The *de novo* design of α-helical peptides for supramolecular self-assembly
Joseph L Beesley¹ and Derek N Woolfson¹,²,³

One approach to designing *de novo* proteinaceous assemblies and materials is to develop simple, standardised building blocks and then to combine these symmetrically to construct more-complex higher-order structures. This has been done extensively using β-structured peptides to produce peptide fibres and hydrogels. Here, we focus on building with *de novo* α-helical peptides. Because of their self-contained, well-defined structures and clear sequence-to-structure relationships, α helices are highly programmable making them robust building blocks for biomolecular construction. The progress made with this approach over the past two decades is astonishing and has led to a variety of *de novo* assemblies, including discrete nanoscale objects, and fibrous, nanotube, sheet and colloidal materials. This body of work provides an exceptionally strong foundation for advancing the field beyond *in vitro* design and into *in vivo* applications including what we call protein design in cells.

Addresses

¹ School of Chemistry, University of Bristol, Cantock’s Close, Bristol BS8 1TS, UK
² School of Biochemistry, University of Bristol, Medical Sciences Building, University Walk, Bristol BS8 1TD, UK
³ BrisSynBio, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol BS8 1TG, UK

Corresponding author: Woolfson, Derek N (D.N.Woolfson@bristol.ac.uk)

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Introduction

*De novo* peptide and protein design refers to the programming of amino-acid sequences to adopt pre-defined three-dimensional structures. This can be guided by studies of natural proteins, but the aim is to achieve minimal, non-natural sequences to realise existing or completely new protein folds [1]. Originally, design approaches were referred to as rational design as they were founded on bioinformatics and empiricism. Increasingly, however, powerful computational tools are emerging that allow *in silico* scoring and optimisation of huge numbers of sequences compatible with the target structure [2,3]. A key area within this maturing field is the design of polypeptide-based systems programmed to self-assemble non-covalently into defined supramolecular structures. This tests our understanding of peptide–peptide and protein–protein interactions and also has the potential to produce biocompatible materials for applications in biotechnology and nanomedicine.

Thus far, a variety of ordered assemblies have been achieved with *de novo* polypeptides, including discrete particles, linear assemblies of fibres or nanotubes, and multi-dimensional arrays such as 2D lattices and crystals (Figure 1) [4]. A founding tenet of the field is the programmed assembly of simple components with rotational symmetry (*C*ₙ) [5]. Assembly via a single interface leads to closed rings or filaments, while using two interfaces enables more-complex supramolecular systems [6]. This approach has been pioneered by Yeates who demonstrates that natural proteins of defined oligomeric states (i.e. symmetries) can be combined to produce fusion proteins that assemble into a variety of architectures [7]. Others have advanced this top–down approach to supramolecular assembly by redesigning symmetric proteins to associate via new interfaces or metal coordination [8–11].

*De novo* peptide self-assembly is a broad field [12]. Currently, much of the work centres on the fibres and hydrogels formed by small β-strand peptides, alternating D-residue and L-residue cyclic peptides, Fmoc-dipeptides and collagens; this subfield is reviewed elsewhere [13,14]. Assembly of more-ordered and discrete systems from the bottom-up is increasingly being achieved with *de novo* α-helical peptides [15]. This is due to several important features of the α helix: 1) its predictable geometry defined by the narrow range of energetically favourable combinations of torsion angles in Ramachandran space; 2) straightforward sequence patterns of hydrophobic and polar residues that lead to amphipathic helices, which then readily associate; and 3) further well-understood sequence-to-structure relationships that allow this association to be directed to form specified oligomeric states and topologies. Here, we discuss recent progress in the design of supramolecular structures that use *de novo* α-helical peptides as building blocks.

Directing α-helical association to make coiled-coil building blocks

α-Helical oligomerisation can be programmed precisely through the formation of coiled coils, which are common
In between ‘holes’ togethertobe occupied ingemein by residues, α-helices are amphipathic as defined by heptad sequence repeats HPPHPPP (where H and P represent hydrophobic and polar residues, respectively), often annotated abcddef. Coiled coils form primarily to bury the hydrophobic residues at a and d positions, which are brought together on one face of the helix. Moreover, the helix-helix interactions are characterised by intimate ‘knobs-into-holes’ (KIH) packing of these residues [17]. The e and g positions that flank the hydrophobic seam are occupied typically by charged residues leading to complementary electrostatic steering and salt bridging between the helices.

In a seminal study, Harbury et al. demonstrate the close relationship between KIH packing and structure, showing that the nature of the hydrophobic residues at a and d directs coiled-coil oligomeric state [18]. Detailed studies of natural proteins and rational design attempts have further established reliable sequence-to-structure relationships [16]. Furthermore, the fold can be geometrically described by a small number of structural parameters, first conceived by Crick, which can be used to build and score sequences in silico rapidly [19,20]. This parameterisable, inherently symmetric and relatively fixed geometry of coiled coils make them ideal modules for supramolecular self-assembly.

A basis set of de novo coiled coils for self-assembly

Towards such building blocks, we have developed a basis set of orthogonal coiled-coil assemblies that robustly adopt specific oligomeric states and \( C_n \) symmetries (Figure 2a). Building on Harbury’s work, Fletcher et al. present completely de novo peptide sequences that homo-oligomerise into parallel dimer (CC-Di), trimer (CC-Tri) and tetramer (CC-Tet) arrangements, with all designs confirmed by high-resolution X-ray crystallography [21]. Thomas et al. demonstrate charge patterning at the e and g positions to design heterodimeric coiled coils (CC-Di-AB) with a range of dissociation constants [22]. Indeed, using rational and computational methods, a swathe of coiled-coil heterodimers are now available [23–27]. Combining rational and computational design, Thomson et al. describe parallel, blunt-ended coiled coils with five (CC-Pent), six (CC-Hex2) and seven (CC-Hept) helical chains [28]. These are α-helical barrels as they have contiguous central channels and are able to bind and discriminate between small molecules [29]. Recently, a thoroughly characterised antiparallel homotetramer with D2 symmetry has been added to the toolkit [30]. From this de novo basis set of characterised components, a range of supramolecular structures have been designed and functionalised towards various applications as follows.

Self-assembling cages (SAGEs) have been constructed by linking CC-Tri-based and CC-Di-AB-based components into hubs, which then assemble into extended hexagonal networks and close to yield particles (Figure 2b) [31]. The structure and assembly of the SAGEs have been modelled by both coarse-grained and all-atom simulations [32,33]. Modification of the component peptides can alter particle diameter and address the surface with small molecules, peptides and proteins to different densities [34,35]. When added to epithelial cells, SAGE particles show no cytotoxicity and are endocytosed at rates that can be controlled by varying surface electrostatics [36]. Furthermore, antigenic peptides can be extended from the homotrimer component to drive selective and assembly-dependent immune responses in vitro and in vivo [37]. Thus, the SAGE system is highly modular, comprises fully de novo peptides assembling into a unique non-natural architecture, and it shows promise for cell-biology and biomedical applications.

The CC-Tri through CC-Hept components have all been adapted to assemble into highly ordered fibres or nanotubes through end-on-end association driven by
complementary charge interactions or native chemical ligation via the peptide termini (Figure 2c) [38,39]. Crucially, these studies show that the superstructure and persistence of the tubular assemblies is a direct product of the individual coiled-coil geometry and surface chemistry. As with the discrete coiled coils, nanotubes built from the α-helical barrels are capable of binding small molecules. Moreover, amphipathic helices of this type can be adapted to bind carbon nanotubes [40,41]. Collectively, this work demonstrates the potential biotechnological value of these proteinaceous fibres in materials and sensing applications.

Outside of our group, the Marsh laboratory has fused CC-Tri, CC-Tet and CC-Pent to the outer face of a natural trimeric protein to produce closed, small and monodisperse particles with tetrahedral, octahedral and icosahedral geometries, respectively (Figure 2d) [42,43,44,45]. Single particle analysis using electron microscopy has generated low-resolution structures that match the design target for all three systems. In addition, the octahedral particles have been decorated with maltose-binding protein through fusion to the coiled-coil domain, further confirming the intended peptide orientation and providing the foundation for application development [44]. To complement these studies, the group have also tested the robustness of the CC-Di to CC-Pent components by fusing these sequences to GFP and investigating the linker length and component orientation to tolerate this large protein [46]. Such studies are critically important in the development of reliable ‘off-the-shelf’ components.

**Accessing discrete nanoparticles more generally**

Beyond the basis set, α helices can be programmed to assemble into discrete, particulate architectures. Ryadnov et al. have designed a homodimeric coiled coil (with \( C_2 \) symmetry) with two additional polar ‘facets’ that are each able to interact with facets on two neighbouring coiled coils (with \( C_3 \) symmetry) to give a hexagonal array that closes [47]. The resulting assemblies are relatively monodisperse and a low-resolution particle reconstruction from cryo-electron microscopy provides evidence of a hollow core, though the designed threefold association between the homodimer subunits has not been verified. The assemblies are able to encapsulate and deliver nucleic acids to cultured mammalian cells.
The Burkhard group has developed self-assembling peptide nanoparticles (SAPNs). This is designed through the fusion of a de novo trimeric coiled coil with tetramer-forming or pentamer-forming sequences adapted from natural proteins to generate particles with octahedral or icosahedral symmetry, respectively (Figure 3a) [48,49]. These assemblies often display a broad size distribution due to the flexibility in the component peptides and consequently, no high-resolution structural data have been obtained. The system has been successfully decorated with peptide epitopes and whole proteins to develop novel vaccines against influenza virus, HIV and Plasmodium falciparum that are highly immunogenic and protective in model murine systems [49,50,51].

The Jerala group have pioneered a different approach where α-helices form the edges, rather than the vertices, of polyhedral nanoparticles (Figure 3b). In their first study, six orthogonal dimeric coiled-coil sequences are concatenated into a single polypeptide that folds as intended into a discrete tetrahedron [52]. The design strategy has been extended to afford four-sided pyramid and triangular prism geometries [53]. Importantly, a new tetrahedron design folds correctly in vivo within the cytosol of murine hepatocytes. Similar approaches using heterodimeric coiled coils to produce discrete 2D polygons have also been reported by the Woolfson and Keating groups [54,55].

Assembling linear filaments and nanotubes

Fibres and nanotubes have also remained key targets for peptide self-assembly. In particular, the Conticello group have contributed two distinct fibrillar materials. A non-blunt-ended heptameric coiled coil was modified to associate longitudinally through terminal electrostatic interactions and shown to encapsulate the small molecule Prodan [56]. The group also describe bifaceted coiled-coil peptides that assemble into spiralling sheets and stack to form wide nanotubes stretching for many microns (Figure 3c) [57]. Two structurally distinct packing modes are observed by cryo-electron microscopy and one or two mutations are sufficient to induce the large structural rearrangement between these forms. The medical potential of this system has been investigated by extending a variety of epitopes from one of the peptide designs to produce immunogenic nanofibers [58]. When injected

**Figure 3**

Coiled coil-based supramolecular systems. (a) Computational model of an icosahedral self-assembling peptide nanoparticle (SAPN) constructed with a pentameric coiled coil (green) and a de novo trimeric coiled coil (blue) presenting a variety of epitopes from P. falciparum (yellow, red and purple) (adapted from Ref. [49]). (b) Computational model of TET12SN, a single-chain tetrahedron composed of orthogonal dimeric coiled coils (adapted from Ref. [53]). (c) Atomic models fitted to cryo-electron microscopy reconstructions for two fibrillar packing modes accessed by bifaceted coiled coils (PDB ID 3J89 (left), adapted from [57]). (d) Computational model of a homotetrameric coiled coil (left) designed to adopt P422 (centre) and P622 (right) space groups as shown by transmission electron microscopy (scale bars: 20 nm) (adapted from Ref. [62]).
into mice, the fibres are internalised by antigen-presenting cells and the epitope triggers a specific immune response without the need for adjuvant. These studies and the related work of Burgess et al. [38] demonstrate the versatility of nanotube assembly through non-covalent coiled-coil stacking.

An entirely new form of α-helical filament has been reported by the De Grado group [59]. Described as a cross-α amyloid-like fibril due to its similarity to the Staphylococcus aureus PSMα3 peptide [60], the structure is composed of two twisted sheets of antiparallel helices that form parallel dimers across the superhelical axis. Mutation to a single interface position impacts assembly kinetics and leads to different structural geometries by X-ray crystallography. As with the SAGEs, these fibres are inherently highly modular, and their functionalisation could be tuned for precise molecular positioning.

Towards multi-dimensional systems

α-Helical building blocks that self-assemble in two or three dimensions will form lattices or crystals, respectively, and may offer new materials for industrial process or therapeutics (Figure 1). The Conticello laboratory describes an 18-residue repeat α-helix using just five amino acids that presents three orthogonal interfaces with pseudo-C3 symmetry [61]. Because of the octadecad repeat, the helix-helix interactions display no superhelical twist and consequently the peptides assemble laterally to form large, highly uniform hexagonal arrays. Lattice parameters, such as interhelical distance and height, are consistent across multiple techniques, supporting the designed structural model and demonstrating a full understanding of the system that will be essential for downstream applications.

The inherent symmetry of coiled coils can also be used to tessellate lateral self-assembly. The Pochan and Saven groups have collaborated to demonstrate that the exterior surface of a D2 symmetric antiparallel homotrimer can be designed computationally to form lattices with targeted space-group symmetries (Figure 3d) [62]. Many of these lattices stretch for over a micron with exceptional uniformity and their morphology and size can be controlled through sequence mutations and assembly conditions. A subsequent study investigates the effect of solution conditions on self-assembly to reveal that the system forms tubes, plates or needles depending on pH [63].

Finally, the computational design of a crystal-forming peptide has been accomplished by the De Grado and Saven laboratories [64]. The C3 symmetric coiled-coil homotrimer arrays laterally with P6 symmetry and stacks through N-termini to-C-termini interactions. As well as material applications, the approach may aid the crystallisation of natural proteins.

Conclusions and future directions: peptide design in the cells

The α helix has proven to be a reliable module for assembling particles, tubes and lattices from the bottom up. However, only a handful of these de novo platforms have gone from design to function, and those that have mostly focus on vaccine development. Moreover, the high cost and low scale of chemical peptide synthesis can hinder the translation of peptide-based materials into real-life applications. A young and exciting area for de novo polypeptide design, where sufficient levels of production can be achieved, is within living cells. For example, designed intracellular proteins could be used to control endogenous pathways or augment them with entirely new and orthogonal functionality. Minimal, de novo scaffolds that do not interfere with natural infrastructure will be critical for the spatiotemporal control of such functional proteins. Engineering biological systems in this way is at the core of synthetic biology and could offer organisms to produce fine chemicals, act as biosensors, or perform bioremediation.

Towards these goals, it has been shown that redesigned proteins can fold and self-assemble as prescribed within the cytoplasm [65–67]. Alongside this, a number of de novo α-helical peptides and small proteins have been presented that operate in cells [26]. From the Woolfson Group’s basis set, homodimer and heterodimer peptides can replace the protein–protein interaction domains in transcription regulation machinery, while CC-Di-AB can direct the intracellular localisation of a synthetic –cytoscaffold’, as well as tethering enzymes to its surface [68,69,70]. The Baker laboratory has designed specific hydrogen-bonding networks within four-helix bundles to generate a suite of heterodimers that assemble orthogonally in Escherichia coli [71]. Furthermore, several groups have engineered protein-based logic circuits within mammalian cells that can rapidly modulate cell behaviour without the need for gene regulation [72,73]. In particular, the system developed by the Jerala group uses de novo coiled-coil dimers as orthogonal protein–protein interaction domains [73]. Using these fast logic networks to control the association of de novo protein assemblies temporally could create highly responsive platforms to present functional biomolecules. We see this emerging area of protein design in cells as one of the next challenges in protein design, which may contribute engineered organisms for multiple applications in basic and applied science.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of particular interest


In this study, a peptide-based self-assembling nanocage system is decorated with functional epitopes to drive-specific immune responses in cell and mouse models.

38. Burgess NC, Sharp TH, Thomas F, Wood CW, Thomson AR, Zaccari NR, Brady RL, Serpell LC, Woolfson DN: Modular design...
Here the basis set de novo coiled-coil peptides are adapted to drive the formation of fibres and nanotubes. The geometry of the building blocks impacts on fibre/nanotube formation and assembly. For one peptide, the nanotubes are highly ordered allowing a high-resolution cryo-electron microscopy structure to be determined. The central channels of these nanotubes bind small molecules.


The authors have developed their own design of hybrid de novo peptide and protein-based polyhedra by decorating the particles with maltose-binding protein.


This paper describes the functionalisation of a de novo peptide nanoparticle with multiple influenza antigens, which protect mice from a normally lethal viral challenge.


This work expands on a previous study to show that orthogonal coiled-coil dimers can form the edges of three different polyhedra. One design fold within cells cultured and murine hepatocytes.


This paper details a completely novel fibre architecture and shows that single mutations to the 29-residue peptide can affect assembly kinetics and fibre geometry.


The authors design α-helices to array laterally into a hexagonal network, which is characterised thoroughly through a variety of experiments.


The authors computationally design antiparallel tetrameric coiled coils to assemble laterally into predefined space groups. Transmission electron micrographs reveal that the resulting lattices are highly uniform over large distances with lattice parameters closely matching the design target.


In this study, de novo coiled-coil heterodimer peptides are appended to a nanotube-forming protein in E. coli. The coiled-coil components can be used to direct the filaments to the bacterial inner membrane, or to recruit enzymes to the surface of nanotubes in cells. Transmission electron tomography of whole cells provides invaluable insight on nanotube organisation within the E. coli cytoplasm.


The authors combine de novo coiled-coil dimers and split proteases to create modular signalling cascades and Boolean circuits within mammalian cells. These rapid networks do not involve gene regulation and could be used to control de novo protein assembly.