Chapter 2
Coiled-Coil Design: Updated and Upgraded

Derek N. Woolfson

Contents

Abstract α-Helical coiled coils are ubiquitous protein-folding and protein-interaction domains in which two or more α-helical chains come together to form bundles. Through a combination of bioinformatics analysis of many thousands of natural coiled-coil sequences and structures, plus empirical protein engineering and design studies, there is now a deep understanding of the sequence-to-structure relationships for this class of protein architecture. This has led to considerable success in rational design and what might be termed *in biro de novo* design of simple coiled coils, which include homo- and hetero-meric parallel dimers, trimers and tetramers. In turn, these provide a toolkit for directing the assembly of both natural proteins and more complex designs in protein engineering, materials science and synthetic biology. Moving on, the increased and improved use of computational design is

D.N. Woolfson (✉)
School of Chemistry, University of Bristol, BS8 1TS Bristol, UK

School of Biochemistry, University of Bristol, BS8 1TD Bristol, UK

BrisSynBio, Life Sciences Building, University of Bristol, BS8 1TQ Bristol, UK
e-mail: d.n.woolfson@bristol.ac.uk

D.A.D. Parry, J.M. Squire (eds.), *Fibrous Proteins: Structures and Mechanisms*,
Subcellular Biochemistry 82, DOI 10.1007/978-3-319-49674-0_2

allowing access to coiled-coil structures that are rare or even not observed in nature, for example α-helical barrels, which comprise five or more α-helices and have central channels into which different functions may be ported. This chapter reviews all of these advances, outlining improvements in our knowledge of the fundamentals of coiled-coil folding and assembly, and highlighting new coiled coil-based materials and applications that this new understanding is opening up. Despite considerable progress, however, challenges remain in coiled-coil design, and the next decade promises to be as productive and exciting as the last.

Keywords Coiled coil • Computational design • *De novo* design • Peptide assembly • Protein design • Protein engineering

2.1 Scope of This Review

Arguably, the α-helical coiled coil is one of the most studied and the best understood of all protein structures (Lupas 1996a; Gruber and Lupas 2003; Woolfson et al. 2012). This review focuses on how our basic knowledge of coiled coils is advancing, and how this understanding is being translated into *de novo* coiled-coil design. The term *de novo design* can be defined as the process of generating completely new peptide and protein sequences that fold and assemble into prescribed 3D structures; this can be done rationally using heuristics, or rules of thumb (Woolfson et al. 2012; Regan et al. 2015), although increasingly it is achieved using computers to sample large swathes of sequence and structural space (Davey and Chica 2012; Feldmeier and Hocker 2013; Regan et al. 2015; Woolfson et al. 2015). It can be distinguished from *peptide / protein engineering* or *redesign*, which may be regarded as the iterative processes of altering usually natural sequences to effect predictable changes to protein structure, stability, and/or function (Magliery 2015). That said, with a large number of successful *de novo* coiled-coil designs now established, these two areas are merging: successful *de novo* coiled-coil designs are being used as templates onto which functions are being grafted (Mocny and Pecoraro 2015), and as building blocks for the construction of more-complex structures, large assemblies, materials and even systems (Bromley et al. 2008; Channon et al. 2008). This review attempts to cover all of these emerging areas. It builds on a previous review (Woolfson 2005), which outlined the basic concepts of coiled-coil design and catalogued *de novo* designs from the preceding 20 years or so.

Although this chapter develops themes from the 2005 article, the latter should be viewed as a basic introduction to the field of coiled-coil design. Because the field has developed considerably over the past 10 years, this new review has been structured differently. Firstly, what can be and has been achieved using established and relatively straightforward sequence-to-structure relationships in *classical coiled* coils is summarised. Secondly, there is a discussion of how the field is moving on in

terms of understanding coiled-coil sequences and structures more generally. This requires us to adjust and expand our traditional view of the sequence repeats and helix packing. Specifically, we have to consider multiple helix-helix interactions within coiled-coil structures. These lead to higher-order oligomer states and more complex coiled-coil assemblies. Thirdly, the development and use of computational methods, which are necessary to explore this larger design space, is discussed. Fourthly, there is a description of what is being done with both the classically-derived and more complex coiled coils, in terms of building elaborate peptide / protein-based architectures and materials, and also incorporating function into these assemblies. Finally, there is a short section on perspectives and where the field might be headed.

2.2 The Basics of Coiled-Coil Sequence and Structure

As the 2005 review covered many of the basic underlying concepts and foundational work in coiled-coil design (Woolfson 2005), this chapter will not dwell on these. Instead, it will discuss only old and new concepts central to the designs being reviewed herein.

However, for completeness and for the avoidance of doubt, coiled-coil units are defined as follows: coiled coils comprise two or more α-helices that wrap around each other to form supercoiled quaternary structures (Lupas 1996a; Lupas and Gruber 2005), Fig. 2.1a, b. As defined by Crick (Crick 1953b), the helix-helix interactions are directed and cemented by so-called knobs-into-holes (KIH) interactions, Fig. 2.1c, d. These are intimate interactions in which a sidechain (the knob) from one helix inserts into a diamond-shaped arrangement (the hole) projecting from another helix. It is generally accepted that helical assemblies must have contiguous stretches of KIH interactions in order to qualify as α-helical coiled-coil structures (Walshaw and Woolfson 2001b), otherwise they are simply globular α-helical domains or bundles, where different and less-intimate packing arrangements operate (Chothia et al. 1981; Walther et al. 1996). KIH-based structures relate back to underlying sequence repeats of hydrophobic (***h***) and polar (***p***) residues (Crick 1953b). Most commonly, these are heptad repeats, ***hpphppp***, often denoted ***abcdefg*** (Lupas 1996a). Variations on this pattern are well known, and these have structural and functional consequences (Brown et al. 1996; Hicks et al. 1997, 2002; Gruber and Lupas 2003). Because the average 3.5-residue spacing of hydrophobic side chains in canonical heptad repeats closely matches the 3.6-residue repeat of the ideal α-helix, they result in amphipathic helices, Fig. 2.2a. In turn, and in what may be termed a *classical coiled coil*, two or more such helices assemble into helical bundles, Fig. 2.1a, b. However, because 3.5 and 3.6 are different, rather than packing like straight and rigid straws, the helices wrap or supercoil around each other.

A *classical coiled coil* is usually considered as one founded on a single, contiguous heptad pattern. To all intents and purposes, these have a single hydrophobic seam defined by residues at the ***a*** and ***d*** sites of the heptad repeat. Largely, this leads

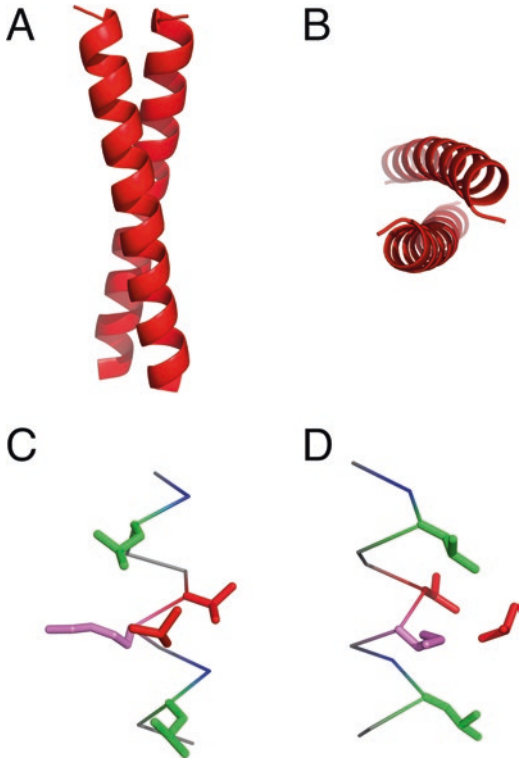

Fig. 2.1 Coiled coils are founded on knobs-into-holes packing of α-helices. (**a** & **b**) Orthogonal projections of the X-ray crystal structure of an α-helical coiled-coil dimer (2ZTA, (O'Shea et al. 1991)). (**c** & **d**) Orthogonal projections of an ***a***-knob projecting into ***d'g'a'd'***-hole from the same structure as panels (**a** & **b**). Key: ***a*** *red*, ***d*** *green*, ***g*** *violet* (Protein-structure images were generated in PyMOL (www.pymol.org))

to only dimeric, trimeric and tetrameric coiled-coil assemblies. However, as introduced and discussed below, our current understanding of coiled coils, and the desire to delve deeper into the structural space possible for coiled coils, requires us to move on from this classical treatment and to consider *more-complex*, or *extended coiled-coil interfaces* (Walshaw and Woolfson 2001a, 2003; Moutevelis and Woolfson 2009; Woolfson et al. 2012).

2.3 Computational Tools for Analysing Coiled-Coil Sequences and Structures

There is now a raft of well-established and reliable web-based bioinformatics tools available:

1. For examining protein sequences to locate coiled-coil regions (Lupas et al. 1991; Lupas 1996b; Delorenzi and Speed 2002)
2. To predict coiled-coil oligomer state (McDonnell et al. 2006; Rackham et al. 2010; Armstrong et al. 2011; Trigg et al. 2011; Vincent et al. 2013; Ramisch et al. 2015)

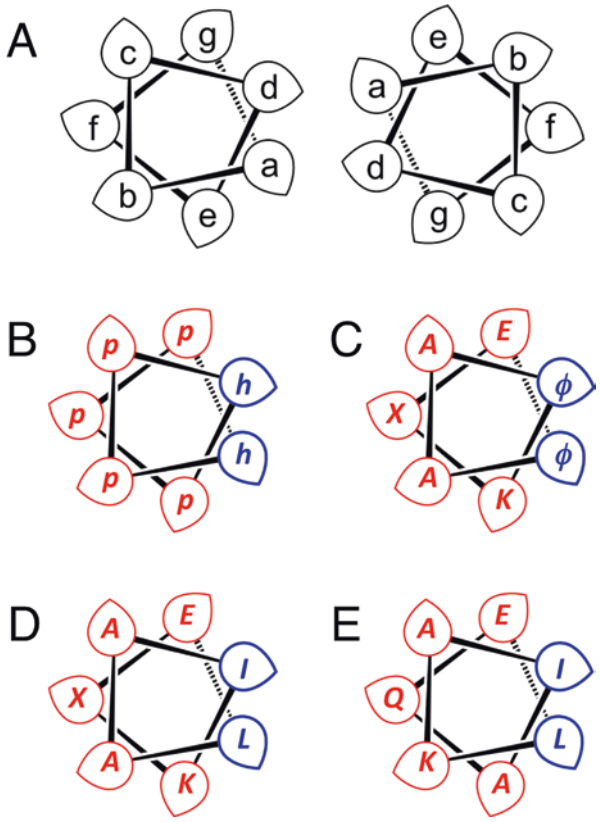

Fig. 2.2 Coiled-coil sequence repeats visualised by helical-wheel diagrams. (**a**) The heptad repeat, ***abcdefg***, spun out onto helical wheels for a classical dimeric coiled coil. The central Cα atom of each residue is represented by "teardrop" with the point directed approximately towards the Cβ atom. (**b**) The repeat of hydrophobic (***h***) and polar (***p***) residues for a classical coiled coil. (**c**) Helical wheel giving the heptad background upon which the CC-series of *de novo* coiled coils were built (Zaccai et al. 2011; Fletcher et al. 2012). (**d** & **e**) Helical wheels showing the sequence relationship between CC-Tet and CC-Hex (Zaccai et al. 2011). For all of these wheels, the number of residues per turn is 3.5, and not 3.6 for a standard α-helix, *i.e.* the helical wheels effectively uncoil the super-helical coil

3. For analysing X-ray crystal and NMR structures of coiled-coil domains that have been deposited in the RCSB Protein Data Bank (PDB; (Rose et al. 2015))

For example, the program SOCKET (Walshaw and Woolfson 2001b) identifies KIH interactions in PDB coordinate files and, along with this, TWISTER can determine geometrical parameters for coiled-coil structures (Strelkov and Burkhard 2002). Moreover, outputs from these programs are being collated into useful relational databases and schemes to visual and interrogate coiled-coil sequences and structures rapidly and *en masse* (Moutevelis and Woolfson 2009; Testa et al. 2009). Specifically, the database (Testa et al. 2009) now has >2500 non-redundant

entries, representing a significant resource for the coiled-coil and wider community. CC+ can be searched using a number of easy-to-use pull-down menus within four over-arching tabs for keywords, structures, sequences and interactions. In this way, a user can readily and quickly create a subset of coiled-coil structures for further manual inspection or computational analysis. Indeed, CC+ is being used in this way by others to search for structure-function and evolutionary relationships of natural coiled coils (Surkont et al. 2015), and to benchmark the performance of coiled-coil modelling and design algorithms (Ramisch et al. 2015).

2.4 Straightforward Rules for Coiled-Coil Prediction and Design

The link between sequence and structure makes coiled coils ideal targets for rational design of peptides and proteins. Indeed, such is the depth of our understanding of classical coiled coils that sequences that adopt parallel dimers, trimers and tetramers can simply be written down (Harbury et al. 1993; Fletcher et al. 2012). This follows from seminal work conducted by Harbury, Alber and Kim on hydrophobic-core variants of the leucine-zipper region of a yeast transcriptional activator, GCN4-p1 (O'Shea et al. 1991), and work that followed (Harbury et al. 1994; Woolfson and Alber 1995; Gonzalez et al. 1996; Woolfson 2005). This furnished us with straightforward sequence-to-structure heuristics for classical coiled coils. In GCN4-p1, which forms a parallel dimer, all ***d*** sites are leucine (Leu, L), and, although more varied, the canonical residue at ***a*** is valine (Val, V). Harbury's work shows that different combinations of Leu and isoleucine (Ile, I; which is β-branched like Val) at the ***a*** and ***d*** sites, lead to different oligomer states being formed. For example, the combination of ***a* = *Ile*** and ***d* = *Leu*** promotes parallel dimers, ***a* = *d* = *Ile*** specifies trimers, and ***a* = *Leu*** plus ***d* = *Ile*** directs tetramers. Although we have learnt over the past decade that things are a little more complicated, these heuristics largely hold up to mutational studies of natural proteins, and completely *de novo* designs (Woolfson 2005; Woolfson et al. 2012). Thus, they allow what might be termed *in biro* design of coiled coils; indeed, this is so well embedded in peptide and protein science that specialist and non-specialist labs can turn out designs of coiled coils and translate these into experiments with ease and, more importantly, with predictable outcomes. In short, *in biro* coiled-coil design is now *de rigueur*.

The *Harbury relationships* work because they link sequence and final structure, in this case the oligomer state of the coiled coil. The variants can be rationalised in structural terms that are fully consistent with Crick's original KIH postulate, Fig. 2.1c, d. Because of helical geometry, the Cα-Cβ bond vector of a sidechain projects out in a defined way from the helical backbone, Fig. 2.3a. As a result, when two helices come together in a parallel dimer, sidechains at ***a*** and ***d*** sites (the knobs) project differently towards the constellation of sidechains on the partnering helix that form their recipient holes, as illustrated in Figs. 2.1c, d and 2.3b. For the ***d*** sites,

the resulting packing is *perpendicular—i.e.*, the knob points directly into the hole—which is favoured by the non-β-branched, hydrophobic alkyl sidechain of Leu. Indeed, this is borne out by analysis of all non-redundant parallel coiled-coil dimers in the PDB, >50 % of the ***d*** sites are Leu (Testa et al. 2009). By contrast, the Cα-Cβ bond vectors of the ***a*** knobs are *parallel* to each other. This type of packing is more accommodating of different sidechain shapes, hence the acceptance of, and indeed preference for β-branched Val and Ile residues. Adding helices to form parallel trimers and tetramers results in different KIH geometries, Fig. 2.3c, d. This can be thought of as the helices rotating as a consequence of the hydrophobic moment of the helix, which falls between the hydrophobic ***a*** and ***d*** sites, being required to point towards the centre of the assembly. As a result, the KIH geometries of how the ***a*** and ***d*** knobs point into their respective holes changes. From dimer to trimer, the ***a*** and ***d*** KIH geometries move from the extremes of parallel and perpendicular packing, respectively, to being more similar, and in between these two states, in so-called *acute packing*. This then explains why in trimers the amino-acid preferences at the two sites are more similar, which is again borne out on inspection of natural sequences and structures. Adding a further helix to make a tetramer rotates the helices relative to one another again, and with it the KIH geometries change. In this case, the ***a*** and ***d*** side chains move through intermediate acute packing to perpendicular and parallel packing, respectively, Fig. 2.3d. Thus, the packing geometries are swapped from those seen with parallel dimers, explaining the residue preferences swap in the GCN4 mutants. Though there are fewer sequences in the CC+ databases for tetramers compared with dimers and trimers, this broad-brush rule for ***a* = *Leu*** plus ***d* = *Ile*** appears to hold in natural tetramers too.

A final point to note here is that whereas the ***a*** and ***d*** KIH interactions are complementary in dimers, they daisy chain in trimers and tetramers; that is, a knob from helix A interfaces with helix B, and the corresponding knob from helix B interfaces with helix C and so on, Fig. 2.3.

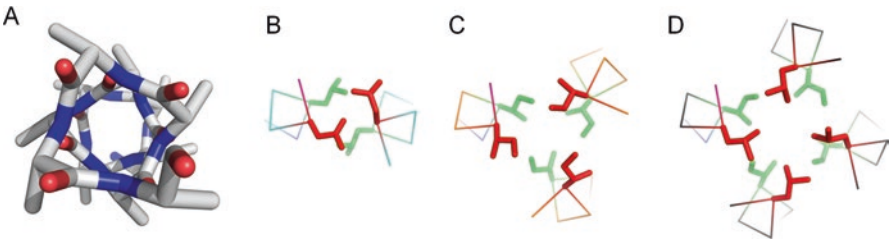

Fig. 2.3 Classical coiled-coil dimers, trimers and tetramers differ in their knobs-into-holes packing. (**a**): The projection of the Cα—Cβ vectors (*grey sticks*) of sidechains from a standard α-helix. (**b**–**d**): Similar diagrams for dimeric, trimeric and tetrameric coiled coils, respectively. These diagrams, which focus on a single heptad repeat and highlight the ***a*** (*red*) and ***d*** (*green*) sites only, show that although the sidechains project in the same ways from each helix, they are directed differently towards neighbouring helices in the three coiled-coil oligomers. This is discussed in detail in the text (Protein-structure images were generated in PyMOL (www.pymol.org))

2.5 A Heptad of Completely de novo Helical Assemblies

2.5.1 Rationally Designed Dimers, Trimers and Tetramers

As elegant as they are, the studies of Harbury *et al.* were performed in the background of a natural coiled coil, namely GCN4-p1, and this sequence may be complicated by biological function and evolutionary pressure. For two reasons Harbury's relationships have been tested in a completely *de novo* background (Fletcher et al. 2012). First, it was important to know if the rules were context-independent or not, *i.e.* if they could be translated to other systems; second, the aim was to generate a *basis set* or *toolkit of coiled-coil modules* for further protein-engineering and synthetic-biology projects. The rationale for the latter was that a set of well-characterised and context-independent coiled-coil modules would have considerable applications for directing protein assembly, and that these could be orthogonal to natural protein-protein-interaction motifs (Bromley et al. 2008; Channon et al. 2008).

To do this, a general background for parallel coiled-coil bundles, *i.e.* EΦAAΦKX, has been designed which maps on to a ***gabcdef*** heptad repeat, Fig. 2.2c. In these sequences, combinations of Ile and Leu are used at the ***a*** and ***d*** sites (denoted Φ) to direct dimer, trimer and tetramer formation as informed by Harbury (Harbury et al. 1993); glutamate (E) and lysine (K) placed at ***g*** and ***e*** sites to encourage inter-helix salt bridges flanking the hydrophobic core; and the ***b, c*** and ***f*** sites are made *vanilla*, with alanine (A) at ***b*** and ***c*** to encourage helicity, and glutamine (Q) and/or lysine at ***f*** (denoted X) for water solubility. Different chromophore and mass tags are used to aid characterisation and, finally, the peptides are four heptads long for stability.

Synthetic peptides for the initial design iterations (CC-pIL, CC-pII and CC-pLI) are all water-soluble, fully α-helical and highly thermally stable (Fletcher et al. 2012). Whilst solution-phase measurements and X-ray crystallography confirm CC-pII and CC-pLI as parallel trimer and tetramer, respectively, CC-pIL is a trimer and not a dimer. Though surprising in the light of Harbury's data, this fits with the broader body work on sequences of the type ***a*** = Ile/Val plus ***d*** = Leu, which can adopt various oligomer states (Ogihara et al. 1997; Ghirlanda et al. 2002; Oshaben et al. 2012). As it is well known that a single asparagine (Asn, N) at ***a*** in natural leucine-zipper and related sequences help specify dimers (Woolfson and Alber 1995; Gonzalez et al. 1996; Lumb and Kim 1998), the centre-most ***a*** in CC-pIL has been mutated from Ile→Asn. This gives the peptide of reduced thermal stability, though it is still a fully folded parallel dimer in solution and by X-ray crystallography. An analogous insertion of Asn at a ***d*** site of the CC-pII sequence, yields a peptide with reduced thermal stability, but remains a parallel trimer state, which is fully consistent with foregoing bioinformatics and experimental data (Hartmann et al. 2009). The net result is a set of fully characterised coiled-coil oligomers—renamed as CC-Di, CC-Tri and CC-Tet—complete with high-resolution X-ray crystal structures, Fig. 2.4a–c.

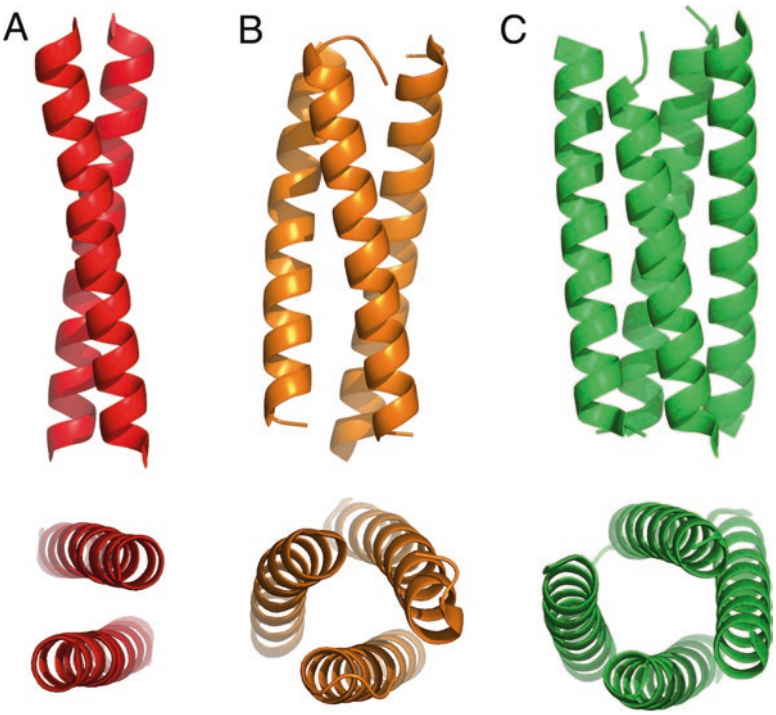

Fig. 2.4 A basis set of de novo coiled coils: dimer, trimer and tetramer. (**a–c**) Orthogonal projections of the X-ray crystal structures of CC-Di, CC-Tri and CC-Tet, respectively. The PDB codes for these are 4DZM, 4DZI and 3R4A, respectively (Zaccai et al. 2011; Fletcher et al. 2012) (Protein-structure images were generated in PyMOL (www.pymol.org))

2.5.2 Expanding de novo Coiled Coils Past Tetramer

Inspection of the CC+ database reveals that the vast majority of natural coiled-coil structures are for dimers, trimers and tetramers, Fig. 2.5. The emphasis and success of the design field on these oligomers states almost certainly reflects this bias in the natural coiled coils observed to date. That said, the door to larger oligomer states is ajar, and has been for a while. There are several pentamers, which include natural and engineered peptides and water-soluble and membrane-spanning proteins (Malashkevich et al. 1996; Liu et al. 2006c; Eshaghi et al. 2006; Payandeh and Pai 2006; Vostrikov et al. 2013; Sastri et al. 2014), one variant of GCN4-p1 forms a heptamer, albeit an unusual spiralling structure (Liu et al. 2006b), there are pore-like natural structures for a membrane-spanning octamer (Dong et al. 2006), a decamer (Sun et al. 2014), and a dodecamer (Koronakis et al. 2000). The interest in pursuing engineering and design of these larger assemblies is twofold. First, it would be interesting to know the limits of coiled-coil assemblies and, second, pentamers and above either have, or are predicted to have contiguous, open-ended,

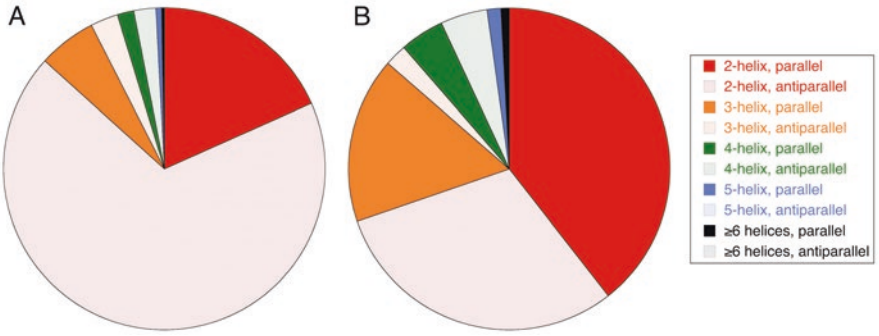

Fig. 2.5 Biased distribution of coiled-coil oligomer states and topologies in the PDB and CC+ database. (**a**) All coiled coil-containing proteins. (**b**) Only intermolecular coiled-coil motifs. The data were culled from the CC+ database of August 2015 (Testa et al. 2009) using the following settings: 70 % sequence redundancy, and canonical heptad repeats of >11 residues long. The raw counts of structures summarised in these plots are: parallel dimers (**a**, 403; **b**, 305); antiparallel dimers (**a**, 1503; **b**, 233); parallel trimers (**a**, 128; **b**, 128); mixed/antiparallel trimers (**a**, 65; **b**, 19); parallel tetramers (**a**, 33; **b**, 33); mixed/antiparallel tetramers (**a**, 50; **b**, 36); parallel pentamers (**a**, 12; **b**, 12); mixed/antiparallel pentamers (**a**, 0; **b**, 0); parallel structures with ≥6 helices (**a**, 5; **b**, 1); mixed/antiparallel with ≥6 helices (**a**, 5; **b**, 1)

central channels or pores; *i.e*, they are *α-helical barrels*. Indeed, the observed naturally-occurring pentamers and the octamer, decamer and dodecamer bind or transport other biomolecules. However, for these structures there are not enough examples or data to inform designs as described above. However, a route into the design of α-helical barrels has been discovered serendipitously as follows.

A permutant of CC-Tet has been made with the residues at the ***b*** and ***e*** positions exchanged; *i.e.*, the ***g*→*f*** heptad repeat was changed from ELAAIKX to ELKAIAX, Fig. 2.2d, e. The resulting peptide is fully α-helical and highly thermally stable. Surprisingly, the solution-phase oligomer state is hexamer, which is confirmed by a high-resolution X-ray crystal structure that reveals a parallel 6-helix supercoiled bundle with regular KIH interactions (Zaccai et al. 2011). This novel structure also has a central ~6 Å channel running completely through its centre. Furthermore, the peptide CC-Hex is somewhat, though not completely, robust to mutations within the lumen of the structure (Burton et al. 2013; Burgess et al. 2015; Thomas et al. 2016). Until that point, there was only one other coiled-coil-based hexamer in the PDB, which is an antiparallel structure buttressed by other helices (Tanaka et al. 2007).

With a view (1) to engineering more-robust versions of CC-Hex, and (2) to designing rare oligomers other than hexamer predictively, a computational design programme has been initiated to generate coiled-coil pentamers, hexamers and heptamers. This has delivered designs and X-ray crystal structures for CC-Pent, CC-Hex2, CC-Hex3, and CC-Hept, Fig. 2.6a–c (Thomson et al. 2014). In parallel, the Baker group has described the parametric computational design of hyperthermostable coiled-coil trimers, tetramers and a pentamer, Fig. 2.6d (Huang et al. 2014). More recently, André and colleagues have reported the design of another

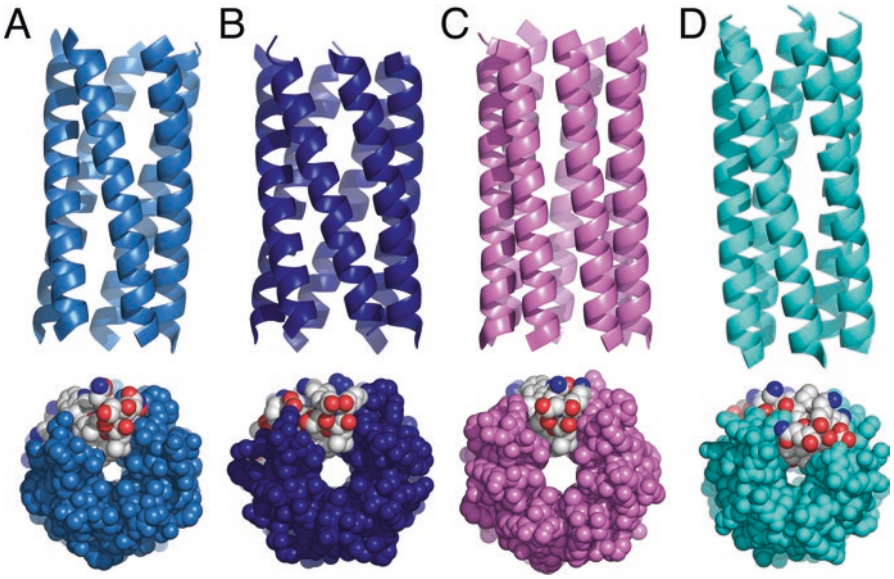

Fig. 2.6 Coiled coils beyond tetramer are possible by design. (**a**–**c**) Orthogonal projections for the X-ray crystal structures of CC-Pent, CC-Hex2 and CC-Hept, respectively. The PDB codes for these are 4PN8, 4PN9 and 4PNA, respectively (Thomson et al. 2014). (**d**) A computationally-designed pentamer from the Baker group (4UOT, (Huang et al. 2014)). *Top*: Ribbon diagrams tracing the polypeptide backbones. *Bottom*: Space-filling representations with one chain in each structure coloured by atom type (Images were generated in PyMOL (www.pymol.org))

pentamer, which switches to a 6-helix bundle upon change of pH (Lizatovic et al. 2016). Details for these studies are given below.

2.5.3 Structural Rationale for Higher-Order Oligomerization

The core-packing arguments learned from classical dimers to tetramers (Harbury et al. 1993; Fletcher et al. 2012) extend to explain the higher-order coiled coils, such as CC-Hex. Above tetramer, apart from small changes with increasing oligomer state, KIH geometries for the traditional ***a*** and ***d*** sites effectively remain fixed at perpendicular and parallel, respectively. As a result, the *largermers*, *i.e.* CC-Pent, CC-Hex and CC-Hept, appear best specified by ***a*** = Leu plus ***d*** = Ile (Thomson et al. 2014; Huang et al. 2014), although variations on this are possible. This is also largely the case for the small number of known natural pentamers, such as COMP (Malashkevich et al. 1996; Testa et al. 2009). Therefore, the controlling influence that selects between tetramers at least through to heptamers must reside elsewhere.

The addition of helices in classical dimers through tetramers leads to the increased inclusion of peripheral residues within the hydrophobic core of the coiled-coil assembly; notably, residues at ***g*** become knobs. These are referred to as Type I

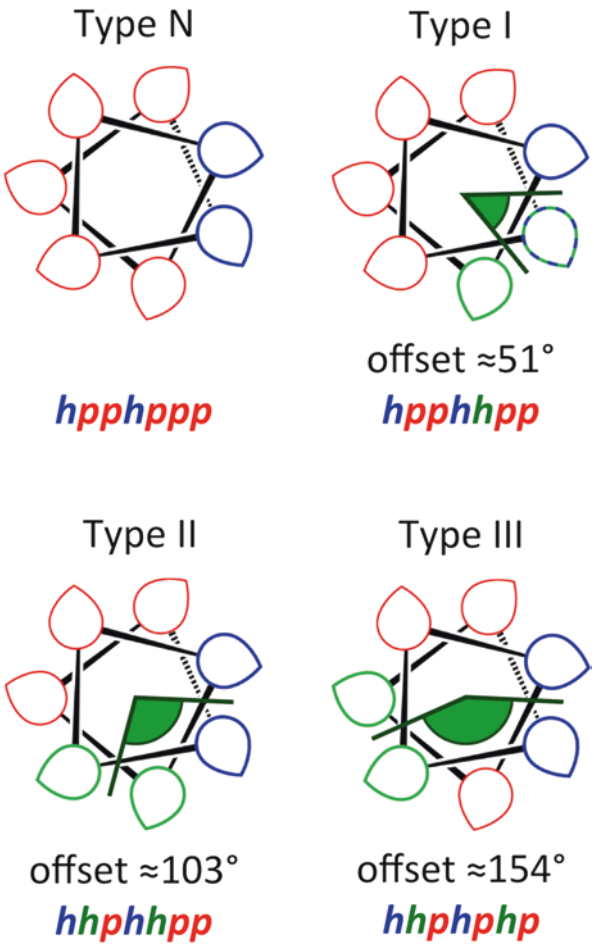

Fig. 2.7 Variations on the classical heptad repeat – different repeats lead to different ranges of oligomer states. Helical wheels for Types N, I, II and III coiled-coil repeats. *Blue* and *green* teardrops represent knob-forming and nominally hydrophobic residues; *red* teardrops represent the remaining and usually polar residues; and the *dark green* angles show the angle subtended between the two resulting hydrophobic seams of Type I – III repeats. Type N repeats are found predominantly in dimers. Based on the offset angles shown, trimers and tetramers are founded on Type I repeats; and higher-order structures on Type II (tetramer – heptamer) and Type III (octomer and above) repeats, although these are rules of thumb rather than hard-and-fast rules. The overall hydrophobic/polar sequence patterns are given below each helical wheel

interfaces, and are distinct from classical Type N interfaces, Fig. 2.7a, b, (Woolfson et al. 2012; Walshaw and Woolfson 2003). Increasing the helix-helix contacts in pentamers and above leads to Type II interfaces, in which sidechains at both ***g*** and ***e*** sites are involved in KIH interactions, Fig. 2.7c. For completeness, Type III interfaces are the final variations in this scheme, and these lead to barrels of the type observed in the 12-helix barrel of TolC, Fig. 2.7d (Koronakis et al. 2000; Walshaw and Woolfson 2001a).

Another way to consider this is that coiled-coil pentamers and above comprise two superposed dimer sequences with the two traditional ***a*** and ***d*** hydrophobic seams falling at ***e*** and ***a***, and ***d*** and ***g*** positions, respectively, Fig. 2.7c. More specifically, and with the traditional heptad nomenclature as a reference, the ***a*** and ***d*** sites are predominantly occupied by Leu and Ile residues, respectively, and the flanking ***e*** and ***g*** sites are occupied by residues that effectively fine-tune oligomer-state selection. For the smaller oligomer, CC-Pent, the flanking residues are large Ile and Glu

sidechains. In the hexameric variants, these sites are occupied by Glu or Ser at ***g*** plus Ala at ***e***. For the heptamer, both residues are Ala. In short, the smaller the volume of the sidechains at ***e*** and ***g***, the more the helix-helix-helix packing angle can open up, and the larger the barrel (Thomson et al. 2014). Baker's designed pentamer conforms to these rules of thumb, with all of the ***a*** sites Leu, the ***d*** sites combinations of Ile, Gln and Leu, and the flanking ***e*** and **g** sites combinations of Leu, Met, Ile and Glu (Huang et al. 2014), although the single β-branched residue at ***d*** is intriguing.

This section promised a heptad of helical folds, but so far has delivered only six (dimer through heptamer). Recently, we have reported a series of highly α-helical, but monomeric peptides (Baker et al. 2015). These indicate that whilst the so-called macrodipole (Hol et al. 1978) of the α-helix may exist, it is outweighed by local electrostatic effects and does not contribute noticeably to helix stability. Though this may seem somewhat of a stretch in this review, it is pertinent to coiled-coil folding and assembly. It is often heard said that parallel arrangements of the helices in coiled coils should be disfavoured over antiparallel forms because of (assumed) unfavourable interactions between adjacent helix macrodipoles in the former. However, if these macrodipoles are masked, or dominated by other forces, and do not contribute significantly to stability, then that debate should be ended.

This does, of course, raise the question: *why are there so many antiparallel pairs of helices in the CC+ database?* This seems best explained in that these tend to be parts of single-chain coiled coil-containing proteins, and as helix-loop-helix topologies. This can be seen by comparing panels A and B of Fig. 2.5. In the former, all coiled-coil structures are counted, including intramolecular coiled coils. Many of the 2-helix structures in this category will be helix-loop-helix structures. By contrast, panel B is based only on the intermolecular coiled coils, and therefore cannot be biased in this way. Indeed, this second panel indicates a much lower proportion of antiparallel dimers in particular, which we argue represent a reduced tendency of intermolecular coiled coils to form antiparallel arrangements.

2.6 Parametric and Computational Coiled-Coil Design

2.6.1 Background: Computational Methods Old and New

The application of computational methods has a long and strong history both in the analysis of natural coiled-coil sequences and structures (*vide supra*) (Lupas 1996b), and the design of *de novo* coiled coils (Harbury et al. 1998). Recent advances that build on these foundations are having a considerable impact in coiled-coil design, and it is predicted that they will increasingly do so; indeed, improved confidence, predictability and ambition in the design of coiled-coil peptides and proteins will likely only come with the increased development and application of computational methods to these problems.

A key aspect of coiled coils is that they are inherently parameterizable; indeed, they were predicted parametrically by Crick (1953a, b) rather than being discovered empirically through X-ray crystallography. Moreover, the number of parameters needed to describe them is small, at least for regular coiled-coil structures. This has led to a number of successful and useful computational resources to model coiled-coil assemblies (Offer et al. 2002; Harbury et al. 1995; Offer and Sessions 1995; Grigoryan and DeGrado 2011; Wood et al. 2014; Ramisch et al. 2015), and, more recently, to design them (Plecs et al. 2004; Harbury et al. 1998; Grigoryan et al. 2011; Huang et al. 2014; Thomson et al. 2014; Lizatovic et al. 2016). In essence, all of these build on Crick's parameterization of the coiled coil: they use the Crick equations to generate backbones, and then add various methods to (i) add sidechains to the models, (ii) assess some *in silico* energy of the generated state, and (iii) search through combinations of structural parameters and sequence space to find solutions for the design target to test experimentally.

Of particular note are two web-based and user-friendly tools—CCCP and CCBuilder (Grigoryan and DeGrado 2011; Wood et al. 2014)—which make coiled-coil modelling and design accessible to all, and expand possibilities for designing coiled-coil structures both to mimic nature and to move into the *dark matter of protein structures* (Taylor et al. 2009).

2.6.2 Parametric Coiled-Coil Designs Achieved to Date

Parametric structure-based designs have been realised before now for coiled-coil proteins, including right-handed structures that incorporate non-natural amino acids, to satisfy unusual packing in the interior of the helical assembly (Harbury et al. 1998; Plecs et al. 2004). However, CCCP, CCBuilder and methods developed in the Baker lab (Grigoryan and DeGrado 2011; Wood et al. 2014; Huang et al. 2014) now allow structure-based designs of coiled coils and helical bundles to be tackled more generally. A key feature of all of these methods is that they implicitly incorporate backbone variations, which has always been a challenge in computational protein design (Harbury et al. 1995; Harbury et al. 1998; Grigoryan and DeGrado 2011; Huang et al. 2014; Wood et al. 2014; Ramisch et al. 2015).

Grigoryan, DeGrado and co-workers have used CCCP to generate helices predicted to make barrel-like assemblies that bind carbon nanotubes (Grigoryan and DeGrado 2011). This opens potential applications requiring solubilised carbon nanotubes. Recently, the same group has applied their approach to design functional membrane-spanning helical bundles called "Rocker" (Joh et al. 2014). This is a zinc-binding four-helix bundle. The idea is that zinc ions presented to one side of the membrane are transported to the other side *via* exchange between two zinc-binding sites, and that this is facilitated both by the rocking of the structure and by the transport of protons in the opposite direction.

Baker's group has achieved computational parametric designs for hyperstable coiled coils (Huang et al. 2014). These water-soluble designs are for single-chain,

3- and 4-helix bundles, which have both parallel and antiparallel helix-helix contacts; and also a parallel 5-helix bundle, Fig. 2.6d. A key and elegant aspect of these designs is the focus on layers of hydrophobic residues that define the hydrophobic core, rather than sequence-based repeats *per se*. By considering 2-, 3- and 5-layer structures, which correspond to 7-, 11- and 18-residue sequence repeats (Hicks et al. 2002; Gruber and Lupas 2003), the X-ray crystal structures of the designs reveal a left-handed supercoil for the pentamer (4UOT), a four-helix bundle with a slight right-handed twist (4UOS), and a three-helix assembly with straight helices (4TQL) (Huang et al. 2014). In an elegant study, Baker's group have used Rosetta, supplemented with new approaches to generate hydrogen-bonded networks of side-chains *in silico*, to design and deliver a series of two-layer helical bundles incorporating intricate networks of buried polar residues (Boyken et al. 2016). Also using Rosetta-based methods, André and co-workers have implemented parametric coiled-coil modelling and scoring to design a peptide that forms a pentamer at pH 8, which surprisingly switches to a collapsed two-layer six-helix bundle (a trimer of antiparallel dimers) at pH 6 (Lizatovic et al. 2016). This last study, in particular, illustrates the complexity of the free-energy landscape for coiled-coil assemblies, and the resulting plasticity of these structures. Thus, it highlights the difficulties in the *de novo* design of coiled coils predictively and reliably.

Finally, and as introduced above, parallel pentameric, hexameric and heptameric α-helical barrels (CC-Pent, CC-Hex and CC-Hept) have all been built (Thomson et al. 2014). The new designs are based on the realization that successive helical interfaces in cyclic coiled-coil structures can be approximated as heterodimeric faces encoded within the same peptide chain, *i.e.* Type II interfaces, Fig. 2.7c. For these designs, potential sequences for the two halves of the heterodimeric interfaces are initially selected from millions of sequence combinations using a scoring algorithm developed by Keating (Fong et al. 2004). The selected sequences are then modelled as coiled-coil bundles and barrels ranging from tetramer through to octamer. The BUDE-based scoring function of CCBuilder is then used to predict the preferred oligomer state. A number of the final sequences are taken through to experimental characterisation in solution and by X-ray protein crystallography. For more than half of the peptides tested, the observed and predicted oligomer states match.

2.7 Improving Heterospecificity in Coiled-Coil Design

Although, *de novo* designs for heterodimeric coiled coils have been with us for some time (O'Shea et al. 1993; Moll et al. 2001; Litowski and Hodges 2002), there has been considerable progress over the past decade in defining and improving these systems. These principles for design and assembly will not be discussed further other than to note that early examples were mostly specified using oppositely charged residues at complementary ***g*** and ***e*** sites of the heptad repeat (Woolfson 2005), and this basic principles remains a key aspect of new designs. Recent

attention has switched to designing or selecting sets of mutually exclusive (orthogonal) heterodimer pairs (Bromley et al. 2009), and controlling specificity and stability in these systems, with Keating and colleagues leading the way here (Thompson et al. 2012; Potapov et al. 2015).

Continuing the theme of generating and populating peptide toolkits for protein engineering and synthetic biology (Bromley et al. 2008; Fletcher et al. 2012), several groups have constructed sets of heterodimeric coiled coils. For example, the aforementioned rules of thumb for coiled-coil dimers with computational methods to deliver orthogonal heterodimers (Bromley et al. 2009) have been combined, and variants made of what is called the CC-Di-AB system, with binding affinities in the μM – nM range (Thomas et al. 2013). Building on foregoing work to make a *coiled-coil interactome* (Reinke et al. 2010), Lim and Keating have introduced SYNZIPs, increasing the number of orthogonal heterodimers in one set to an impressive 27 pairs (Thompson et al. 2012). Some of these pairs have been used in synthetic biology to control gene activation in a model system. Other contributions from Keating's group include demonstrating that non-interfacial, surface-exposed residues influence binding anti-bZIP peptides designed to natural targets: specifically, that increasing the helix propensity at these sites improves binding (Kaplan et al. 2014); and, notably, the considerable advance of using knowledge-based computational methods to predict heterodimer stability (Potapov et al. 2015). This method has clear applications not only for generating highly specified coiled-coil pairings, but also in developing peptides to target and disrupt natural protein-protein interfaces. Additional sets of heterodimers have been contributed by Jerala (Gradisar and Jerala 2011), Aili (Aronsson et al. 2015), and Mason (Crooks et al. 2016).

It remains to be seen how orthogonal all of these sets are to each other. Nonetheless, they provide valuable additions to peptide toolkits for applications in protein engineering and synthetic biology. Specifically, the rigorous characterizations of some of these peptide designs, which has delivered internal orthogonality, experimentally determined K_D values, and in some cases X-ray crystal structures for a designed heterodimers (Reinke et al. 2010; Sharp et al. 2012). All of these attributes will improve the predictability of using the toolkits and the development of future designs.

Compared to this progress on heterodimers, new heterospecific systems for larger oligomers have progressed more modestly, which is perhaps understandable given the increased demands on design. As of 2005, there were designs for heterotrimers (Nautiyal et al. 1995; Nautiyal and Alber 1999; Kashiwada et al. 2000; Kiyokawa et al. 2004; Schnarr and Kennan 2002) and a heterotetramer (Fairman et al. 1996). Since then, Kennan has used polar contacts in place of previously used complementary hydrophobic sidechains (Schnarr and Kennan 2002) to make another heterotrimeric system (Diss and Kennan 2008); Fairman has extended his studies of the Lac repressor (Fairman et al. 1996) to a GCN4-p1 background, to give an alternative A_2B_2 design (Root et al. 2009); Tanaka's group have applied their IZ peptides to switch protein-complex structure and function (Mizuno et al. 2009); and a heterohexamer based on CC-Hex has been delivered (Zaccai et al. 2011).

2.8 Adding Antiparallel Coiled Coils to the Mix

The elephants in the room of coiled-coil structure and design are assemblies with antiparallel, or mixed arrangements of helices. This is apparent from Fig. 2.5, which indicates that there are many natural antiparallel structures. However, emphasis in the design community, at least as judged from publications, has been for small oligomers with parallel arrangements of helices. As noted in the 2005 review (Woolfson 2005) and Oakley's earlier review (Oakley and Hollenbeck 2001), there are notable exceptions, with successful designs of antiparallel dimers from Hodges, Chaiken, Regan, Oakley and from Woolfson (Monera et al. 1993, 1994; Myszka and Chaiken 1994; Ghosh et al. 2000; McClain et al. 2001; Gurnon et al. 2003; Pandya et al. 2004). Nonetheless, with the exception of the Coiled Serine 1cos, which came much earlier and is a 3-helix structure (Lovejoy et al. 1993), no *de novo* antiparallel coiled-coil structures had been fully verified by X-ray crystal structures.

There are signs that this is about to change. Over the past decade, there has been considerable progress in our fundamental understanding of antiparallel arrangements of helices in coiled coils, and in our abilities to model these structures (Apgar et al. 2008; Grigoryan and DeGrado 2011; Wood et al. 2014). Sequence-to-structure relationships in antiparallel coiled coils have been probed using bioinformatics for some time (Walshaw and Woolfson 2001b), using experimental approaches more recently (Hadley and Gellman 2006), and with combinations of the two (Hadley et al. 2008; Steinkruger et al. 2012a). Notably, Gellman has applied his backbone thioester exchange (BTE) method to determine energies of interaction between residue pairs or groups of three across synthetic, designed antiparallel interfaces (Hadley and Gellman 2006; Hadley et al. 2008; Steinkruger et al. 2012a). His group has also extended these studies to models for parallel dimers (Steinkruger et al. 2012b).

Negron and Keating report what should be considered the current state-of-the-art in antiparallel coiled-coil design (Negron and Keating 2014). Their paper describes the computational design and solution-phase experimental validation of *de novo* and, importantly, orthogonal antiparallel homodimers, using the group's CLASSY and DFIRE computational methods (Negron and Keating 2013, 2014). In addition, the paper puts the work in a broader context, and impressively so, by conducting deep computational analyses of the designs using aforementioned computational methods such as Rosetta, CCBuilder and LOGICOIL.

2.9 Building with Coiled-Coil Modules: Protein Origami, Synthetic Biology and Materials

The availability of defined and well-characterised coiled-coil building blocks, and indeed peptide and protein units in general, paves the way for the reliable construction of more-complex assemblies and materials (Sinclair 2013; Kocar et al. 2015;

Quinlan et al. 2015). In the language of supramolecular assembly, these might be referred to as *tectons*, and in synthetic biology as *modules* or *parts* (Bromley et al. 2008; Channon et al. 2008). As outlined below, module-based assembly brings advantages of speed, more predictable outcomes, and the exchangeability of parts to the design and construction process. Roughly in order of increasing complexity, the following have been achieved using coiled coils as tectons.

CC-Tri to direct the assembly of difficult-to-fold bacterial collagen sequences (Yoshizumi et al. 2011). The two peptides of CC-Di-AB-based heterodimer have been joined with flexible linkers to make A-linker-B systems that form different assemblies, depending on the intervening linker (Boyle et al. 2012). Short linkers give fibrous materials and large colloidal assemblies, whereas longer linkers provide flexibility, allowing closure to smaller, possibly square and triangular, assemblies. Jerala and colleagues have taken this *peptide-origami* approach an elegant step further (Gradisar et al. 2013), combining eight coiled-coil pairs in a single polypeptide chain to direct the folding of a novel tetrahedron. Whilst this assembly resists high-resolution structure determination, split-protein constructs and AFM studies are fully consistent with the design.

Kros has developed a SNARE-inspired concept for peptide-directed vesicle fusion using coiled-coil peptides (Marsden et al. 2009, 2011). Through a strong and impressive series of papers, this team has shown that the early designs for "EK" heterodimer peptides from Hodges (Litowski and Hodges 2002) can be appended with lipid anchors, inserted into two different populations of lipid vesicles, which can then be mixed to effect membrane fusion in anything from simple *in vitro* systems through to live-animal models. In addition, the peptide and lipid-based components can be varied to alter and better understand the membrane-fusion events (Versluis et al. 2013a, b; Zheng et al. 2013; Schwenen et al. 2015; Kong et al. 2016; Mora et al. 2016; Zheng et al. 2016).

In the context of more-traditional synthetic biology, as mentioned above, Lim and Keating have used designer coiled coils to control transcription (Thompson et al. 2012); CC-D-AB systems with variable association constants (Thomas et al. 2013) are being used to similar effect (Smith, Savery and Woolfson, unpublished); and Swainsbury and Jones have used the CC-Di, CC-Tri and CC-Tet to bring together parts of the photosynthetic reaction centre in membranes (unpublished).

Moving onto peptide-based materials:

First, although there has been considerable progress in the development and application of coiled coil-based fibrous materials and hydrogels (Dong et al. 2008; Banwell et al. 2009; Sharp et al. 2012) this is a heavily reviewed area (Boyle and Woolfson 2011; Dasgupta et al. 2013; Woolfson and Mahmoud 2010), and will not be dwelt on further here.

Conticello has demonstrated that coiled coils can be used to make peptide nanotubes (PNTs) (Xu et al. 2013). Specifically, these assemblies use the aforementioned GCN4-based heptamer (Liu et al. 2006a), which has spiralling "stepped ends" that facilitate end-to-end assembly, in what Conticello refers to as a "lock-

washer" model. His redesign promotes assembly further, with oppositely charged groups and partly exposed hydrophobic surfaces at the termini. Inspired by this, the coiled-coil toolkit, with the exception of CC-Di—*i.e.*, the CC-Tri through CC-Hept structures—can be engineered to make peptide fibres (for CC-Tri and a variant of CC-Tet) and PNTs (CC-Pent, CC-Hex and CC-Hept) (Burgess et al. 2015). However, in these cases, the redesigned coiled coils can be blunt-ended, apparently negating the needed for ragged ends. The CC-Hex-based assemblies are particularly well ordered, allowing detailed structural studies by cryoTEM (Burgess et al. 2015). Both studies show that the PNTs can bind long-aspect ratio dyes within their lumens (Xu et al. 2013; Burgess et al. 2015), and it has been demonstrated that tubes corresponding to a single coiled-coil hexamer in diameter can be engineered (Thomas et al. 2016). Between these pieces of work, Montclare described fibres made from variants of the natural pentameric COMP, that also bind small molecules (Hume et al. 2014). These new materials hold promise for the construction of materials that could sequester and/or deliver appropriately sized small molecules.

On a related theme, Conticello and Egelman have shown that larger-diameter tubes of designed coiled coils, possibly comprising sheet-like assemblies, can be made and probed to high resolution using electron microscopy (Egelman et al. 2015). This is somewhat related to the aforementioned studies of Grigoryan and DeGrado on the decoration of carbon nanotubes with computationally designed amphipathic coiled-coil peptides (Grigoryan et al. 2011).

More complex still, a concept for Self-Assembled peptide caGEs (SAGEs) using the coiled-coil basis set has been developed as follows (Fletcher et al. 2013): first, two hubs are created (Hub-A and Hub-B). Each of these has a central CC-Tri module, with one of the outer ***f*** positions of each peptide functionalised as cysteine. The latter are used to couple either CC-Di-A or CC-Di-B, which have corresponding ***f*** sites as Cys, via disulphide bonds. In solution, these hubs behave as discrete and partly folded trimeric units. When mixed, the two hubs combine to form peptide arrays, which are assumed to be hexagonal, and then fold over and close to make spherical objects of approximately 100 nm diameter. Biophysical and microscopic analyses, and computational atomistic modelling conducted thus far indicate that the SAGEs are hollow, unilamellar, cage-like objects with defined inner and outer surfaces. They provide a potential platform for developing functional designs for the active encapsulation and delivery of drugs and biologics, and for the presentation of multiple antigenic peptides and proteins. Related to this, Marsh has constructed smaller cage-like protein objects by combining symmetry axes of coiled-coil units and natural proteins (Patterson et al. 2014). This is also reminiscent of the broader area of what might be termed directed assembly of protein-based objects, lattices and materials first proposed by Padilla and Yeates (Padilla et al. 2001), and more recently developed by Sinclair and Noble (Sinclair et al. 2011) and Yeates and Baker (King et al. 2012, 2014; Bale et al. 2015), which has been reviewed by others (Sinclair 2013; Kocar et al. 2015; Norn and Andre 2016).

2.10 Concluding Remarks

The initial aim for this chapter was to provide a comprehensive update to my 2005 review on coiled-coil design (Woolfson 2005). However, topics not covered in detail, or indeed not covered at all, include: the design and application of coiled coil-based fibrous materials (Woolfson and Mahmoud 2010; Boyle and Woolfson 2011; Dasgupta et al. 2013); the incorporation of functional metal sites into coiled-coil scaffolds (Mocny and Pecoraro 2015; Tebo and Pecoraro 2015; Slope and Peacock 2016); and the development of dynamic coiled-coil systems, such as switches and self-replicating peptides (Ambroggio and Kuhlman 2006; Pagel and Koksch 2008; Wagner and Ashkenasy 2009; Bromley and Channon 2011). These topics are covered superbly well by others in the reviews cited in the preceding sentences. What this chapter does highlight, however, is that the field of coiled-coil design is brimming with scope and ideas, and that there is the talent to realise the many ambitions that come with these. If the advances that have been made over the past decade or so are anything to judge by, the future of field is looking very rosy indeed.

On this, some speculation is justified. It is anticipated that rational designs for much more-complex coiled-coil architectures will occur in the near future—that is, there will be designed assemblies with more helices, more layers, different orientations of helices, bigger barrels, and multi-component materials. It is also envisaged that useful functions will be incorporated, including specified binding sites and enzyme-like activities, into these *de novo* coiled-coil folds (Burton and Woolfson, 2016); and more-dynamic designs, with coiled coils that can switch state on cue in response to particular stimulus, are foreseen (Lizatovic et al. 2016). The hope is that all of this is done in a collaborative and increasingly computational-based environment, where software is developed to meet challenges in both basic and applied science. For instance, an ability to not only predict the most likely structure for a designed coiled-coil sequence, but also its free energy with more confidence—or at least a free energy difference from the nearest off-target design—would be extremely useful; as would computational coiled-coil and, more generally, protein-design tools that are accessible to non-experts, for example to synthetic biologists, who will have different ambitions than structural biologists.

Acknowledgements I thank Gail Bartlett, James Ross and Drew Thomson for help with making some of the figures. I would like to thank current and past members of my research group who have added, both knowingly or unwittingly, to my understanding and knowledge of coiled-coil folding, assembly and design. I am grateful to the European Research Council (340764) for funding, and to the Royal Society and the Wolfson Foundation for the gift of a Royal Society Wolfson Research Merit Award.

References

Ambroggio XI, Kuhlman B (2006) Design of protein conformational switches. Curr Opin Struct Biol 16(4):525–530

Apgar JR, Gutwin KN, Keating AE (2008) Predicting helix orientation for coiled-coil dimers. Proteins 72(3):1048–1065

Armstrong CT, Vincent TL, Green PJ, Woolfson DN (2011) SCORER 2.0: an algorithm for distinguishing parallel dimeric and trimeric coiled-coil sequences. Bioinformatics 27(14):1908–1914

Aronsson C, Danmark S, Zhou F, Oberg P, Enander K, Su H, Aili D (2015) Self-sorting heterodimeric coiled coil peptides with defined and tuneable self-assembly properties. Sci Rep:5

Baker EG, Bartlett GJ, Crump MP, Sessions RB, Linden N, Faul CFJ, Woolfson DN (2015) Local and macroscopic electrostatic interactions in single alpha-helices. Nat Chem Biol 11(3):221–228

Bale JB, Park RU, Liu YX, Gonen S, Gonen T, Cascio D, King NP, Yeates TO, Baker D (2015) Structure of a designed tetrahedral protein assembly variant engineered to have improved soluble expression. Protein Sci 24(10):1695–1701

Banwell EF, Abelardo ES, Adams DJ, Birchall MA, Corrigan A, Donald AM, Kirkland M, Serpell LC, Butler MF, Woolfson DN (2009) Rational design and application of responsive alpha-helical peptide hydrogels. Nat Mater 8(7):596–600

Boyken SE, Chen Z, Groves B, Langan RA, Oberdorfer G, Ford A, Gilmore JM, Xu C, DiMaio F, Pereira JH, Sankaran B, Seelig G, Zwart PH, Baker D (2016) De novo design of protein homo-oligomers with modular hydrogen-bond network-mediated specificity. Science 352(6286):680–687

Boyle AL, Woolfson DN (2011) De novo designed peptides for biological applications. Chem Soc Rev 40(8):4295–4306

Boyle AL, Bromley EHC, Bartlett GJ, Sessions RB, Sharp TH, Williams CL, Curmi PMG, Forde NR, Linke H, Woolfson DN (2012) Squaring the circle in peptide assembly: from fibers to discrete nanostructures by de novo design. J Am Chem Soc 134(37):15457–15467

Bromley EHC, Channon KJ (2011) Alpha-helical peptide assemblies: giving new function to designed structures. Prog Mol Biol Transl Sci 103:231–275

Bromley EHC, Channon K, Moutevelis E, Woolfson DN (2008) Peptide and protein building blocks for synthetic biology: from programming biomolecules to self-organized biomolecular systems. ACS Chem Biol 3(1):38–50

Bromley EHC, Sessions RB, Thomson AR, Woolfson DN (2009) Designed alpha-helical tectons for constructing multicomponent synthetic biological systems. J Am Chem Soc 131(3):928–930

Brown JH, Cohen C, Parry DAD (1996) Heptad breaks in alpha-helical coiled coils: stutters and stammers. Proteins 26(2):134–145

Burgess NC, Sharp TH, Thomas F, Wood CW, Thomson AR, Zaccai NR, Brady RL, Serpell LC, Woolfson DN (2015) Modular design of self-assembling peptide-based nanotubes. J Am Chem Soc 137(33):10554–10562

Burton AJ, Thomas F, Agnew C, Hudson KL, Halford SE, Brady RL, Woolfson DN (2013) Accessibility, reactivity, and selectivity of side chains within a channel of de novo peptide assembly. J Am Chem Soc 135(34):12524–12527

Burton AJ, Thomson AR, Dawson WM, Brady RL, Woolfson DN (2016) Installing hydrolytic activity into a completely de novo protein framework. Nat Chem 8(9):837–844.

Channon K, Bromley EHC, Woolfson DN (2008) Synthetic biology through biomolecular design and engineering. Curr Opin Struct Biol 18(4):491–498

Chothia C, Levitt M, Richardson D (1981) Helix to helix packing in proteins. J Mol Biol 145(1):215–250

Crick FHC (1953a) The Fourier transform of a coiled-coil. Acta Crystallogr 6(8-9):685–689

Crick FHC (1953b) The packing of alpha-helices – simple coiled-coils. Acta Crystallogr 6(8-9):689–697
Crooks RO, Baxter D, Panek AS, Lubben AT, Mason JM (2016) Deriving heterospecific self-assembling protein-protein interactions using a computational interactome screen. J Mol Biol 428(2):385–398
Dasgupta A, Mondal JH, Das D (2013) Peptide hydrogels. RSC Adv 3(24):9117–9149
Davey JA, Chica RA (2012) Multistate approaches in computational protein design. Protein Sci 21(9):1241–1252
Delorenzi M, Speed T (2002) An HMM model for coiled-coil domains and a comparison with PSSM-based predictions. Bioinformatics 18(4):617–625
Diss ML, Kennan AJ (2008) Heterotrimeric coiled coils with core residue urea side chains. J Org Chem 73(24):9752–9755
Dong CJ, Beis K, Nesper J, Brunkan-LaMontagne AL, Clarke BR, Whitfield C, Naismith JH (2006) Wza the translocon for E-coli capsular polysaccharides defines a new class of membrane protein. Nature 444(7116):226–229
Dong H, Paramonov SE, Hartgerink JD (2008) Self-assembly of alpha-helical coiled coil nanofibers. J Am Chem Soc 130(41):13691–13695
Egelman EH, Xu C, DiMaio F, Magnotti E, Modlin C, Yu X, Wright E, Baker D, Conticello VP (2015) Structural plasticity of helical nanotubes based on coiled-coil assemblies. Structure 23(2):280–289
Eshaghi S, Niegowski D, Kohl A, Molina DM, Lesley SA, Nordlund P (2006) Crystal structure of a divalent metal ion transporter CorA at 2.9 angstrom resolution. Science 313(5785):354–357
Fairman R, Chao HG, Lavoie TB, Villafranca JJ, Matsueda GR, Novotny J (1996) Design of heterotetrameric coiled coils: evidence for increased stabilization by Glu(-)-Lys(+) ion pair interactions. Biochemistry 35(9):2824–2829
Feldmeier K, Hocker B (2013) Computational protein design of ligand binding and catalysis. Curr Opin Chem Biol 17(6):929–933
Fletcher JM, Boyle AL, Bruning M, Bartlett GJ, Vincent TL, Zaccai NR, Armstrong CT, Bromley EHC, Booth PJ, Brady RL, Thomson AR, Woolfson DN (2012) A basis set of de novo coiled-coil peptide oligomers for rational protein design and synthetic biology. ACS Synth Biol 1(6):240–250
Fletcher JM, Harniman RL, Barnes FRH, Boyle AL, Collins A, Mantell J, Sharp TH, Antognozzi M, Booth PJ, Linden N, Miles MJ, Sessions RB, Verkade P, Woolfson DN (2013) Self-assembling cages from coiled-coil peptide modules. Science 340(6132):595–599
Fong JH, Keating AE, Singh M (2004) Predicting specificity in bZIP coiled-coil protein interactions. Genome Biol 5(2): R11
Ghirlanda G, Lear JD, Ogihara NL, Eisenberg D, DeGrado WF (2002) A hierarchic approach to the design of hexameric helical barrels. J Mol Biol 319(1):243–253
Ghosh I, Hamilton AD, Regan L (2000) Antiparallel leucine zipper-directed protein reassembly: application to the green fluorescent protein. J Am Chem Soc 122(23):5658–5659
Gonzalez L, Woolfson DN, Alber T (1996) Buried polar residues and structural specificity in the GCN4 leucine zipper. Nat Struct Biol 3(12):1011–1018
Gradisar H, Jerala R (2011) De novo design of orthogonal peptide pairs forming parallel coiled-coil heterodimers. J Pept Sci 17(2):100–106
Gradisar H, Bozic S, Doles T, Vengust D, Hafner-Bratkovic I, Mertelj A, Webb B, Sali A, Klavzar S, Jerala R (2013) Design of a single-chain polypeptide tetrahedron assembled from coiled-coil segments. Nat Chem Biol 9(6):362–366
Grigoryan G, DeGrado WF (2011) Probing designability via a generalized model of helical bundle geometry. J Mol Biol 405(4):1079–1100
Grigoryan G, Kim YH, Acharya R, Axelrod K, Jain RM, Willis L, Drndic M, Kikkawa JM, DeGrado WF (2011) Computational design of virus-like protein assemblies on carbon nanotube surfaces. Science 332(6033):1071–1076

Gruber M, Lupas AN (2003) Historical review: another 50th anniversary – new periodicities in coiled coils. Trends Biochem Sci 28(12):679–685

Gurnon DG, Whitaker JA, Oakley MG (2003) Design and characterization of a homodimeric antiparallel coiled coil. J Am Chem Soc 125(25):7518–7519

Hadley EB, Gellman SH (2006) An antiparallel alpha-helical coiled-coil model system for rapid assessment of side-chain recognition at the hydrophobic interface. J Am Chem Soc 128(51):16444–16445

Hadley EB, Testa OD, Woolfson DN, Gellman SH (2008) Preferred side-chain constellations at antiparallel coiled-coil interfaces. Proc Natl Acad Sci U S A 105(2):530–535

Harbury PB, Zhang T, Kim PS, Alber T (1993) A switch between 2-stranded, 3-stranded and 4-stranded coiled coils in Gcn4 leucine-zipper mutants. Science 262(5138):1401–1407

Harbury PB, Kim PS, Alber T (1994) Crystal-structure of an isoleucine-zipper trimer. Nature 371(6492):80–83

Harbury PB, Tidor B, Kim PS (1995) Repacking protein cores with backbone freedom – structure prediction for coiled coils. Proc Natl Acad Sci U S A 92(18):8408–8412

Harbury PB, Plecs JJ, Tidor B, Alber T, Kim PS (1998) High-resolution protein design with backbone freedom. Science 282(5393):1462–1467

Hartmann MD, Ridderbusch O, Zeth K, Albrecht R, Testa O, Woolfson DN, Sauer G, Dunin-Horkawicz S, Lupas AN, Alvarez BH (2009) A coiled-coil motif that sequesters ions to the hydrophobic core. Proc Natl Acad Sci U S A 106(40):16950–16955

Hicks MR, Holberton DV, Kowalczyk C, Woolfson DN (1997) Coiled-coil assembly by peptides with non-heptad sequence motifs. Fold Des 2(3):149–158

Hicks MR, Walshaw J, Woolfson DN (2002) Investigating the tolerance of coiled-coil peptides to nonheptad sequence inserts. J Struct Biol 137(1-2):73–81

Hol WGJ, Vanduijnen PT, Berendsen HJC (1978) Alpha-helix dipole and properties of proteins. Nature 273(5662):443–446

Huang PS, Oberdorfer G, Xu CF, Pei XY, Nannenga BL, Rogers JM, DiMaio F, Gonen T, Luisi B, Baker D (2014) High thermodynamic stability of parametrically designed helical bundles. Science 346(6208):481–485

Hume J, Sun J, Jacquet R, Renfrew PD, Martin JA, Bonneau R, Gilchrist ML, Montclare JK (2014) Engineered coiled-coil protein microfibers. Biomacromolecules 15(10):3503–3510

Joh NH, Wang T, Bhate MP, Acharya R, Wu YB, Grabe M, Hong M, Grigoryan G, DeGrado WF (2014) De novo design of a transmembrane Zn2+-transporting four-helix bundle. Science 346(6216):1520-1524.

Kaplan JB, Reinke AW, Keating AE (2014) Increasing the affinity of selective bZIP-binding peptides through surface residue redesign. Protein Sci 23(7):940–953

Kashiwada A, Hiroaki H, Kohda D, Nango M, Tanaka T (2000) Design of a heterotrimeric alpha-helical bundle by hydrophobic core engineering. J Am Chem Soc 122(2):212–215

King NP, Sheffler W, Sawaya MR, Vollmar BS, Sumida JP, Andre I, Gonen T, Yeates TO, Baker D (2012) Computational design of self-assembling protein nanomaterials with atomic level accuracy. Science 336(6085):1171–1174

King NP, Bale JB, Sheffler W, McNamara DE, Gonen S, Gonen T, Yeates TO, Baker D (2014) Accurate design of co-assembling multi-component protein nanomaterials. Nature 510(7503):103–108

Kiyokawa T, Kanaori K, Tajima K, Kawaguchi M, Mizuno T, Oku JI, Tanaka T (2004) Selective formation of AAB- and ABC-type heterotrimeric alpha-helical coiled coils. Chem Eur J 10(14):3548–3554

Kocar V, Abram SB, Doles T, Basic N, Gradisar H, Pisanski T, Jerala R (2015) TOPOFOLD, the designed modular biomolecular folds: polypeptide-based molecular origami nanostructures following the footsteps of DNA. Wiley Interdiscip Rev Nanomed Nanobiotechnol 7(2):218–237

Kong L, Askes SHC, Bonnet S, Kros A, Campbell F (2016) Temporal control of membrane fusion through photolabile PEGylation of liposome membranes. Angew Chem Int Ed 55(4):1396–1400

Koronakis V, Sharff A, Koronakis E, Luisi B, Hughes C (2000) Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. Nature 405(6789):914–919

Litowski JR, Hodges RS (2002) Designing heterodimeric two-stranded alpha-helical coiled-coils – effects of hydrophobicity and alpha-helical propensity on protein folding, stability, and specificity. J Biol Chem 277(40):37272–37279

Liu J, Zheng Q, Deng Y, Cheng C-S, Kallenbach NR, Lu M (2006a) A seven-helix coiled coil. Proc Natl Acad Sci U S A 103(42):15457–15462

Liu J, Zheng Q, Deng YQ, Kallenbach NR, Lu M (2006b) Conformational transition between four and five-stranded phenylalanine zippers determined by a local packing interaction. J Mol Biol 361(1):168–179

Lizatovic R, Aurelius O, Stenstrom O, Drakenberg T, Akke M, Logan DT, Andre I (2016) A de novo designed coiled-coil peptide with a reversible pH-induced oligomerization switch.

Lovejoy B, Choe S, Cascio D, McRorie DK, Degrado WF, Eisenberg D (1993) Crystal-structure of a synthetic triple-stranded alpha-helical bundle. Science 259(5099):1288–1293

Lumb KJ, Kim PS (1998) A buried polar interaction imparts structural uniqueness in a designed heterodimeric coiled coil (vol 34, pg 8642, 1995). Biochemistry 37(37):13042–13042

Lupas A (1996a) Coiled coils: new structures and new functions. Trends Biochem Sci 21(10):375–382

Lupas A (1996b) Prediction and analysis of coiled-coil structures. Methods Enzymol 266:513–525

Lupas AN, Gruber M (2005) The structure of alpha-helical coiled coils. Adv Protein Chem 70:37–78

Lupas A, Vandyke M, Stock J (1991) Predicting coiled coils from protein sequences. Science 252(5009):1162–1164

Magliery TJ (2015) Protein stability: computation, sequence statistics, and new experimental methods. Curr Opin Struct Biol 33:161–168

Malashkevich VN, Kammerer RA, Efimov VP, Schulthess T, Engel J (1996) The crystal structure of a five-stranded coiled coil in COMP: a prototype ion channel? Science 274(5288):761–765

Marsden HR, Elbers NA, Bomans PHH, Sommerdijk NAJM, Kros A (2009) A reduced SNARE model for membrane fusion. Angew Chem Int Ed 48(13):2330–2333

Marsden HR, Tomatsu I, Kros A (2011) Model systems for membrane fusion. Chem Soc Rev 40(3):1572–1585

McClain DL, Woods HL, Oakley MG (2001) Design and characterization of a heterodimeric coiled coil that forms exclusively with an antiparallel relative helix orientation. J Am Chem Soc 123(13):3151–3152

McDonnell AV, Jiang T, Keating AE, Berger B (2006) Paircoil2: improved prediction of coiled coils from sequence. Bioinformatics 22(3):356–358

Mizuno T, Suzuki K, Imai T, Kitade Y, Furutani Y, Kudou M, Oda M, Kandori H, Tsumoto K, Tanaka T (2009) Manipulation of protein-complex function by using an engineered heterotrimeric coiled-coil switch. Org Biomol Chem 7(15):3102–3111

Mocny CS, Pecoraro VL (2015) De novo protein design as a methodology for synthetic bioinorganic chemistry. Acc Chem Res 48(8):2388–2396

Moll JR, Ruvinov SB, Pastan I, Vinson C (2001) Designed heterodimerizing leucine zippers with a range of pIs and stabilities up to 10(-15) M. Protein Sci 10(3):649–655

Monera OD, Zhou NE, Kay CM, Hodges RS (1993) Comparison of antiparallel and parallel 2-stranded alpha-helical coiled-coils – design, synthesis, and characterization. J Biol Chem 268(26):19218–19227

Monera OD, Kay CM, Hodges RS (1994) Electrostatic interactions control the parallel and antiparallel orientation of alpha-helical chains in 2-stranded alpha-helical coiled-coils. Biochemistry 33(13):3862–3871

Mora NL, Bahreman A, Valkenier H, Li HY, Sharp TH, Sheppard DN, Davis AP, Kros A (2016) Targeted anion transporter delivery by coiled-coil driven membrane fusion. Chem Sci 7(3):1768–1772

Moutevelis E, Woolfson DN (2009) A periodic table of coiled-coil protein structures. J Mol Biol 385(3):726–732
Myszka DG, Chaiken IM (1994) Design and characterization of an intramolecular antiparallel coiled-coil peptide. Biochemistry 33(9):2363–2372
Nautiyal S, Alber T (1999) Crystal structure of a designed, thermostable; heterotrimeric coiled coil. Protein Sci 8(1):84–90
Nautiyal S, Woolfson DN, King DS, Alber T (1995) A designed heterotrimeric coiled-coil. Biochemistry 34(37):11645–11651
Negron C, Keating AE (2013) Multistate protein design using CLEVER and CLASSY. Methods Enzymol 523:171–190
Negron C, Keating AE (2014) A set of computationally designed orthogonal antiparallel homodimers that expands the synthetic coiled-coil toolkit. J Am Chem Soc 136(47):16544–16556
Norn CH, Andre I (2016) Computational design of protein self-assembly. Curr Opin Struct Biol 39:39–45
Oakley MG, Hollenbeck JJ (2001) The design of antiparallel coiled coils. Curr Opin Struct Biol 11(4):450–457
Offer G, Sessions R (1995) Computer modeling of the alpha-helical coiled-coil – packing of side-chains in the inner-core. J Mol Biol 249(5):967–987
Offer G, Hicks MR, Woolfson DN (2002) Generalized Crick equations for modeling noncanonical coiled coils. J Struct Biol 137(1-2):41–53
Ogihara NL, Weiss MS, Degrado WF, Eisenberg D (1997) The crystal structure of the designed trimeric coiled coil coil-V(a)L(d): implications for engineering crystals and supramolecular assemblies. Protein Sci 6(1):80–88
Oshaben KM, Salari R, McCaslin DR, Chong LT, Horne WS (2012) The native GCN4 leucine-zipper domain does not uniquely specify a dimeric oligomerization state. Biochemistry 51(47):9581–9591
O'Shea EK, Klemm JD, Kim PS, Alber T (1991) X-ray structure of the Gcn4 leucine zipper, a 2-stranded, parallel coiled coil. Science 254(5031):539–544
O'Shea EK, Lumb KJ, Kim PS (1993) Peptide velcro – design of a heterodimeric coiled-coil. Curr Biol 3(10):658–667
Padilla JE, Colovos C, Yeates TO (2001) Nanohedra: using symmetry to design self assembling protein cages, layers, crystals, and filaments. Proc Natl Acad Sci U S A 98(5):2217–2221
Pagel K, Koksch B (2008) Following polypeptide folding and assembly with conformational switches. Curr Opin Chem Biol 12(6):730–739
Pandya MJ, Cerasoli E, Joseph A, Stoneman RG, Waite E, Woolfson DN (2004) Sequence and structural duality: designing peptides to adopt two stable conformations. J Am Chem Soc 126(51):17016–17024
Patterson DP, Su M, Franzmann TM, Sciore A, Skiniotis G, Marsh ENG (2014) Characterization of a highly flexible self-assembling protein system designed to form nanocages. Protein Sci 23(2):190–199
Payandeh J, Pai EF (2006) A structural basis for Mg2+ homeostasis and the CorA translocation cycle. EMBO J 25(16):3762–3773
Plecs JJ, Harbury PB, Kim PS, Alber T (2004) Structural test of the parameterized-backbone method for protein design. J Mol Biol 342(1):289–297
Potapov V, Kaplan JB, Keating AE (2015) Data-driven prediction and design of bZIP coiled-coil interactions. PLoS Comput Biol 11(2) UNSP e1004046
Quinlan RA, Bromley EH, Pohl E (2015) A silk purse from a sow's ear – bioinspired materials based on alpha-helical coiled coils. Curr Opin Cell Biol 32:131–137
Rackham OJL, Madera M, Armstrong CT, Vincent TL, Woolfson DN, Gough J (2010) The evolution and structure prediction of coiled coils across all genomes. J Mol Biol 403(3):480–493
Ramisch S, Lizatovic R, Andre I (2015) Exploring alternate states and oligomerization preferences of coiled-coils by de novo structure modeling. Proteins 83(2):235–247
Regan L, Caballero D, Hinrichsen MR, Virrueta A, Williams DM, O'Hern CS (2015) Protein design: past, present, and future. Biopolymers 104(4):334–350

Reinke AW, Grant RA, Keating AE (2010) A synthetic coiled-coil interactome provides heterospecific modules for molecular engineering. J Am Chem Soc 132(17):6025–6031
Root BC, Pellegrino LD, Crawford ED, Kokona B, Fairman R (2009) Design of a heterotetrameric coiled coil. Protein Sci 18(2):329–336
Rose PW, Prlic A, Bi CX, Bluhm WF, Christie CH, Dutta S, Green RK, Goodsell DS, Westbrook JD, Woo J, Young J, Zardecki C, Berman HM, Bourne PE, Burley SK (2015) The RCSB Protein Data Bank: views of structural biology for basic and applied research and education. Nucleic Acids Res 43(D1):D345–D356
Sastri NP, Viskovska M, Hyser JM, Tanner MR, Horton LB, Sankaran B, Prasad BVV, Estes MK (2014) Structural plasticity of the coiled-coil domain of rotavirus NSP4. J Virol 88(23):13602–13612
Schnarr NA, Kennan AJ (2002) Peptide tic-tac-toe: heterotrimeric coiled-coil specificity from steric matching of multiple hydrophobic side chains. J Am Chem Soc 124(33):9779–9783
Schwenen LLG, Hubrich R, Milovanovic D, Geil B, Yang J, Kros A, Jahn R, Steinem C (2015) Resolving single membrane fusion events on planar pore-spanning membranes. Sci Rep 5
Sharp TH, Bruning M, Mantell J, Sessions RB, Thomson AR, Zaccai NR, Brady RL, Verkade P, Woolfson DN (2012) Cryo-transmission electron microscopy structure of a gigadalton peptide fiber of de novo design. Proc Natl Acad Sci U S A 109(33):13266–13271
Sinclair JC (2013) Constructing arrays of proteins. Curr Opin Chem Biol 17(6):946–951
Sinclair JC, Davies KM, Venien-Bryan C, Noble MEM (2011) Generation of protein lattices by fusing proteins with matching rotational symmetry. Nat Nanotechnol 6(9):558–562
Slope LN, Peacock AFA (2016) De novo design of xeno-metallo coiled coils. Chem Asian J 11(5):660–666
Steinkruger JD, Bartlett GJ, Hadley EB, Fay L, Woolfson DN, Gellman SH (2012a) The d′-d-d′ vertical triad is less discriminating than the a′-a-a′ vertical triad in the antiparallel coiled-coil dimer motif. J Am Chem Soc 134(5):2626–2633
Steinkruger JD, Bartlett GJ, Woolfson DN, Gellman SH (2012b) Strong contributions from vertical triads to helix-partner preferences in parallel coiled coils. J Am Chem Soc 134(38):15652–15655
Strelkov SV, Burkhard P (2002) Analysis of alpha-helical coiled coils with the program TWISTER reveals a structural mechanism for stutter compensation. J Struct Biol 137(1-2):54–64
Sun L, Young LN, Zhang XZ, Boudko SP, Fokine A, Zbornik E, Roznowski AP, Molineux IJ, Rossmann MG, Fane BA (2014) Icosahedral bacteriophage Phi X174 forms a tail for DNA transport during infection. Nature 505(7483):432–435
Surkont J, Diekmann Y, Ryder PV, Pereira-Leal JB (2015) Coiled-coil length: size does matter. Proteins 83(12):2162–2169
Tanaka Y, Sasaki T, Kumagai I, Yasutake Y, Yao M, Tanaka I, Tsumoto K (2007) Molecular properties of two proteins homologous to PduO-Type ATP: cob(I)alamin adenosyltransferase from Sulfolobus tokodaii. Proteins 68(2):446–457
Taylor WR, Chelliah V, Hollup SM, MacDonald JT, Jonassen I (2009) Probing the "Dark Matter" of protein fold space. Structure 17(9):1244–1252
Tebo AG, Pecoraro VL (2015) Artificial metalloenzymes derived from three-helix bundles. Curr Opin Chem Biol 25:65–70
Testa OD, Moutevelis E, Woolfson DN (2009) CC plus: a relational database of coiled-coil structures. Nucleic Acids Res 37:D315-D322.
Thomas F, Boyle AL, Burton AJ, Woolfson DN (2013) A set of de novo designed parallel heterodimeric coiled coils with quantified dissociation constants in the micromolar to sub-nanomolar regime. J Am Chem Soc 135(13):5161–5166
Thomas F, Burgess NC, Thomson AR, Woolfson DN (2016) Controlling the assembly of coiled-coil peptide nanotubes. Angew Chem Int Ed 55(3):987–991
Thompson KE, Bashor CJ, Lim WA, Keating AE (2012) SYNZIP protein interaction toolbox: in vitro and in vivo specifications of heterospecific coiled-coil interaction domains. ACS Synth Biol 1(4):118–129

Thomson AR, Wood CW, Burton AJ, Bartlett GJ, Sessions RB, Brady RL, Woolfson DN (2014) Computational design of water-soluble alpha-helical barrels. Science 346(6208):485–488
Trigg J, Gutwin K, Keating AE, Berger B (2011) Multicoil2: predicting coiled coils and their oligomerization states from sequence in the twilight zone. PLoS One 6(8) e23519
Versluis F, Dominguez J, Voskuhl J, Kros A (2013a) Coiled-coil driven membrane fusion: zipper-like vs. non-zipper-like peptide orientation. Faraday Discuss 166:349–359
Versluis F, Voskuhl J, van Kolck B, Zope H, Bremmer M, Albregtse T, Kros A (2013b) In situ modification of plain liposomes with lipidated coiled coil forming peptides induces membrane fusion. J Am Chem Soc 135(21):8057–8062
Vincent TL, Green PJ, Woolfson DN (2013) LOGICOIL-multi-state prediction of coiled-coil oligomeric state. Bioinformatics 29(1):69–76
Vostrikov VV, Mote KR, Verardi R, Veglia G (2013) Structural dynamics and topology of phosphorylated phospholamban homopentamer reveal its role in the regulation of calcium transport. Structure 21(12):2119–2130
Wagner N, Ashkenasy G (2009) Systems chemistry: logic gates, arithmetic units, and network motifs in small networks. Chem-Eur J 15(7):1765–1775
Walshaw J, Woolfson DN (2001a) Open-and-shut cases in coiled-coil assembly: alpha-sheets and alpha-cylinders. Protein Sci 10(3):668–673
Walshaw J, Woolfson DN (2001b) SOCKET: a program for identifying and analysing coiled-coil motifs within protein structures. J Mol Biol 307(5):1427–1450
Walshaw J, Woolfson DN (2003) Extended knobs-into-holes packing in classical and complex coiled-coil assemblies. J Struct Biol 144(3):349–361
Walther D, Eisenhaber F, Argos P (1996) Principles of helix-helix packing in proteins: the helical lattice superposition model. J Mol Biol 255(3):536–553
Wood CW, Bruning M, Ibarra AA, Bartlett GJ, Thomson AR, Sessions RB, Brady RL, Woolfson DN (2014) CCBuilder: an interactive web-based tool for building, designing and assessing coiled-coil protein assemblies. Bioinformatics 30(21):3029–3035
Woolfson DN (2005) The design of coiled-coil structures and assemblies. Adv Protein Chem 70:79–112
Woolfson DN, Alber T (1995) Predicting oligomerization states of coiled coils. Protein Sci 4(8):1596–1607
Woolfson DN, Mahmoud ZN (2010) More than just bare scaffolds: towards multi-component and decorated fibrous biomaterials. Chem Soc Rev 39(9):3464–3479
Woolfson DN, Bartlett GJ, Bruning M, Thomson AR (2012) New currency for old rope: from coiled-coil assemblies to alpha-helical barrels. Curr Opin Struct Biol 22(4):432–441
Woolfson DN, Bartlett GJ, Burton AJ, Heal JW, Niitsu A, Thomson AR, Wood CW (2015) De novo protein design: how do we expand into the universe of possible protein structures? Curr Opin Struct Biol 33:16–26
Xu C, Liu R, Mehta AK, Guerrero-Ferreira RC, Wright ER, Dunin-Horkawicz S, Morris K, Serpell LC, Zuo X, Wall JS, Conticello VP (2013) Rational design of helical nanotubes from self-aof coiled-coil lock washers. J Am Chem Soc 135(41):15565–15578
Yoshizumi A, Fletcher JM, Yu ZX, Persikov AV, Bartlett GJ, Boyle AL, Vincent TL, Woolfson DN, Brodsky B (2011) Designed coiled coils promote folding of a recombinant bacterial collagen. J Biol Chem 286(20):17512–17520
Zaccai NR, Chi B, Thomson AR, Boyle AL, Bartlett GJ, Bruning M, Linden N, Sessions RB, Booth PJ, Brady RL, Woolfson DN (2011) A de novo peptide hexamer with a mutable channel. Nat Chem Biol 7(12):935–941
Zheng TT, Voskuhl J, Versluis F, Zope HR, Tomatsu I, Marsden HR, Kros A (2013) Controlling the rate of coiled coil driven membrane fusion. Chem Commun 49(35):3649–3651
Zheng TT, Bulacu M, Daudey G, Versluis F, Voskuhl J, Martelli G, Raap J, Sevink GJA, Kros A, Boyle AL (2016) A non-zipper-like tetrameric coiled coil promotes membrane fusion. RSC Adv 6(10):7990–7998