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Structural specificity in coiled-coil interactions

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Abstract

Coiled coils have a rich history in the field of protein design and engineering. Novel structures, such as the first 7-helix coiled coil, continue to provide surprises and insights. Large-scale data sets quantifying the influence of systematic mutations on coiled-coil stability are a valuable new asset to the area. Scoring methods based on sequence and/or structure can predict interaction preferences in coiled-coil-mediated bZIP transcription factor dimerization. Experimental and computational methods for dealing with the near-degeneracy of many coiled-coil structures appear promising for future design applications.

Introduction

Coiled coils remain beguiling after more than two decades of close scrutiny. Their supercoiled structures are encoded by a seven-residue repeat that can often be detected in sequence data [1,2]. The heptad repeat, denoted [**abcdefg**]$_n$, typically has hydrophobic residues at **a** and **d**, and polar/charged residues at **e** and **g** (Figure 1). Many protein engineers have introduced some variant of this pattern into *de novo* peptide sequences, often with surprising results. Despite an apparent simplicity at the sequence level, a large number of structural variations are observed among coiled coils. Dimers, trimers, tetramers, pentamers and at least one heptamer have been reported, and these can vary in their helix orientations and alignments, as well as in whether they form homo- or hetero-complexes. This poses an interesting specificity problem. How, within the confines of the heptad sequence repeat, are such diverse structures encoded? Design studies, serendipitous discoveries and systematic analyses continue to provide new insights.

Coiled coils offer attractive features to the protein designer and were among the first rationally designed structures [3]. Hydrophobic-polar patterning imposes association of helices, and charge patterning and other features can be used to confer specificity. For example, heterodimerization can be introduced by making one helix basic and another acidic, and a preference for asparagine residues to pair at **a-a'** positions can influence oligomerization state and helix orientation [4]. High symmetry simplifies the description of coiled-coil structure, and mathematical methods for describing ideal coiled-coil backbones have been developed [5,6]. Coiled coils present experimental advantages as well. Short peptides of ~30 residues fold to give stable complexes, allowing for facile introduction of both native and non-native amino acids via peptide synthesis. Circular dichroism can report on cooperative folding and association, which are usually reversible.

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Interest in coiled coils is heightened by their prevalence in biology, and by their potential applications in materials science and synthetic biology [7,8]. Coiled coils are predicted in ~10% of all eukaryotic proteins and are associated with widely ranging functions [9–11]. In materials science and nanotechnology, the rod-like shapes and distinct folded-to-disordered transitions of coiled coils have inspired numerous applications. The utility of charge-pairing rules and other simple principles for controlling helix association are also valuable for engineering nanomaterials with defined structures [12].

Reviews have summarized much of the accumulated knowledge regarding coiled coils [1,4, 7,13]. Here, we focus on recent results in three areas. First, coiled coils continue to surprise us with their variability and sensitivity to sequence changes. Second, we describe database analyses, systematic experiments and computational modeling studies that elucidate principles controlling specificity and stability in coiled coils. Finally, experiments and calculations demonstrate progress in identifying synthetic peptide ligands for native sequences that can form coiled coils.

Novel structures, switches and functions

Altering the hydrophobic/polar patterning of the coiled-coil heptad repeat can generate a variety of structures. For example, the Lu group has reported variants of the yeast GCN4 transcription factor coiled coil with hydrophobic substitutions at the **e** or **g** positions, generating 3-3-1 hydrophobic repeats. Four peptides with this pattern form homotetramers, although the structures show orientations and axial alignments that differ according to the substitutions made (Figure 2A–B) [14–16]. The GCN4 variants all contain an asparagine residue at one of the hydrophobic positions, and the hydrogen-bonding potential of this group is accommodated in a variety of ways. The resulting topologies reflect the combined influence of the 3-3-1 hydrophobic repeat, the Asn residue that disrupts this repeat, and perhaps other features. Right-handed helices can be encoded by 11-residue 3-4-4 repeats, as in a classic design study by Harbury et al. [17]. The Harbury tetramer contained alloisolucine to satisfy core packing requirements, but more recently the structure of a designed right-handed tetramer with only biological amino acids was solved (Figure 2C) [18].

A fascinating new topology, the first 7-helix coiled coil, is formed when both **e** and **g** positions of the GCN4 leucine zipper are replaced with non-polar alanine (Figure 2D) [19]. The structure has parallel helices, a large superhelical radius and a continuous channel through the middle of the coil. Interestingly, the rotational symmetry typical of parallel coiled coils is broken, and a 1-residue axial shift per helix gives a screw axis instead. The seven-helix coiled coil accommodates the **a**-position asparagine residue through a continuous pattern of Asn-Asn hydrogen bonding that is disrupted only at one helix-helix interface. The buried Asn residues may be as critical as the hydrophobic patterning for determining the structure.

Many coiled-coil structures lie close together in sequence and/or energy space, and this has led to the discovery and design of peptides that switch topology given small changes in sequence or conditions. Liu et al. report a parallel five-helix coiled coil with Phe at all **a** and **d** positions. When a single Phe is mutated to Met, a tetramer is formed instead (Figure 2E and F) [20]. Yadav et al. similarly identified a tetrameric GCN4 variant in which a single amino-acid change alters topology [21]. Substitution of glutamate with cysteine at an **e** position switched a parallel tetramer to an anti-parallel one. Strikingly, substitution with serine led to a sequence that crystallized both as parallel and anti-parallel tetramers. Molecular dynamics simulations reproduced the parallel preference of the parent peptide and the anti-parallel preference of the cysteine mutant.

It is not yet possible to systematically predict the structures that form, or the changes in structure that occur, in cases such as those discussed above. Predicting structure is complicated by an

enormous diversity of possible topologies. At least five different packing geometries have been reported for antiparallel tetramers alone [16], and continuous axial shifts, as seen in the parallel heptamer, present a new parameter to be considered. Coiled-coil structures are not determined by simple rules for hydrophobic packing. For example, whereas Lu and colleagues found a pentameric structure for a peptide with Phe at all **a** and **d** positions, Yoder et al. report a shorter sequence with the same core residues that forms a trimer [20,22]. Good agreement of calculations with experimental data in the study by Yadav et al. suggests that explanations for some unexpected changes may be accessible via computation [21]. The modeling in this case was aided by the availability of x-ray structures of both relevant topologies, however, and using these types of computational approaches to make predictions will be more difficult.

Coiled coils can also act as conformational switches in response to various stimuli. Kuhlman and colleagues used a computational approach to design a sequence that forms a coiled-coil homotrimer yet switches to the α/β zinc-finger fold in the presence of metals [23]. A peptide with similar properties was designed by hand in 2005 [24], and both designs show solution properties consistent with the intended folds. Coiled-coil peptides have also been designed to form fibers (both amyloid and non-amyloid) upon external triggering by pH or temperature, or simply upon mixing [25–28].

Interesting applications are suggested by studies where function is switched on or off using coiled coils. Loh and colleagues fused the bZIP domain of GCN4 and the enzyme barnase such that a topological constraint prevented them from existing in their folded states simultaneously. While folding of barnase normally prevailed, addition of AP-1 DNA drove GCN4 dimerization and switched off barnase activity [29]. Tanaka and colleagues inserted two out of three helices from a hetero-specific coiled-coil trimer into a circularly permuted version of RNaseT1. They showed that adding the missing helix could constitute the trimer, and with it the enzyme [30]. Reversible photo-activation of coiled-coil transcription factor binding to DNA has also been demonstrated [31]. These applications illustrate the potential utility of coiled coils for biomedical or materials applications, which will be enhanced as we understand sequence-structure relationships better.

Uncovering relationships between sequence, structure and stability

Interactions among **a**, **d**, **e** and **g** residues account for most structural specificity in coiled coils. Important determinants include electrostatic interactions involving **e** and **g** sites, packing complementarity of **a** and **d** positions, and hydrogen bonding requirements of buried or partially buried polar groups [4,13]. The influence of **e**/**g** electrostatics on coiled-coil stability has long been controversial and continues to attract attention [32,33]. A review by Bosshard and colleagues now clarifies many of the issues that have caused confusion in the literature, mostly due to poor definitions of reference states in energy comparisons [34].

Uncovering specificity-determining features by mining the PDB is becoming feasible now that the database is large and can be automatically surveyed for coiled coils [35]. Issues related to sampling remain severe, however, as the crystallized structures have many biases, and homo-oligomers are especially over-represented. Straussman et al. report that anti-parallel coiled coils in the PDB contain more charged residues in core positions than parallel ones, although it is not yet clear how to interpret this difference [36]. Kammerer et al. previously described a short sequence motif that is common in parallel trimers in the PDB but rare in other types of coiled coils, and mutational data support this motif favoring trimer formation [37].

Kennan and colleagues used non-natural amino acids to explore the influence of side-chain length on coiled-coil stability. Adding methylene groups to lysine and glutamate side chains at **e** and **g** positions in an ACID/BASE heterodimer significantly increased coiled-coil stability [38]. Modulating the length of guanidinium-functionalized side chains at a central **a** position

had less effect. However, a difference in stability between pairing asparagine at **a** with aspartate vs glutamate at the opposing **a'** was used to engineer four sequences that specifically segregated when mixed to give primarily two heterodimers [39]. Helix orientation in these designed dimers was not established.

Exciting advances relating coiled-coil sequences to stabilities and specificities come from systematic sets of quantitative measurements. Acharya et al. report a tour de force of 100 measurements of homo and heterotypic substitutions at **a-a'**-position pairs in a model bZIP-like coiled-coil heterodimer [40]. These data illustrate homotypic preferences for most hydrophobic residues and Asn, and heterotypic preferences for other polar or charged residues: Thr, Lys, Arg and Glu. The 100 measurements allow calculation of double-alanine coupling energies. Asparagine is particularly important for imparting specificity and shows large unfavorable coupling energies with all residues tested except Lys, Arg and itself. The **a-a'** data can now be combined with measured preferences at **g-e'** positions and relative stabilities of homotypic **d-d'** interactions, generating a powerful dataset for analyzing sequence-stability relationships in leucine zippers [41].

An efficient method for measuring relative stabilities in anti-parallel coiled-coil dimers has now been developed as well [42]. Relative stabilities for all pair-wise interactions among Leu, Ile, Val, Asn and Ala interacting at **a-d'** positions have been reported. Further, strong sequence dependencies are apparent in the contributions of "vertical" interactions, i.e. interactions within the core along the coiled-coil axis, such as **a'-a-a'** [43]. These measurements, along with earlier studies of anti-parallel interaction preferences [44], should be valuable for understanding determinants of anti-parallel coiled-coil dimer stability and specificity.

In another large-scale study, a coiled-coil peptide from GCN4 was tested for interaction with 589 single-mutation variants and 4320 double-mutation variants using cellulose-membrane arrays [45]. The utility of this very large data set for advancing our understanding is somewhat limited because the stoichiometries and helix orientations of most of the heterocomplexes formed are not known.

Calculation of specificity from sequence and structure

Principles governing coiled-coil stability and specificity would ideally be encoded in predictive models, and several such models have been proposed. A benchmark to assess performance is provided by a study from our lab that measured all pair-wise interactions among the leucine-zipper regions of most of the 53 human bZIP transcription factors [46]. These proteins homo and heterodimerize via a parallel dimeric coiled coil [41].

A machine-learning model trained on coiled-coil data not including the human bZIP interactions performs very well at predicting these, although it is somewhat difficult to interpret mechanistically [47]. A simpler sequence-based model for scoring different coiled-coil dimers involves summing up relevant interhelical coupling energies [40,41]. However, this does not work well for predicting bZIP pairs [48]. The most likely reason is that not all important coupling energies have been measured and, in particular, coupling of **d**-position residues is likely significant. With an artificial weight introduced to favor Leu-Leu pairing at **d-d**' over all other possibilities, we found that a coupling-energy model does very well (G.G. and A.K., unpublished). Another simple model that also performs very well was proposed by Mason et al. [49]. Weights in this model were hand-selected based on the Vinson data and the authors' experience.

Although it is noteworthy that extremely simple models can perform well, there are a number of caveats. For example, in the model by Mason et al., some unrealistic assumptions are made: **a-a'** and **d-d'** residue pair weights were assumed to be equivalent, and many weights were

empirical. Although the model predicts bZIP association preferences well, this certainly does not mean that **a**-**a**’ and **d**-**d**’ interactions are equivalent or that the weights in the model are "correct". The risk of such models is that they will not perform well outside of the sequence space where they were validated or trained.

There has been progress in using structure-based modeling to understand coiled-coil interactions. We employed an ideal-backbone side-chain packing approach to model bZIP coiled-coil interaction preferences [48]. This performed similarly to the best sequence-based approaches, but only after several limitations of this type of modeling were addressed. We used a similar method to predict parallel vs. anti-parallel orientation for coiled-coil dimers that is ~81% accurate on known structures [50]. Ramos *et al*. used a molecular dynamics approach to dissect the oligomerization preferences of four coiled-coil sequences [51]. Each sequence was modeled in each of four oligomerization states. Impressively, the calculations correctly identified the native oligomerization state as having the lowest free energy for each sequence. However, the correct crystal-structure templates were used when modeling the native oligomers. Although some of the “memory” of the correct backbone was erased by molecular dynamics, the general applicability of this approach for cases where experimental structures are not available is not yet clear.

Coiled-coil partners for native proteins

It is highly desirable to be able to engineer custom peptides that interact with native coiled coils in a specific manner. Vinson and colleagues have described an elegant “A-ZIP” method for creating peptides that bind and inhibit native bZIP transcription factors [52,53]. A-ZIPs are based on bZIPs, but their basic DNA-binding region is replaced with an acidic extension. One limitation of the A-ZIP strategy is that the interaction specificity of the designs is inherited from the bZIP on which they are based.

In an attempt to make bZIP inhibitors by modifying the affinity and interaction specificity of existing bZIP coiled coils, Mason *et al*. used a selection approach. Peptides were selected from a combinatorial library to interact with cJun and cFos [49]. The best binders formed strong associations with their targets. However, other undesired complexes were also very stable. To address this, the authors modified their assay such that library members were selected in the presence of competitor peptides [54]. The resulting peptides bound to cJun and cFos stronger than they homo-dimerized or interacted with the each other’s target.

These experiments illustrate a need for methods that optimize both affinity and specificity. An appealing approach to this problem was pioneered by Havranek and Harbury [55], but applying this broadly confronts obstacles. We recently developed a framework for specificity design that is flexible and computationally efficient. Our method, called CLASSY, uses the technique of cluster expansion to dramatically accelerate design calculations [56]. CLASSY is based on optimizing the stability of a design•target complex while imposing a constraint on the stability difference between the design•target complex and design•undesired partner complexes. We have applied it to design peptides intended to bind each of the 20 families of human bZIP coiled coils while interacting minimally with the remaining 19 families. Many designs indeed bind their targets with high specificity (G.G., A. Reinke and A.K., unpublished).

Conclusions

The coiled-coil energy landscape is degenerate, in that small changes in sequence can lead to large changes in structure. This presents challenges and opportunities to protein engineers. Despite a great body of knowledge, we cannot yet predict coiled-coil topologies from sequence, or reliably design coiled-coil complexes other than the simplest folds. Future coiled-coil designs and structures will surely continue to surprise us. As a model for studying structural

specificity, and as a scaffold with high functional potential, coiled coils remain as compelling as ever. New experimental and computational methods with the capacity to consider multiple states will advance both our basic understanding of coiled-coil structural biology and the practical application of coiled-coil engineering to problems in biomedical and materials science.

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different contexts. Significant differences between energies measured in two environments implicated a role for "vertical" interactions (here **a'-a-a'**) in affecting anti-parallel coiled-coil stability. These results are supported by studies in a different, longer anti-parallel coiled coil, and by striking differences in the conformational heterogeneity of side chains in energetically preferred vs non-preferred combinations.

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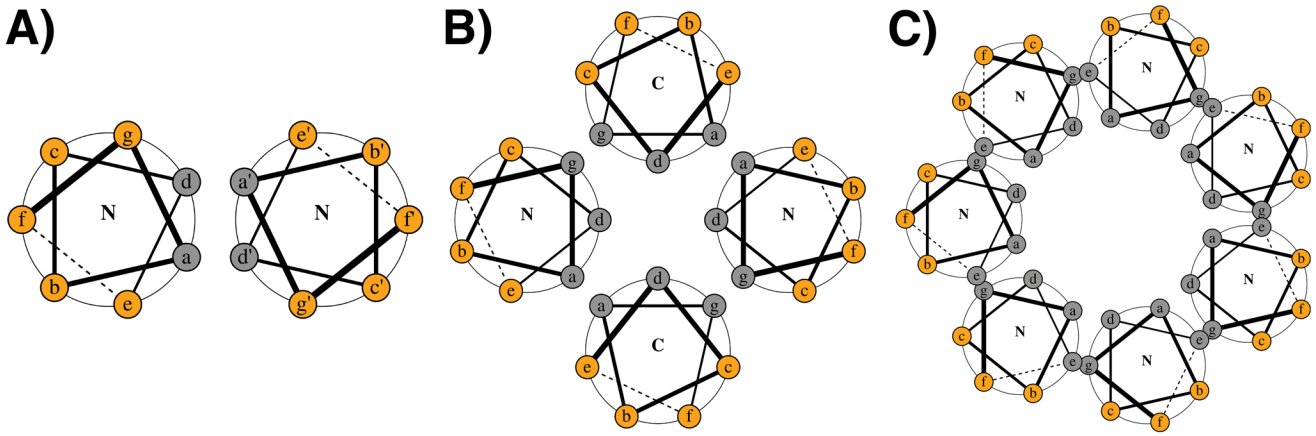

Figure 1.

Helical wheels for coiled coils of varying topology. Heptad positions are shown in small letters, with gray and orange circles indicating predominantly hydrophobic and predominantly polar/charged residues, respectively. **A** The canonical 3–4 heptad repeat, in which hydrophobic residues are 3 and 4 amino acids apart, is found for many coiled coils including dimers (shown in the figure), trimers and tetramers. Prime notation (e.g. **a'**) in this figure and throughout the main text is used to indicate a residue on the opposite chain. **B** An antiparallel tetramer with a 3-3-1 repeat, as in Figure 2B and reference [14]. **C** A parallel seven-helix coiled coil with a 3-1-2-1 hydrophobic pattern, as in Figure 2D and reference [19]. Note that these hydrophobic patterns do not uniquely specify these structures. Other features, including **a-a**' asparagine hydrogen bonding, can be important. Helical wheels were made using DrawCoil 1.0 (http://www.gevorggrigoryan.com/drawcoil/)

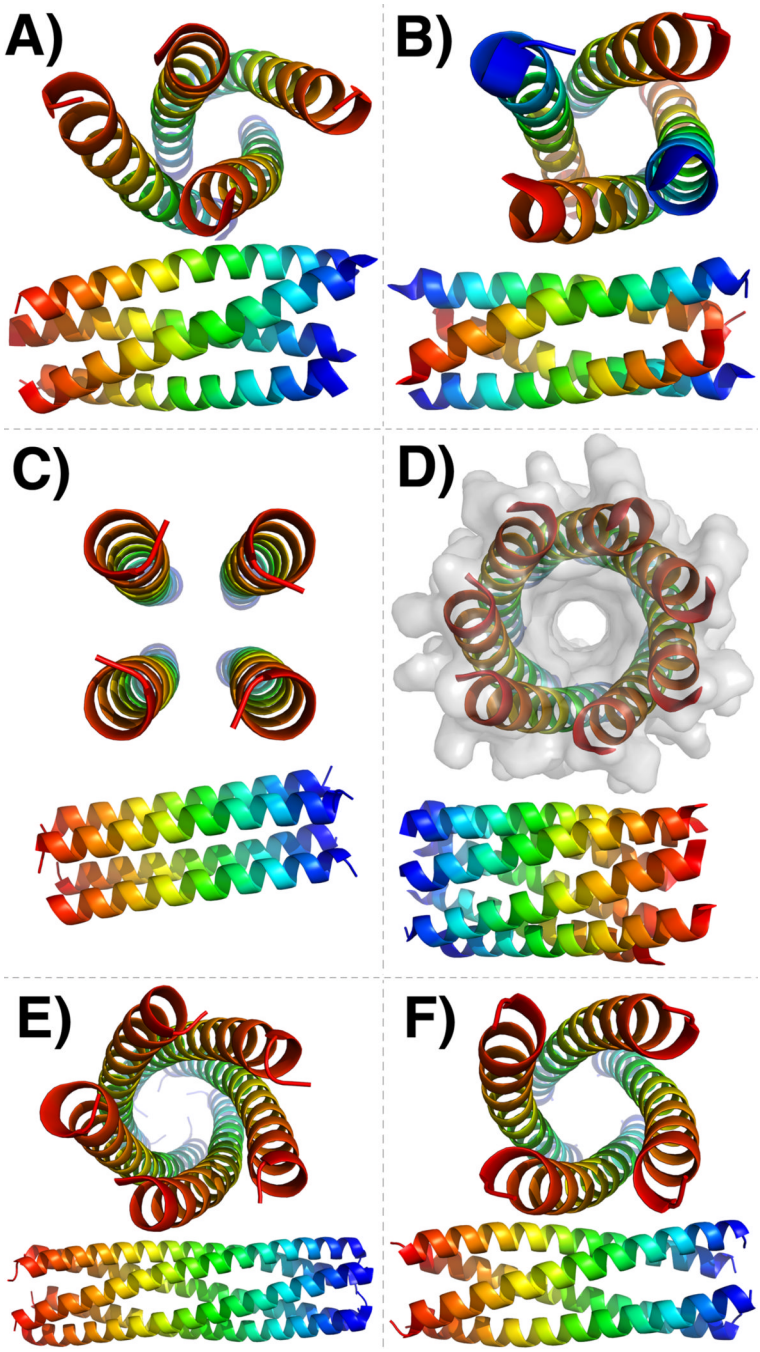

Figure 2.

New and interesting coiled-coil structures. Each coiled coil is shown axially and from the side. Color indicates helix orientation: blue – N-terminus, red – C-terminus. **A**, **B** and **D** Show variants of a GCN4-derived coiled coil with altered hydrophobic-polar patterning. **A** When all **e** positions are mutated to Val, departing from the canonical 3–4 repeat and creating a 3-3-1 hydrophobic repeat, the resulting sequence gives a parallel tetramer [15]. **B** When all **g** positions are substituted with either Val or Ala, also producing a 3-3-1 repeat, the result is an anti-parallel tetramer [14]. **C** A right-handed parallel coiled-coil tetramer [18]. **D** A parallel heptamer is formed when both **e** and **g** positions of GCN4 are substituted with Ala [19]. **E–F** A coiled coil with phenylalanine at all **a** and **d** positions folds as a parallel pentamer (**E**), while if just one

of these phenylalanines is substituted by methionine the resulting structure is a tetramer (**F**) [20]