, we disagree with their results since their formulation is inconsistent with Fraser & MacRae's formula. Note also that Eqs. (8) and (9) yield good agreement with experiments performed on elastic filaments [11] provided $\hat{\tau}$ is identified with a pretwist. Third, we remark that super-helical geometry implies a fundamental coupling between extension and rotation. Pulling on the structure changes its super-helical angle θ , which in turn changes both its extension and its overall rotation. An analysis of the deformation of coiled-coils can be performed by solving the equilibrium equations (8), which is done graphically in Fig. 3.

Structurally, the heptad repeat is the best characterized motif in the class of coiled-coils, in part because of the availability of numerous crystal structures of variant leucine zipper proteins. In order to test the validity of approximation (10), we use the crystal data provided in [12] to compare the experimental super-helical angle θ (as given by X-ray data) to the one computed by Eq. (10). The comparison in Table I shows good quantitative agreement.

This analysis of coiled-coils is also directly applicable to other super-helical structures stabilized through other mechanisms. For instance the triple helix of collagen, a right-handed super-helix, is held together by hydrogen bonds between the primitive collagen helices. Nevertheless, from a geometric and mechanical perspective, these

GCN4	X-ray data				model	
	res./turn	rise/res.	R	2θ	$\hat{\tau}$ (rad/Å)	2θ
dimer	3.62	1.51	4.9	-23.4°	-0.039	-22°
trimer	3.60	1.53	6.7	-26.8°	-0.033	-25°
tetramer	3.59	1.52	7.6	-26.0°	-0.030	-26°

TABLE I: Comparison of the super-helical crossing angle (2θ) from X-ray data (see [12]) with the one given by Eq. (10). Radii and rises are given in Å.

super-helical structures can be treated in the same way as coiled-coils. Each individual strand is a left-handed helix with a repeating motif of $p = 3$ residues: Gly-X-Y. The glycine residues have to face the interior of the structure (in this sense they play the same role as the hydrophobic residues in coiled-coils). There are 10 residues per 3 turns, that is $\alpha = -3\pi/5$, which implies a positive shift of $\pi/5$ rad between glycine residues [13]. Equation (3) with a rise h per residue of 2.86 Å yields $\hat{\tau} = 0.0732$ rad/Å whose positive sign imposes that the triple-helix is indeed right-handed.

We have introduced a continuum elastic model reconciling the mechanical and structural properties of coiled-coils. The coiled-coil is considered to comprise two or more elastic filaments that are uniform and isotropic in their elastic properties. The model explains how the observed chirality of the coiled-coils is due to *both* the location of specific residues and the requirement that the constituent helices (i.e. the filaments) are at equilibrium in the coiled-coil configuration.

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