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Coiled Coils

Self-Assembly of Coiled Coils in Synthetic Biology: Inspiration and Progress

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Biological self-assembly is very complex and results in highly functional materials. In effect, it takes a bottom-up approach using biomolecular building blocks of precisely defined shape, size, hydrophobicity, and spatial distribution of functionality. Inspired by, and drawing lessons from self-assembly processes in nature, scientists are learning how to control the balance of many small forces to increase the complexity and functionality of self-assembled nanomaterials. The coiled-coil motif, a multipurpose building block commonly found in nature, has great potential in synthetic biology. In this review we examine the roles that the coiled-coil peptide motif plays in self-assembly in nature, and then summarize the advances that this has inspired in the creation of functional units, assemblies, and systems.

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1. Introduction

Synthetic biology aims to understand and harness the emergent properties of complex biological systems. As discussed here, one approach towards this is the use of biological, or biologically inspired modules, for the directed self-assembly of functional synthetic systems. In this review we draw attention to the versatility in nature of one of these biological modules, the simple coiled-coil peptide structure, and then highlight recent efforts towards meeting the synthetic biological challenge this presents: attempts to use coiled-coil-forming peptides to assemble functional units, assemblies, and systems of increasing complexity (Figure 1).

Both in nature and in the laboratory, α-helical coiled coils are formed by the binding of two or more α-helical peptides in a specific manner to produce a stable complex in aqueous solution. The specificity of binding results from the amino acid sequences: the majority of coiled-coil-forming peptides are characterized by a heptad repeat, denoted "a-b-c-d-e-f-g"; apolar amino acids occur at most of the a and d positions, and an amphiphilic) helix results (Figure 2). The packing of the hydrophobic a,d face against that of another coiled-coil-forming peptide produces most of the binding energy. The apolar face of the helix is not parallel to the helical axis but winds around the helix roughly once every 15 nm,[2] such that the packing of the hydrophobic strips against one another leads to the coiling of individual α-helical "coils". Amino acids with charged side chains are often located at positions e and g, which border the hydrophobic core when the peptides are in the coiled-coil conformation; they contribute to the specificity of binding. Coiled coils have a ropelike structure, with each heptad extending the length of the complex by approximately 1 nm. Many aspects of coiled-coil binding are determined by the amino acid sequence: the oligomerization state (two or more peptides), size (2 nm–200 nm long), direction of binding (parallel or antiparallel), homo- or heterobinding, stability, and rigidity. The noncovalent association of these peptides is sensitive to changes in the environment, for example, pH, temperature, ionic strength, and metal ions, which affect the electrostatic or hydrophobic interactions. This versatility arising from a simple helix has resulted

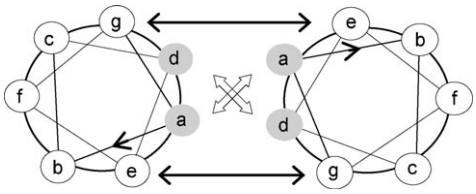

Figure 2. Helical-wheel representation of a parallel dimer with a heptad repeat of amino acids. The heptad repeat positions are labeled from a to g and the α helices propagate into the page. The a,d surface (shaded gray) is predominantly hydrophobic, and residues at positions e and g are often charged.

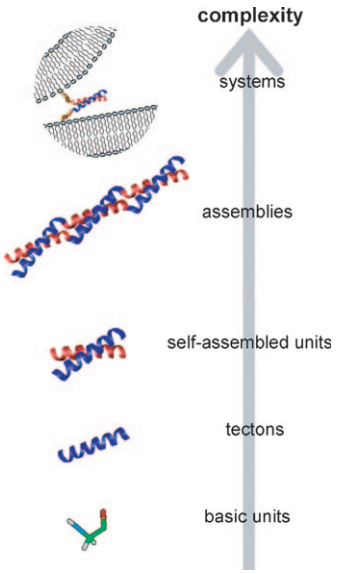

Figure 1. An overview of the use of the coiled-coil peptide motif in directed self-assembly. In synthetic biology there are a range of natural and synthetic basic units, and for each there is a progression from basic units, to tectons, to self-assembled units, to assemblies. As the final goal, multiple assemblies combine to yield functional systems.[1]

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in many functions of coiled coils in nature, and has inspired many advances in synthetic biology.[3]

2. Coiled Coils in Nature

Predictions based on analyses of primary sequences suggest that 2.5–10% of all protein residues are in α-helical coiled-coil motifs.[4,5] α-Helical coiled coils are remarkable not only for their ubiquity, but for the range of functions that they exhibit in vivo. The very definition of coiled coils—two or more α helices binding together in a specific manner—means that wherever they are found—in every compartment of plant cells, in every eukaryote and prokaryote cell—they have a common feature: the molecular recognition between two or more α helices causes the peptide segments to function as "cellular velcro" that holds together the molecules and subcellular structures to which they are covalently attached.[6] The specific amino acid sequences modulate the velcro binding properties, and can also give rise to other, more specific functions of coiled coils. Shorter coiled coils function primarily as highly specific cellular velcro, whereas longer coiled coils act as binding domains and simultaneously take on a wider variety of tasks in the cell.[7] In vivo many coiled-coil domains are long, containing several hundred amino acids. The respective proteins are often composed of a long coiled-coil domain flanked at one or both ends by a globular domain. In contrast to short coiled-coil domains, where binding leads to lateral positioning of protein segments, the binding of long coiled-coil domains results in rodlike supramolecular structures. Only few long coiled-coil proteins have been characterized in prokaryotes (organisms without a cell nucleus). In contrast eukaryotic organisms (containing a nucleus and with highly compartmentalized cells) contain more types of long coiled-coil proteins, such as motor proteins and membrane-tethering and vesicle-transport proteins, many of which are eukaryote-specific; this suggests that coiled-coil proteins have gained functions in the increasingly complex processes of the eukaryotic cell.[6] Although thousands of proteins are known to contain coiled-coil domains, in the majority of cases the function of these coiled coils is not known. The functions that have been elucidated to date have been predominantly binding, structural, and dynamic. All of the identified functions are summarized in the following sections, and for each function one or two proteins are discussed as illustrative examples.

2.1. Protein Binding

Short coiled-coil domains are most commonly found as oligomerization segments, where by means of molecular recognition they bring together proteins or protein segments, mediating a large number of specific protein interactions.[7] These coiled-coil domains can contain as little as two heptad repeats (roughly 2 nm long),[8] but often have six or seven heptad repeats (6–7 nm long). The folding of these domains into a stable complex can result in intramolecular binding, for example that contributing to the assembly of the hydrophobic core of globular proteins.[9] Examples of intermolecular binding include the assembly of ion channel signaling complexes and transcription factors (proteins that bind to specific sequences of DNA to either activate or repress gene transcription).[7,10] The most widely studied coiled-coil-containing proteins are the bZIP transcription factors. Proteins in this family consist of a "basic region leucine zipper" (bZIP) domain, and an activation domain, which modifies the gene transcription. The protein complexes are formed by coiled-coil dimerization of the leucine zipper and are anchored in position by a basic DNA-binding sequence (Figure 3). Homo- or heterodimerization of coiled-coil-forming domains on different bZIP-containing proteins determines which activation domains are in the protein complex, and hence precisely modulates the transcription of genes.

An example of how sensitive the coiled-coil function is to amino acid sequence is the large extent to which a single amino acid modification can modulate the level of transcription. A serine in the e position of a 31-residue coiled-coil domain of a bZIP transcription factor was phosphorylated, leading to additional intra- and interhelical electrostatic interactions. This stabilized the protein dimer, and as a consequence the phosphorylated protein bound to DNA with a 15-fold higher affinity.[11] Although the binding even of short coiled coils is specific, it is not necessarily exclusive, and it is thought that the coiled-coil sequence of some signaling complexes allows for different coiled-coil partners at different stages of the signaling process.[12]

2.2. Structural Functions

As coiled coils have a rodlike morphology, it is not surprising that they play a role as structural components of the cell. In some proteins the long coiled-coil domain

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Alexander Kros completed his PhD in physical organic chemistry in 2000 at Nijmegen University, the Netherlands, with Prof. R. Nolte. After a period of postdoctoral research at Caltech, USA, with Prof. Tirrell he returned to the Netherlands and became an Assistant Professor at Leiden University. His scientific interests are in the design and assembly of lipidated peptides, peptide-based polymers, and hydrogel-based drug-delivery systems. Very recently his group developed a synthetic model system for membrane fusion.

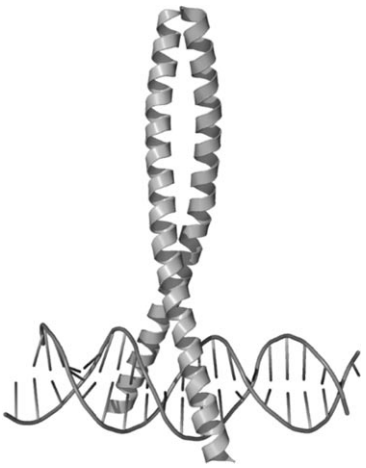

Figure 3. Crystal structure of the heterodimeric bZIP domain of the transcription factor c-Fos-c-Jun.[13,14] The upper eleven helix turns constitute the coiled coil that acts as a pincher to attach the proteins onto the DNA strand. The activation domains are not shown.

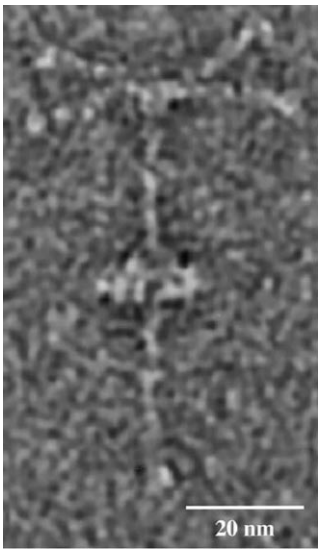

Figure 4. Negative-stained TEM image of the tetrabrachion protein.[19] The 70 nm long coiled-coil stalk is stable up to 130 °C and 6 M guanidine hydrochloride.[20] There are four β-sheet arms at the top of the coiled coil and two proteases noncovalently bound around the center of the stalk.[21]

functions as a rod that connects, spaces, and orients functional head and tail domains,[7] leading to the assembly of multiple bioactive components that are positioned with nanometer precision.[1] One example of a long coiled-coil spacer rod is the 8.3 nm long parallel homotrimer that separates the outer membrane from the bacterial cell wall in *Escherichia coli*.[15] Another example is found in the yeast spindle pole body, where the distance between the plaques is determined by the length of a parallel homodimer in the connecting proteins.[6,16] The amino acid sequence of spacer rods varies considerably between species, with positions a and d showing the least variation.[17] The sequence divergence is constrained only by the need to maintain the coiled-coil structure, which is predominantly determined by positions a and d. Coiled-coil rods are often homooligomers, in which maximal apolar and/or ionic interactions[18] account for rigidity.

A remarkably stable coiled-coil stalk forms a structural edifice at the cell surface of the bacterium *Staphylothermus marinus*, which inhabits geothermally heated marine environments and has an optimum growth temperature of 92 °C.[19] The bacterium is coated with umbrella-like tetrabrachion proteins consisting of four identical subunits. The protein forms a 70 nm long coiled-coil stalk that is anchored to the cell membrane at its C terminus and branches into four β-sheet arms each 24 nm long at its N terminus (Figure 4).[20] The arms form a canopylike meshwork by end-to-end contacts that create a semi-isolating sheath around the bacterium.[21] The coiled-coil domain sequence is such that the tetramer is remarkably stable; it remains folded at temperatures of 130 °C and in the presence of strong denaturants such as 6 M guanidinium hydrochloride.[20] The core positions contain an almost flawless pattern of aliphatic residues, mainly leucine and isoleucine, which contributes to its extreme stability.[19] This surface meshwork presumably serves as a sort of cytoskeleton[22] and stabilizes the lipids and proteins of the cytoplasmic membrane.[23]

The protein family of intermediate filaments has high sequence divergence, but all contain a roughly 45 nm long coiled-coil rod.[24] Intermediate filaments dimerize through the formation of homodimer or heterodimer coiled-coils. These parallel coiled-coil dimers pack together into filaments that are approximately 10 nm wide and several micrometers long.[25] The filaments have a persistence length of about 1 μm and can be stretched to 3.5 times their original length. Both the properties of the coiled-coil dimers[24] and axial slipping between dimers[26] lead to the flexibility of intermediate filaments, and they are thought to function as stress absorbers in animal cells, which lack a cell wall.[24]

Many coiled-coil proteins utilize long coiled coils to create ordered two-dimensional networks and three-dimensional scaffolds that support the cell.[27] Like the intermediate filaments, these two- and three-dimensional structures can span micrometers. One such protein is spectrin, a cytoskeletal protein that forms a planar layer on the inner surface of the cell membrane of all animal cells (Figure 5a).[28] Spectrin is a fibrous protein largely made up of multiple 106-residue coiled-coil domains that fold into repeats of intramolecular coiled-coil trimers (Figure 5b). Four folded spectrin proteins associate end-on-end and side-to-side in a manner that, though not yet fully elucidated, does not seem to be through coiled-coil interactions.[29] Multiple spectrin tetramers bind at actin junctions such that a membrane skeleton composed of ordered mosaics is formed (Figure 5a). These mosaics link to both membrane proteins and to proteins in the cytoplasm.[30] The coiled-coil binding is dynamic, and coiled-coil rearrangements (the switching of one section of the protein between a loop and an α helix, Figure 5b) and variations in binding between two spectrin chains can rapidly change the length and flexibility of the molecule; this in turn affects the organization of proteins that are bound to each mosaic, and the membrane shape and mechanical resilience.[30,31] An equivalent coiled-coil protein found in a bacterial cell was found to be essential for the shape of the cell.[32]

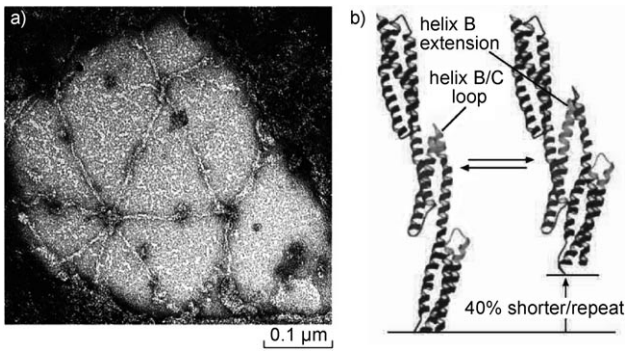

Figure 5. a) TEM image of a membrane skeletal network showing the actin hubs and linking spectrin network. The darker spots along the spectrin spokes are where spectrin is cross-linked to membrane proteins.[33] b) Spectrin mosaics are largely composed of intramolecular antiparallel heterotrimers. The coiled-coil trimer repeats are depicted in dark gray, and the section that switches between loop and α-helical conformations is depicted in light gray.[30]

The protein NuMa contains the longest known coiled-coil domain (1485 residues, 207 nm long) which forms the major component of this fibrous nucleoskeletal protein. In vitro it self-assembles into multiarm oligomers, and when overexpressed in vivo it induces a three-dimensional nuclear scaffold with a quasi-hexagonal organization that can fill the nuclei (Figure 6). This indicates that its function is related to building up the architecture of the nuclear matrix.[34]

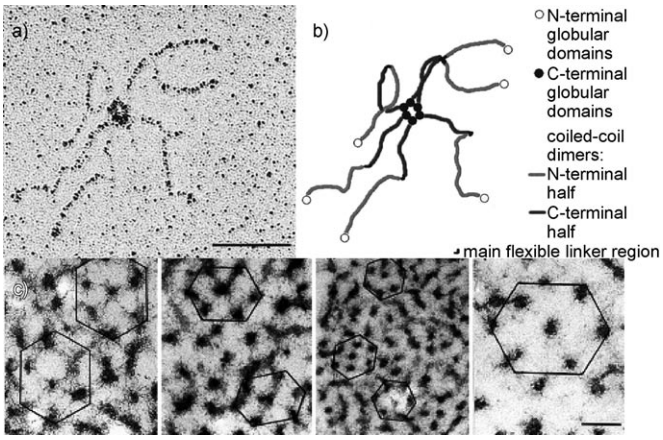

Figure 6. a) TEM image and b) schematic representation of multi-armed NuMa oligomers in vitro in which each arm is a homodimeric coiled coil. Scale bar: 100 nm. The globular N-terminal domains (rings) can bind to the centers of neighboring oligomers, resulting in a coiled-coil scaffold. c) When NuMa is overexpressed in vivo it forms a three-dimensional scaffold in which the mesh size is determined by the length of the coiled-coil domain. Scale bar: 200 nm.[34]

2.3. Dynamic Functions

Directly interacting with the cytoskeleton are the cytoskeletal motor proteins. Three classes of cytoskeletal motor proteins have been identified—myosins, kinesins, and dyneins—all of which contain coiled-coil domains.[7] These "movement" proteins undergo large conformational changes in which the dynamic nature of the coiled-coil domains plays a key role. During each movement cycle of the proteins, which lasts tens of milliseconds,[35–38] the coiled-coil packing changes in response to applied force.

In muscle cells, myosin II is responsible for producing the contractile force by pulling along actin filaments. Myosin has a globular head domain and a coiled-coil-forming tail roughly 150 nm long.[39] Parallel homodimers lead to two globular head domains, the motor units being positioned adjacent to one another. Multiple coiled-coil tail domains associate laterally and longitudinally, in a very precise manner, forming thick filaments. The force-producing head domains that protrude from the side of the thick filament are arranged helically around the filament with an axial sepration of 144 Å (Figure 7a). The packing of many coiled-coil domains

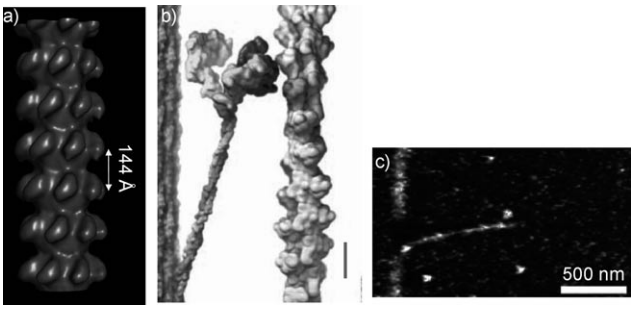

Figure 7. a) 3D reconstruction from single-particle electron-microscopic analysis of a myosin thick filament from a relaxed muscle illustrating the regular configuration of the myosin headgroups brought about by the packing of coiled-coil dimers.[41] b) Model of myosin dimer flexing out from the thick filament and binding to an actin filament on the right of the image. The elasticity of the coiled-coil domain allows the motor head group to "walk" along actin filaments. Scale bar: 60 Å.[42] c) Atomic force microscopic (AFM) image of a myosin thick filament that has been stretched and torn by lateral pushing by the AFM tip.[40]

together means that not only the a/d interface directs the packing, but the outer residues as well. In fact in myosin the positions b, c, e, f, and g are more constrained between species than the residues in positions a and d are.[17] The amino acid sequences of myosin coiled-coil domains are such that the N termini of the coiled-coil dimers extend out from the filament (Figure 7b). Thus the packing of the coiled-coil domains keeps the myosin heads in the required orientation and spacing along the thick filament,[22] and the flexibility of coiled-coil domain allows movement of the head groups along the adjacent actin filaments, creating tension.[7] In vitro the myosin thick filaments have been shown to bend and to reversibly and quickly extend to more than 3.5 times their original length (Figure 7c).[39,40] Bending is dominated by shearing between the coiled-coil dimers within the thick filament, whereas the stretching behavior is explained by shearing between coiled-coil dimers and unfolding of the coiled coils and α helices.[40] The storage of elastic energy has been proposed as an important mechanism for minimizing the energetic cost of insect flight, and these elastic properties of myosin thick filaments in muscle may constitute part of this mechanism.[40]

In myosin the coiled-coil dimer must be flexible in order to bend out from the thick filament to allow the head domains to "walk" along actin filaments. In another motor protein, kinesin, the coiled coil plays a more direct mechanical role in the "foot-over-foot" movement of the molecular motor along microtubules. Kinesin contains a central coiled coil with a motor domain at one end and a cargo-binding domain at the other; it forms a dimer by coiled-coil binding (Figure 8a). A

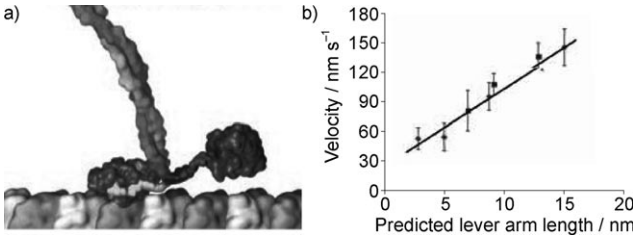

Figure 8. a) Conformational changes in the kinesin motor domain are amplified by the coiled-coil lever, causing the second "foot" to swing forward (cargo not shown).[42] b) The velocity of kinesin's movement along the microtubules depends on the length of the coiled-coil domain.[43]

small conformational change at the forward-most "foot" is conveyed and amplified by the coiled-coil "lever" to the trailing motor domain, thrusting it forward, and pulling the cargo 8 nm along the microtubule.[42] The length of the coiled-coil "lever" determines the velocity of the gliding motion (Figure 8b).[43] In order for the motor domains to walk along the microtubules it is essential that the strands of the coiled-coil dimer adjacent to the motor domains can unwind. To demonstrate this, the dynamic native domain was replaced with a more stable coiled coil, and the motility of the protein was effectively eliminated.[44]

Dynein, the third class of motor proteins, is composed of a roughly 12 nm long[45] antiparallel coiled-coil stalk domain that binds to microtubules through a small globular domain, a central globular head, and a cargo-binding stem (Figure 9a). Like kinesin, dynein also moves along microtubules in 8 nm steps.[46] The movement of dynein is not as well understood as that of the other cytoskeletal motor proteins, but the micro-

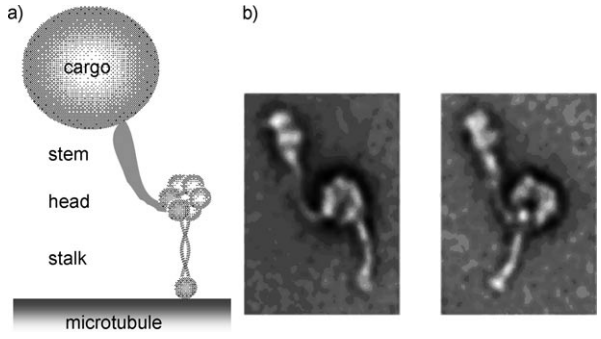

Figure 9. a) Dynein carries cargo along microtubules. b) Composite images of dynein from negative-stained TEM images. The coiled-coil stalk before the power stroke (left) is more flexible than after the power stroke (right).[49]

tubule-binding domain at one end of the coiled coil changes its affinity for microtubules depending on events at the headgroup, which is at the other end of the coiled coil (and vice versa). Therefore structural changes must be transmitted along the length of the coiled coil. This implies a requirement for dynamic changes to helix–helix interactions.[47] It has been found recently that sliding the strands in the coiled-coil stalk by four amino acids couples the microtubule-binding and headgroup activity.[48] It has also been observed that before the movement phase of each cycle, when dynein is tightly bound to the microtubule, the coiled-coil stalk is more flexible than after the power stroke. At this point in the cycle the coiled coil is straighter and has a lower standard deviation in its relative position, and is therefore thought to be more stable (Figure 9b). It is proposed that this flexibility may render the coiled coil capable of storing elastic energy when the molecule develops force against a load.[49] The length of the coiled-coil domain is highly conserved and is thought to be optimal for its role in force transduction.[22]

The motor proteins discussed above all transport cargo along intracellular "cables". Another method for intracellular transport that takes place in all eukaryotic cells is by means of transport vesicles. SNARE proteins are key components of this form of transport, as the dynamic coiled coil that forms between different SNARE proteins facilitates the docking and fusion of transport vesicles with organelles or the cell membrane. The SNARE proteins are a large family, with 27 SNARE proteins identified in a single unicellular parasite.[50] Although these proteins vary considerably in their structure and size, the coiled-coil domains are highly conserved, and it is thought that they all operate by way of the same mechanism. The SNARE proteins involved in the exocytosis of neurotransmitters from neurons are the best characterized. One type of SNARE protein is connected to the transport vesicle membrane, another to the target membrane (in this case the neuronal membrane), and a third SNARE protein is found in the cytoplasm. A very stable coiled-coil complex forms between these three proteins, bringing the membranes together (Figure 10).[51] Assembly proceeds spontaneously from less structured monomers and results in a 6.5 nm long

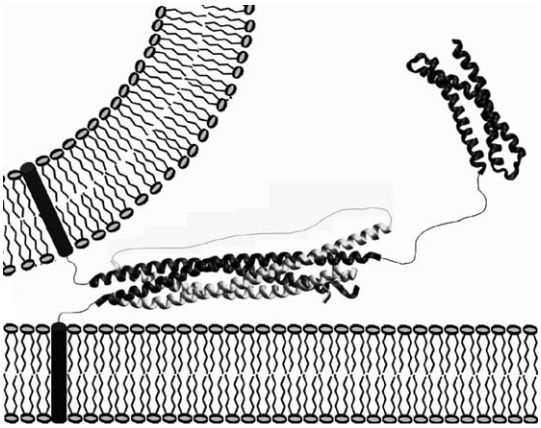

Figure 10. Crystal structure of a SNARE protein complex[55] featuring a coiled-coil tetramer that docks a transport vesicle to the target membrane and leads to membrane fusion and contents transfer.

coiled-coil heterotetramer.[18,52,53] The energy released by the formation of the stable four-helix bundle is greater than the free energy barrier for fusion. Enough force is produced to disrupt the lipid bilayers, leading to membrane fusion, although the exact mechanism is unknown.[18,53] The bundle is then "unzipped" with the aid of four proteins and energy from ATP hydrolysis so that the SNARE proteins can be used again.[54]

Another group of proteins, Rab proteins, are thought to act upstream of the formation of the SNARE coiled-coil complexes to organize the fusion site.[56] Rab proteins are switched between active and inactive forms through conformational changes that are catalyzed by specific guanine nucleotide exchange factors (GEFs). Coiled-coil proteins have recently been found to function as GEF catalysts, a role usually carried out by structurally much more complex proteins. The Sec2p GEF domain forms a 22 nm long parallel coiled-coil homodimer that makes use of the coiled-coil motif for catalysis in a very simple manner. A small midsection of 25 amino acids of the coiled-coil hydrophobic core packing is disrupted, and this region binds specifically to a Rab protein (Figure 11). The binding interface is mostly hydrophobic and

Figure 11. Crystal structure of the homodimeric coiled-coil GEF domain of Sec2p in complex with the Rab GTPase Sec4p.[57]

covers approximately 30 nm^2 of solvent-accessible surface. The binding induces extensive structural rearrangements in the Rab protein, which activates the protein. The amino acids from both helices of Sec2p that are involved in this binding interface are highly conserved in other GEFs whose mode of function is currently unknown, indicating that they also operate similarly to Sec2p.[57]

Besides eukaryotic cells, viruses also make use of a dynamic aspect of coiled coils to transfer their contents across membranes; however, the mechanisms are rather dissimilar. Enveloped viruses (which are surrounded by a lipid membrane) such as influenza, Ebola, or HIV fuse their membrane coats to cell membranes to import their genomes into cells by way of pH-mediated coiled-coil extension.[58,59] An extensively studied example is the entry of the influenza virus, which displays a parallel coiled-coil trimer surrounded by globular head domains as an 8 nm long "spike" on the surface of the viral envelope at normal physiological pH (Figure 12a). In the initial steps of cell entry, viruses are internalized by endosomes, where the pH is gradually lowered to about 5. The pH change causes the globular head units to dissociate from the spike, triggering what was previously a loop region to rearrange into a coiled-coil configuration and irreversibly extending the coiled-coil "spike" to a length of 13.5 nm (Figure 12b).[60–62] The folding of the coiled coil propels a

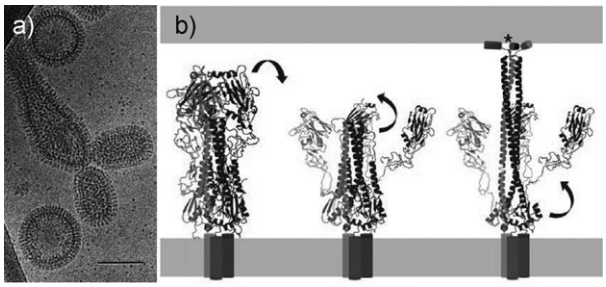

Figure 12. a) Cryo-TEM image of influenza viruses at 30 °C, pH 7.4; the coiled-coil-containing protein complexes are visible as spikes protruding from the surface of the viruses.[61] b) When a virus is encapsulated in an endosome, the pH drops and the globular head domains dissociate from the coiled-coil bundle (left); a loop domain folds (center), thereby extending the coiled coil and projecting a fusion peptide towards the endosome membrane (right). The crystallographically determined components are in ribbon representation.[62]

hydrophobic fusion peptide from a buried, basal position 10 nm towards the target membrane,[60] inducing membrane fusion and hence the release of the viral RNA into the cell.[63] In effect the central coiled coil provides a spring-loaded hinge that is set off by a drop in pH.

Recent results indicate that the means of membrane entry of non-enveloped viruses also involves a coiled-coil spring-loaded hinge that brings a fusion sequence close to the target membrane; however, the stimulus that releases the spring (i.e. that leads to coiled-coil formation) is not clear.[64]

Whereas the coiled coils in motor proteins are dynamic in response to applied force, and enveloped viruses form a coiled coil in response to a pH drop, some proteins make use of the temperature-dependent dissociation of coiled coils. Virulent bacteria experience many changes in pH, temperature, and osmolarity as they advance along their route of invasion.[65] As coiled coils respond to changes in the environment they may act as sensors to variations in the intracellular environment. Salmonella contains a protein, TlpA, with an N-terminal DNA-binding region and a coiled-coil domain of 250 amino acids.[65] This is similar to the b-ZIP domains of transcription factors, except that these coiled-coil domains function not only by molecular recognition but also by temperature "recognition". At temperatures below 37 °C TlpA forms a homodimer that can bind sequence-specifically to DNA, repressing its activity. When the bacterium enters warm bodies, that is, with temperatures above 37 °C, the homodimer is destabilized,[66] releasing the DNA, which is then available for replication.[67] Circular dichroism spectroscopy demonstrates that the temperature-induced dimer-to-monomer transition of TlpA is reversible; upon cooling, both function and full α helicity are regained.[67]

It is evident that variations in the interfaces between α helices (through different amino acid sequences) lead to a remarkable assortment of properties, and coiled coils are used in numerous ways in the cell. Coiled-coil structures provide mechanical stability in one, two, and three dimensions to the interior and surfaces of cells by means of rods, mosaics, and scaffolding. The supramolecular structures are also involved in movement processes for which particular degrees of

flexibility are essential. Natural coiled coils are utilized for their extreme thermostability in some cases, and their relative lability in others, as they switch structure in response to temperature or pH. Furthermore, coiled coils act as molecular recognition systems, catalyzing cell activities. The biological function of the coiled-coil motifs in many other proteins is not clear, and it is expected that several other functions will be elucidated in the future.

3. Coiled Coils in Synthetic Biology

The functions of natural coiled coils discussed above evolved over the last 3.8 billion years.[68] Since the 1950s scientists have been reverse-engineering nature: they have studied the form and function of proteins and traced these back to amino acid sequences to obtain the "rules" for protein self-assembly,[69,70] which then allows de novo peptide design, yielding structures of novel form and function. In a synthetic sense this means that molecules can be designed that organize into well-defined structures with specific functions.

Coiled coils are good candidates for the self-assembly of functional biosynthetic nanostructures for many reasons: 1) they have precisely defined size and shape (i.e. rods 2 nm in diameter with each heptad roughly 1 nm long) and surface functionality; 2) the intra- and interhelical noncovalent interactions are relatively well understood; 3) they can self-assemble into stable structures at low concentrations (below nanomolar[71]); 4) coiled coils can be functionalized at the N or C terminus or on solvent-exposed amino acids; and 5) the affinity and specificity of the binding of coiled coils are very sensitive to the amino acid sequence. This rich array of controllable properties means that there is a coiled-coil "building block" to suit many castles in the (supramolecular) sky.

We discuss self-assembly that is inspired by α-helical coiled-coil peptides in terms of a progression in synthetic biology, in which basic units bind covalently to form tectons, which hierarchically self-assemble by means of units and functional assemblies, and combine with other functional assemblies to culminate in systems.[1,72] In this quadrant of synthetic biology the basic units are amino acids, sequences of which covalently bind to form the tectons, α helices. The α helices bind noncovalently to form the units, coiled coils, which organize further into assemblies, and finally develop into entire systems. The mapping and exploration of coiled-coil units in synthetic biology up to the current date is reviewed below. First units are discussed, then assemblies, and finally, the first uses of coiled coils in systems are charted, and parallels are drawn between these advances and the sophistication of naturally occurring coiled-coil motifs.

3.1. Coiled-Coil Units

The initial aim of research on coiled coils was to understand the structures and binding of natural coiled coils. Peptides derived from transcription factors and other natural coiled coils have been mutated in order to delve into their binding properties. Once the rules correlating peptide primary sequence to intermolecular interactions had started to emerge, scientists widened their focus from changing isolated residues in a natural sequence to include designing completely de novo sequences. The units have become more removed from native form and function as the possibilities of the units are explored. Many aspects have been tailored in coiled-coil units, namely coiled-coil length, stability, specificity of molecular recognition, oligomerization number, and strand orientation and conformation. We will touch upon highlights in the following section.

To date, the majority of the peptide units whose sequence-to-structure relationships have been investigated and modified have been short, usually with three to five heptad repeats. Generally peptides with more heptad repeats form more stable coiled-coil complexes. Very short homodimers with only two heptad repeats have been created by optimizing design criteria, that is, by enhancing the hydrophobic packing and intra- and intermolecular salt bridges, utilizing amino acids with high α-helix propensity, and using suitable capping moieties.[73,74] The shortest identified coiled coils in nature also contain two heptad repeats,[8] and this appears to be the lower size limit.

A common goal in this area is to design coiled coils with greater binding stability while retaining the other properties of coiled coils. This feature of unit self-assembly has primarily been targeted by optimizing the primary sequence. As an example, amino acid substitutions in the 37-residue coiled-coil domain of the c-Jun transcription factor[75] caused an increase in the melting temperature the Fos–Jun heterodimer of 37 K. After analyzing different amino acid sequences, researchers concluded that mutations that increase the buried hydrophobic area and improve helix stability accelerate the formation of a partially folded dimeric intermediate, and that after this intermediate is formed, improved intermolecular Coulombic interactions increase the thermodynamic stability of the final coiled-coil structure.[76] In another example the replacement of two amino acids in position a of the 34-residue coiled-coil domain of another DNA-binding protein decreased the dissociation constant for homodimers by a factor of 10^5.[77]

Non-natural fluorinated amino acids, which have a large hydrophobic area, have been incorporated into recombinant coiled-coil peptides, leading to increased stability with minimal structural perturbation of the final complex.[78,79] For instance, isoleucine residues in core positions of the bzip domain of peptides derived from the transcription factor GCN4 were replaced with 5,5,5-trifluoroisoleucine, resulting in an increase of 27 K in the melting temperature; the affinity and specificity for DNA binding of the mutant was similar to that of the hydrogen-containing counterparts.[79]

Another "non-natural" approach to increasing coiled-coil stability is the modification of amino acids. In one example an azobenzene moiety was attached as an intramolecular cross-linker between two residues in position f of a heterodimer, that is, solvent exposed and parallel to the helix length. Irradiation of the peptide reversibly changed the conformation of the azobenzene cross-linker from *trans* to *cis*, thereby decreasing the cross-linker length such that it was comparable

with the natural helical repeat length of the peptide, which increased the peptide's helicity and promoted coiled-coil folding.[80]

The binding of metal ions to histidine and cysteine residues can also affect the stability of coiled coils. The divalent binding of metal ions to residues at positions i and $(i+4)$ can be used to stabilize coiled coils, while binding at i and $i+2$ destabilizes coiled coils. Coordination of two peptides to a single metal ion can induce the coiled-coil complex. This effect has been demonstrated with the metal ion at both solvent-exposed and internal positions.[81]

Coiled-coil complexes are specific in terms of the sequences of the peptides that will bind, the number of strands that associate, and the orientation of the binding partners. The high degree of binding specificity that can be designed into the coiled-coil interaction has been exemplified by the formation of three distinct heterodimers in solutions of six peptides.[82,83] In one case the four-heptad-repeat peptides were based upon natural coiled-coil domains from transcription factors, and the selectivity was introduced by replacing a single amino acid in a core position. Besides the replacement of natural amino acids, non-natural, urea-derived side chains were utilized to improve selectivity.[82]

Amino acids with charged side chains are important determinants of which peptides will form a coiled-coil complex, and controlling inter- or intramolecular Coulomb interactions through pH change or addition of salt can be used to modulate coiled-coil formation by destabilizing certain complexes. Many heterocoiled coils gain their specificity by having charged strips bordering the hydrophobic core such that one helix is positively charged and the other negatively charged, hence preventing homocoils forming. In this way pH can be used to influence Coulomb interactions such that heterocoiled coils form at neutral pH, and homocoiled coils at low[84] and high pH.[85]

This concept of pH-controlled strand exchange has been developed further with iterative cycles in which one, two, or all three initial helices of a coiled-coil trimer are replaced specifically.[86] The strand exchange can also be programmed to be accompanied by a switch from a parallel to antiparallel trimer.[87]

Besides the electrostatic destabilization of particular helix combinations, the number of α helices in a coiled-coil bundle can be changed by the stabilizing effect of steric packing in the hydrophobic core, which is the major driving force for coiled-coil formation. For example, an engineered form of a native coiled coil is predominantly two-stranded, but the coiled-coil trimer becomes the most stable arrangement when one benzene molecule is bound in the hydrophobic core to increase buried hydrophobic surface.[88]

The oligomerization state can also be varied by tuning the hydrophobicity by way of substituting amino acids in positions within the coiled-coil hydrophobic core. This was investigated by systematically replacing the 20 natural amino acids in the central a and d positions of a five-heptad-repeat peptide that forms homocoiled coils. The β-branched residues isoleucine, valine, and threonine, which have side chains with large hydrophobic areas, promote trimer formation, whereas amino acids with charged side chains favor two-stranded coiled coils.[89,90] The substitution of amino acids with different hydrophobic side chains can also determine the oligomerization state owing to packing changes within the coiled-coil core. The GCN4 coiled coil was selectively substituted with valine, isoleucine, and leucine at positions a and d of the heptad repeat. These substitutions resulted in two-, three-, and four-stranded coiled coils, since the different hydrophobic side chains led to variations in core packing.[91]

Small changes in peptide sequence can also lead to different binding orientations. For instance, the five-heptad-repeat coiled-coil domain from an osmosensory transporter binds as an antiparallel homodimer. When two charged residues in position a of the heptad repeat are replaced with isoleucine, the hydrophobic packing is altered, interchain salt bridges are eliminated, and the dimer changes orientation from antiparallel to parallel, rendering the protein inactive in vivo.[92] A single amino acid sequence can also be induced to fold into both parallel and antiparallel coiled coils. An intramolecular antiparallel dimer was stabilized by a disulfide bridge, and it was demonstrated that upon reduction of the disulfide bond the peptide refolded into a parallel coiled-coil dimer.[93]

As a result of the distribution and range of functions of coiled coils in cells, there are many potential ways in which controlling existing coiled-coil binding can influence in vivo function. For example, there are research groups investigating coiled-coil-forming peptides to specifically bind to the coiled-coil bundles essential to viral entry. Their aim is to inhibit viral entry into cells;[94,95] other researchers are designing coiled-coil peptides to bind to specific transcription factors to modulate the replication of DNA.[96]

Another aspect of the self-assembly of coiled-coil units that has been investigated is switching the secondary structure of the peptides, which can be programmed to fold into different structures in different environments.[97] The most common conformational switch (other than coiled coil → random coil) is between coiled coils and β sheets. This is generally achieved by incorporating amino acids with high β-sheet propensity or hydrophobic character into the solvent-exposed f positions of coiled coils; upon heating, the α helices rearrange into β sheets, which aggregate into amyloid-like fibers.[98–100] In another approach, a peptide that forms homodimers at neutral pH was modified such that there was a lysine or glutamic acid face next to the hydrophobic core of the coiled-coil complex. When the pH was changed, these faces became charged, the coiled-coil structure was destabilized, and the peptide rearranged into random-coil or β-sheet structures.[101] A more readily reversible type of secondary-structure switch is between coiled coils and zinc fingers. When aspects of the two folds are merged into one sequence, stable coiled coils can refold into the more globular zinc-finger conformation upon metal binding.[97,102]

The final examples in this section on coiled-coil units demonstrate that even without any larger scale assembly the units can be highly functional. Self-replicating complexes have been developed in which coiled-coil folding catalyzes peptide bond formation, producing replicates of the coiled-coil-forming peptide. Two peptide fragments fold onto a full-

length peptide template, and an amide bond is formed between the two fragments via a cysteine thioester intermediate (Figure 13).[103] Subsequent developments included enhanced catalysis at reduced pH[104] or at high ionic

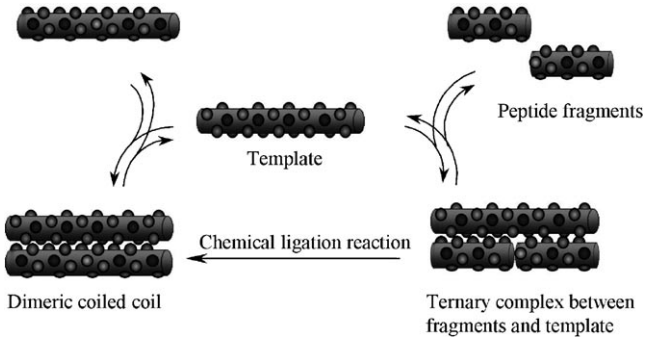

Figure 13. Diagram of an autocatalytic coiled-coil self-replication cycle. The binding of peptide fragments to a full-length template promotes ligation of the fragments, thereby producing more of the template, and catalyzing the replication.[112]

strength,[105] the formation of heterodimeric complexes,[106–108] and the development of a hypercycle, in which two self-replicating peptides catalyze each other's replication,[109] and a chirality-dependent self-replication cycle. In order for the cycle to continue the peptides must dissociate once the chemoselective ligation has occurred. To speed this up peptides one heptad repeat shorter were used,[110] or alternatively a proline kink was introduced to destabilize the coiled-coil complex.[111,112] An interesting advance is a system with two self-assembling groups: peptides with nucleobases introduced as side chains. The interaction of complementary nucleobases (through hydrogen bonding) enhanced the peptide self-replication reaction.[113]

These examples demonstrate how researchers have taken the coiled-coil motif as a natural structural unit, deconstructed and rearranged it in many permutations to elucidate the mechanisms and subtleties of its formation, and in the process explored the wide variety of functions that can be chemically programmed into coiled-coil units. The advantage of these designed peptides over natural peptides is that the chemical, physical, and biological properties of the complex can be precisely determined over a broader range. For this reason it is predominantly designed peptides that are used to create higher order structures and systems. In the following sections an overview is given of the use of these functional building blocks to create one-,two-, and three-dimensional assemblies.

3.2. Coiled-Coil Assemblies

Since 1997 coiled-coil-based synthetic biology has been extended by the self-assembly of coiled-coil units into larger structures that contain multiple coiled-coil units.[114] As in the previous section on the self-assembly of coiled-coil units, all of the examples of coiled-coil assembly described here are based on peptides that would be considered short in nature, three to six heptads in length. For the study of isolated coiled-coil units it is convenient to elucidate the binding properties with small molecules because they can be readily synthesized on solid support and because the effect of, for example, changing a single amino acid can be identified more readily. As the functional possibilities of coiled-coil assemblies are explored, it is likely that there will be an expansion (through protein engineering) to longer coiled coils and de novo proteins with coiled-coil domains. In this section we give an overview of coiled-coil assemblies; the structures are categorized into those for which coiled-coil formation is the sole driving force for material organization, and those that contain two self-assembling entities.

3.2.1. Materials Formed Solely by the Folding of Coiled Coils

The first examples are of materials composed entirely of coiled coils, and the following examples are assemblies formed from coiled-coil hybrids, which nevertheless derive their higher order structure from coiled-coil folding. Fibers and fibrils are well-established aggregation forms.[1,115–120] Work in this field takes inspiration from nature, in which coiled-coil proteins often occur in the form of fibers, such as spacer rods or intermediate filaments (see Section 2.2). One method of accessing long coiled-coil rods has been to covalently link multiple coiled-coil-forming peptides such that larger scale assemblies result upon complex formation.[121,122] Helix–loop–helix peptides have been linked into four-arm dendrimers by means of a sulfide bridge between cysteine residues in the loop region. These assemble into fibers with diameters of only 5 nm, which are postulated to be one complex wide and are many microns long. Heterofibers or homofibers can be formed depending on the pH-dependent charge of the peptides (Figure 14).[122]

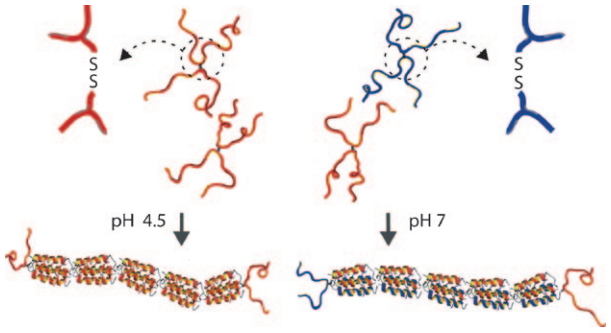

Figure 14. Two helix-loop-helix polypeptides are dimerized at cysteine residues and assemble into either homo- or heteroassociated fibers upon folding, depending on the pH value.[122]

The rodlike structure of long native coiled coils has also been mimicked by using multiple short homo- or hetero-coiled-coil-forming peptides. These associate laterally and in a staggered way such that each peptide is involved in two coiled-coil interactions simultaneously, leading to fibers, some up to hundreds of micrometers long.[123–125] The fibers are generally composed of a bundle of coiled coils as a result of interactions between the amino acids on the outside of the

coiled coil. To control this higher order structure, the design of the amino acids in positions b, c, and f of the heptad repeat is important. This would be analogous to the decreased sequence variation in buried native coiled coils in comparison to that of solvent-exposed motifs, for example, in myosin filaments. Although the native rod structures can be emulated, their functions have by and large not been mimicked yet. Current efforts in this direction are geared towards the controlled design of fiber morphology and related properties, for instance, the formation of thinner and more flexible peptide fibers (Figure 15a).[126] The functionality of the fibers has been increased by conjugating additional molecules to the coiled-coil-forming peptides, resulting in fibers coated with recuiting agents. These molecules on the surface of the fibers were able to bind to and hence localize proteins from solution (Figure 15b).[127] An additional dimension can be introduced by engineering kinks and branches into the fibers (Figure 15c).[128,129] These coiled-coil fibers have been used to template silica layers at ambient temperature and physiological pH. Upon removal of the peptide (achieved most effectively by a protease), hollow silica tubes nanometers wide and microns long result which are straight, kinked, or branched depending on the peptide template.[130] Alternatively, the fibrils can be induced to change to spherical objects at neutral pH[131] or to reversibly dissociate at low pH.[132]

By redesigning the amino acids in the exposed b, c, and f positions of the heptad repeat such that all are cooperatively involved in binding interactions between coiled coils, three-dimensional hydrogel networks have been obtained. The physical properties of the hydrogels could be varied through the design of different mechanisms of gelation mediated by the amino acids in positions b, c, and f. When alanine was placed at each of these positions, the network formed by hydrophobic interactions between fibrils, and the gel was stable up to at least 95 °C. Alternatively, when glutamine was incorporated in these positions, the binding between coiled coils was based on hydrogen bonds, and the gels melted at room temperature.[133]

The majority of hierarchical coiled-coil structures are fibrous.[134] When dendrimer structures are introduced, three-dimensional assemblies can result. Relatively complex self-assembly has been programmed with coiled-coil dendrimers: each peptide of a three-armed dendrimer forms a dimer with a complementary peptide monomer, and the six-helix bundle then binds to three other dendrimer complexes through electrostatic interactions. In this way supramolecular porous submicron- to micron-sized spheres self-assembled. Silver colloids could be formed within these "nanoreactors" having diameters that matched the pore sizes (Figure 16). As thiols have been shown to have a size-stabilizing effect on metallic colloids, a cysteine residue was placed at position f in the coiled coil such that the cysteine residues were orientated into

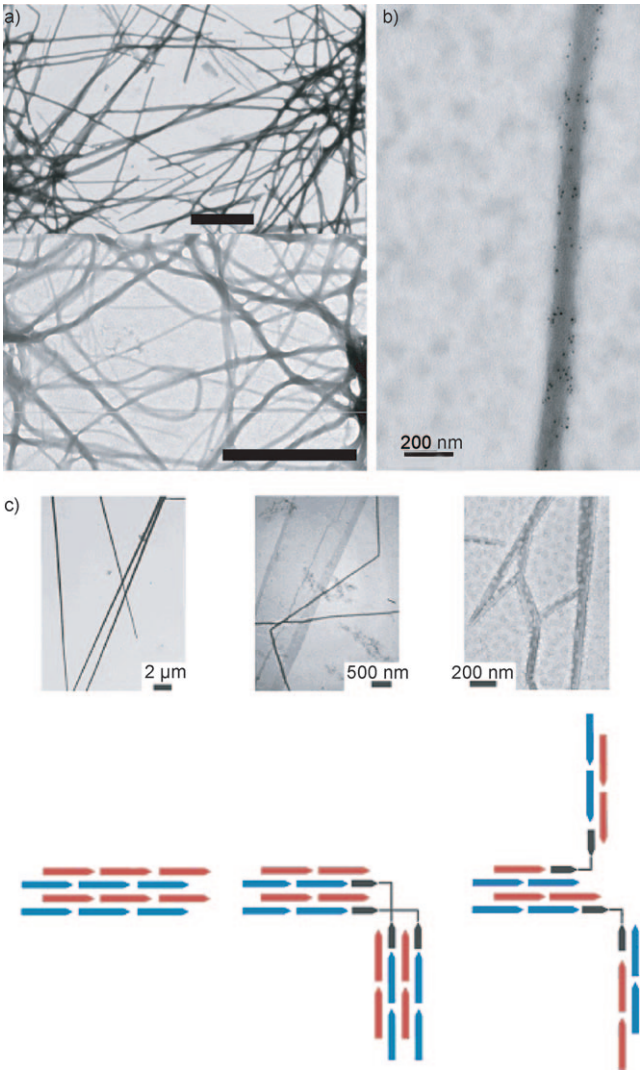

Figure 15. a) The rigidity of coiled-coil fibers can be programmed into the amino acid sequence. Negative-stained TEM images; scale bars: 1 μm.[126] b) TEM image of a peptide fiber coated with recruited proteins. Gold particles (5 nm in diameter) were bound to the protein to enable visualization.[127] c) TEM images of straight, kinked, and branched coiled-coil fibers; the modes of assembly are shown schematically.[120]

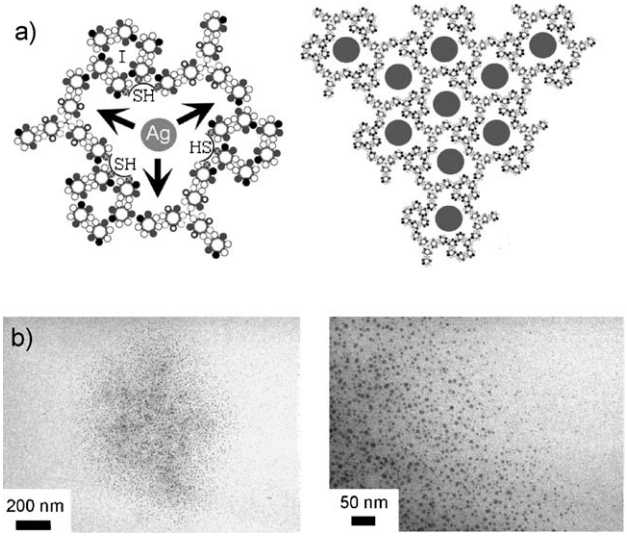

Figure 16. a) Schematic representations of coiled-coil dendrimers that form mesoscopic spheres which have pores that can serve as reactors for the formation of nanoscopic silver particles. b) TEM images of the colloidal silver clusters formed in these cavities.[134]

the cavities.[134] These assemblies have certain parallels with the coiled-coil protein NuMa, which also forms dendrimers that self-assemble into well-defined three-dimensional networks, creating a porous structural support (see Figure 6, Section 2.2.),[34] although NuMa contains the longest known coiled coil, while this assembly is built up from the shortest known heterocoil length.

Most strategies for self-assembly using coiled coils are targeted at controlling the hydrophobic core and the charged residues bordering the core. The previous example deviated from this in that the charged residues were designed for interactions between coiled coils. The next example, in which this concept was extended, complete departed from standard coiled-coil formation. Amphipathic α-helical coiled-coil-forming peptides were located at water–air interfaces, with the hydrophobic face of the helix oriented towards the air. Intra- and intermolecular metal-ion cross-links between histidine residues stabilized the helices, which created a film at the interface, strengthening the foams. The films could be disrupted by the addition of a metal chelator or by changing the pH to break the peptide–metal bond.[135] This is the only case in which the self-assembly of coiled-coil-forming peptides is utilized not for the specific binding properties but for their more general amphiphilicity. This parallels the recently discovered coiled-coil GEF catalyst, in which the hydrophobic interface of the coiled-coil is temporarily disrupted and binds to a hydrophobic patch on a Rab protein (see Figure 11, Section 2.3.).[57] An important difference is that the protein–protein interface remains highly specific, in contrast to this synthetic example.

The remainder of the assemblies described in this section are composed of coiled-coil hybrids. The biological role of coiled coils in linking larger molecules and subcellular structures has been mimicked in the many instances of coiled-coil-induced aggregation of nanoparticles. The first demonstration of this use of coiled coils was the decoration of gold nanoparticles with two different three-heptad-repeat peptides. Upon introduction of a complementary six-heptad-repeat peptide to the solution, coiled coils formed and reversible networks of gold nanoparticles resulted.[136] The sensitivity of coiled coils to their environment was utilized to investigate the dependence of nanoparticle aggregation on reaction conditions. Gold particles decorated with coiled-coil-forming peptides have been induced to aggregate only at low pH or in the presence of metal ions, conditions that reduce the charge on multiple glutamic acid side chains (by protonation or chelation), allowing homocoils to form.[137–139] The same peptide also forms a heterocoil with a complimentary peptide dendrimer, which when added to solution induced the aggregation of gold particles with well-defined spacing (Figure 17). The four-armed dendrimer linker has a central disulfide bridge, which could be reduced in solution, redispersing the gold particles.[138]

The responsiveness of coiled-coil-based assemblies most frequently results from directly disrupting the binding. A recent example where the binding is indirectly targeted involves a heterocoiled coil (with a $T_m > 85\,^{\circ}C$) attached to gold nanocapsules, which aggregated through the formation of coiled coils. Upon exposure to infrared radiation, the gold

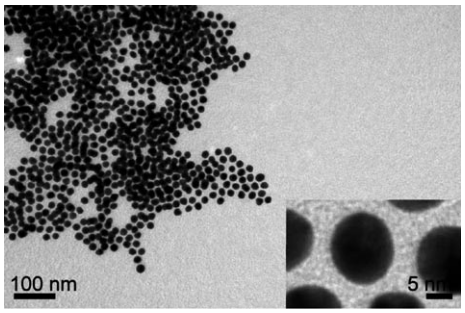

Figure 17. The aggregation of gold nanoparticles can be controlled by coiled-coil association/disassociation.[138]

nanoshells, which have a large photothermal response, produced enough heat to denature the coiled-coil complex, separating the nanoshells. When individual nanoshells were decorated in the same way with quantum dots, irradiation caused a large increase in quantum-dot fluorescence, but the heat produced by a single nanocapsule did not dissociate the coiled coil (Figure 18).[140] This indirect photothermal control over coiled-coil assembly has no known parallels in nature.

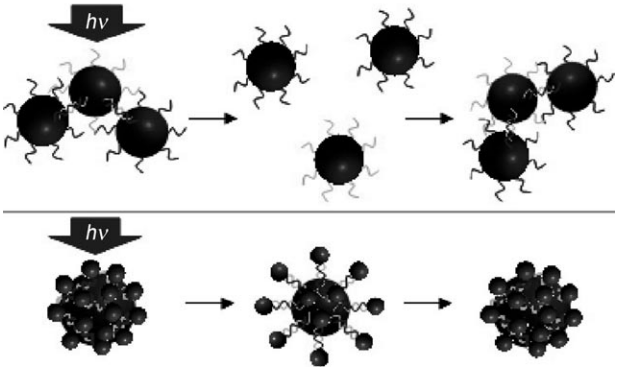

Figure 18. Coiled coils dissociate or stretch as a result of the heat released by gold nanoshells upon irradiation.[140]

Coiled coils can also be used to link other objects; for example, carbon nanotubes can be decorated with gold nanoparticles when each component is functionalized with complementary heterodimer-forming peptides (Figure 19). In addition, the dimers were able to chelate cobalt by means of histidine residues.[71] The aim of this research is to produce an interface for electrically conducting carbon nanotubes that will sense soluble biomolecular targets.

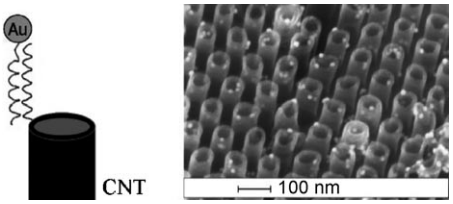

Figure 19. Reversible decoration of carbon nanotubes (CNTs) with gold nanoparticles by way of coiled-coil recognition; illustration of the concept (left) and SEM image (right).[71]

Since 1998 assemblies of coiled coils coupled with large water-soluble polymer blocks have been investigated.[141] Currently the only materials assembled from hybrids of coiled coils and hydrophilic polymers have been hydrogels. Like the assemblies constructed entirely of coiled coils, these materials have parallels to the structural cyto- and nucleoskeletal coiled-coil networks. In these constructs coiled-coil motifs flank a water-soluble protein or polymer segment, and the coiled-coil interaction creates a randomly connected network. In the first example of this kind two coiled-coil-forming peptides were linked by a long genetically engineered random-coil polypeptide.[141] These artificial proteins form hydrogels through the formation of homodimers.[141–143] Shortly after this a more synthetic equivalent was demonstrated: a peptide–poly(ethylene glycol)–peptide hybrid that forms a hydrogel through the formation of homodimers.[144] Because the coiled coil responds to changes in temperature, pH, and metal ion concentration, the triblock hybrids can be switched between solution and gel states. Coiled-coil-mediated hydrogels have also been created in which the arms consist of another water-soluble polymer, *N*-(2-hydroxypropyl)methacrylamide. The coiled coil forms in either a parallel orientation[145] or an antiparallel orientation, which reduces the steric crowding of the polymer arms.[146] A recent review of the peptide-directed self-assembly of hydrogels gives more details on hydrogels generated through the formation of coiled coils.[147]

In contrast to the coiled-coil networks and scaffolds in nature, in which coiled coils constitute the structure or "arms" of the network (for example, the protein spectrin described in Section 2.2), in the examples of synthetic networks mentioned above the coiled coils are used to connect the arms of the network to each other. There is one example in synthetic biology toward the biological end of the spectrum of a hydrogel with coiled-coil arms. A long α helix from the intermediate filament keratin (a fibrous coiled-coil structural protein) was expressed fused to a globular cell-binding domain, and this hybrid was coassembled with extracted keratins that form hydrogels through intermolecular coiled-coil association of α-helical segments (Figure 20). It was found that neurosphere-forming cells specifically adhered to the modified keratin hydrogel and actively proliferated with a high survival rate.[148]

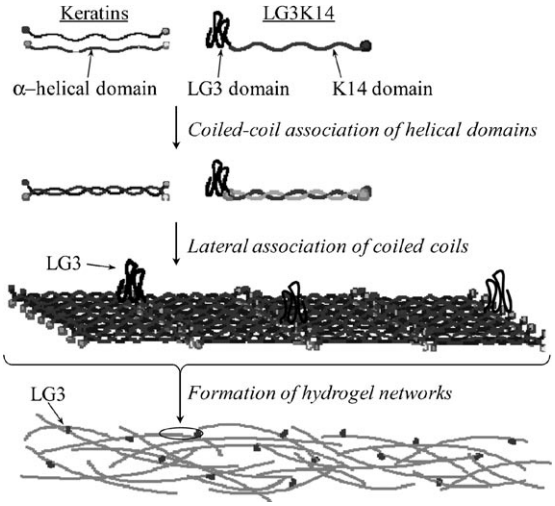

Figure 20. The fusion of a natural keratin peptide with a cell-binding domain modified the properties of the keratin hydrogel.[148]

3.2.2. *Coiled-Coil Assemblies with Orthogonal Self-Assembly*

Proteins, themselves hybrids of many self-assembled units, do not operate in isolation; they are embedded in cells, which are composed of self-assembled lipid compartments, self-assembled nucleotides etc. The complexity in coiled-coil-based synthetic biology can be extended by the hierarchical self-assembly of functional nanostructures in which both coiled-coil formation and the properties of other blocks play an essential role.

An interesting hydrogel uses star-shaped poly(ethylene glycol) (PEG) functionalized with a lysine-rich peptide that folds into a coiled-coil homodimer. This in turn binds to a polysaccharide segment (heparin) on a second star-shaped PEG by electrostatic interactions, hence leading to a hydrogel (Figure 21). This is one of the few examples in which coiled coils mediate a mode of self-assembly other than coiled-coil formation.[149]

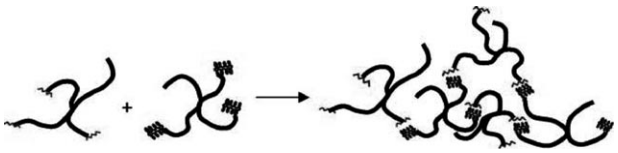

Figure 21. Formation of a hydrogel through coiled-coil–polysaccharide binding.[149]

Hydrogels have been constructed in which random-coil polypeptide spacers are connected by two types of hubs: coiled-coil-forming peptides and an enzyme (SLAC) that dimerizes. Each protein-based hub has an additional function: the coiled coils are chelated at the hisidine residues with osmium moieties, rendering the hydrogels conductive. In the dimeric form the enzyme uses electrons for the bioelectrocatalytic reduction of dioxygen to water. Thus, the preparation of hydrogels on electrodes could find possible application in fuel cells (Figure 22).[150]

The driving force for the self-assembly of coiled coils and the enzyme dimerization described in the previous paragraph results from the shielding of hydrophobic residues, but these amphipathic blocks are still water soluble and do not affect one another. The enzyme binding is the same as without the coiled coil, and the coiled-coil binding is not influenced by the enzyme. Ten years after the first synthetic polymer was attached to a coiled coil,[144] the possibility of combining coiled coils with hydrophobic polymer blocks was investigated.[151] In this case self-assembly of the coiled coil and the hydrophobic block coexist and influence the final structures that form. One peptide of a heterodimeric pair was coupled with polystyrene, and the other with poly(ethylene glycol) such that their coiled-coil formation resulted in a noncovalent amphiphilic triblock copolymer, which further assembled into

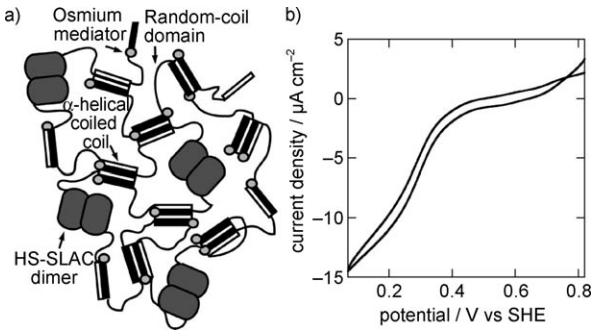

Figure 22. a) Diagram of a supramolecular hydrogel that relies on coiled-coil folding and enzyme dimerization to gelate. b) The mixed hydrogel produces a catalytic current during the reduction of dioxygen to water.[150]

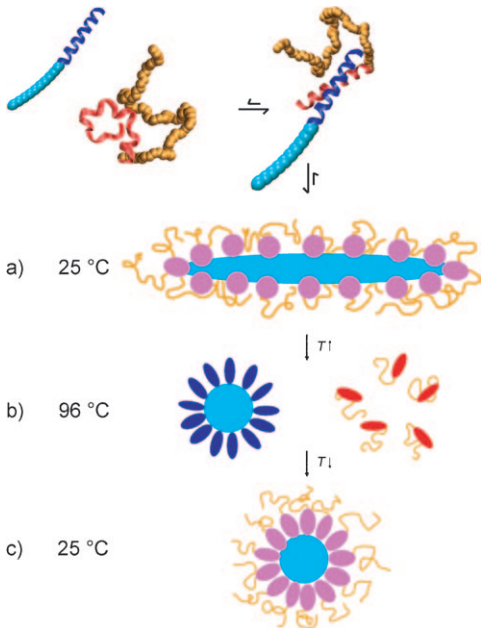

Figure 23. Hierarchical self-assembly of a coiled-coil-containing block copolymer. Coiled-coil folding creates an amphiphilic noncovalent block copolymer (light blue: polystyrene, red and blue: coiled-coil-forming peptides, yellow: poly(ethylene glycol)). The folding of the peptides is juxtaposed with aggregation of the hydrophobic polymer block, leading to rodlike micelles (a). Heating results in dissociation of the coiled coil, leading to a change in morphology to spherical micelles (b), which are retained when the coiled coil refolds upon cooling (c).[151]

rodlike micelles that were temperature dependent (Figure 23).

3.3. Coiled-Coil Systems

The goal of synthetic biology is to create functional systems, which implies the interaction of multiple self-assembled components. The aim is therefore to incorporate units or assemblies, such as those discussed in the previous sections, with other self-assembled structures, such that functional systems emerge from the combination of the properties of the components and the effects that they exert on one another. All of the systems developed to date have been rather simple, based on coiled-coil units rather than assemblies. Additionally, each system has been developed as a modification or a model of a natural process: there has not yet been a synthetic biology system with an original purpose.

Biologists have made use of coiled-coil synthetic biology for some time. One technique used to visualize protein complexes in living cells is biomolecular fluorescence complementation. The concept is that moieties with highly specific associations are fused to protein fragments, and the interaction of these moieties in vivo causes the protein fragments to form a functional and fluorescent complex. Coiled-coil peptides that bind in a stable and specific manner, for example, the coiled-coil region from the GCN4 transcription factor and designed peptides, have been used for such applications.[152–154] Alternatively the interactions of native coiled-coil-containing proteins can be visualized by fusing them with fragments of small fluorescent proteins.[155] In one example of a specific tag–probe complex, a heterodimeric coiled-coil pair was utilized to label proteins in living cells. One of the peptides was recombinantly attached to the surface-exposed terminus of a transmembrane receptor protein. The corresponding peptide was synthesized with a fluorescent label and added to the culture medium. Within one minute the fluorescently labeled peptide had coated the cell surface as the heterocoiled coils formed. The formation of the coiled coil did not affect the receptor function, hence they were an efficient small tag–probe pair (Figure 24).[156]

In other examples with more synthetic character coiled-coil units and lipid assemblies are combined. In one case different coiled-coil-forming peptides were added to solutions of liposomes. The positively charged peptides adsorbed to the surfaces of the liposomes and caused aggregation of the vesicles (Figure 25). Although the lipid packing was disturbed, there was no liposome fusion or leakage. This model system could be used to study the interrelated effects of lipid membranes and coiled-coil peptides on one another.[157]

As explained in Section 2.3, enveloped viruses enter cells by way of a pH-triggered conformational change involving a coiled-coil complex. Peptides that form an extremely stable complex with the viral envelope proteins may be effective in reducing viral infection. Such inhibitors could be screened with an efficient sensor platform. To that end, a coiled-coil trimer based on a native viral protein was anchored to supported lipid bilayers, and peptide binding to the coiled coil was monitored. The concept was demonstrated with two known inhibitor peptides, and binding was monitored with AFM and elipsometry. Further developent is needed to make this a practical system.[158]

Another synthetic biology system has been developed that is intended not to prevent, but to mimic viral membrane fusion. A peptide that forms an α-helical trimer at low pH was anchored in liposome bilayers at its C terminus and displayed a tryptophan residue at the N terminus. At low pH, when the peptides have a helical configuration, the liposomes fuse, albeit very slowly and with contents leakage. The fusion is proposed to occur through tryptophan insertion into a nearby

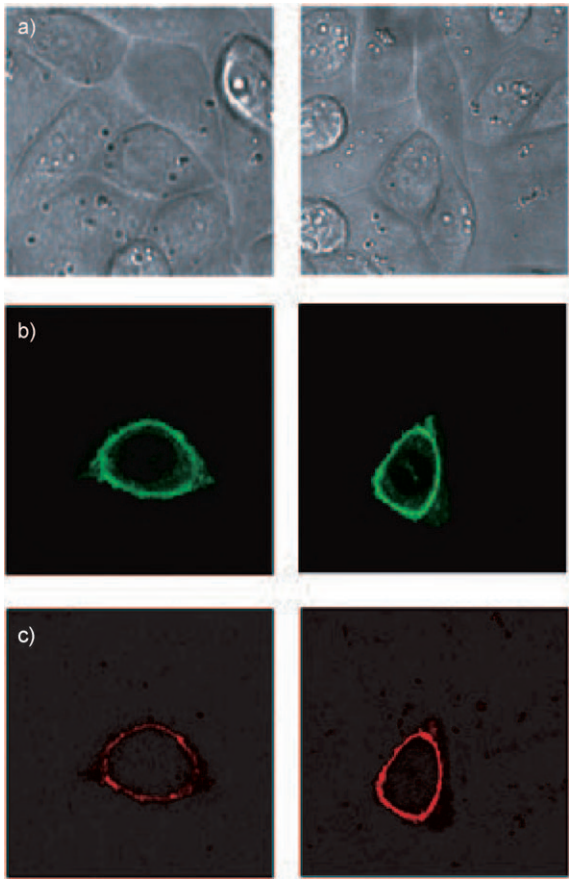

Figure 24. Visualization of coiled-coil interactions. a,b) Cells were expressed bearing a coiled-coil-tagged surface protein (green). c) Upon addition of the complementary peptide (left: three heptads, right: four heptads) the specific molecular recognition localized the peptide to the surface (red). The labeling was more effective with four heptad repeats on the probe peptide.[156]

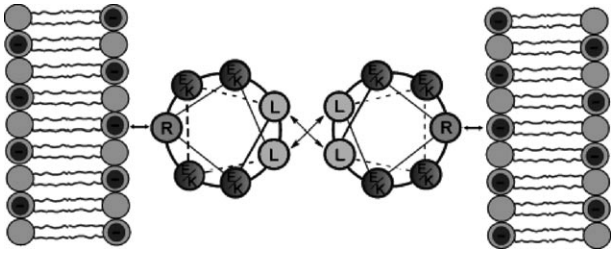

Figure 25. Coiled-coil folding and the interaction of the cationic coiled coil with negatively charged lipid membranes result in vesicle aggregation.[157]

liposome, analogous to the fusion sequence in viral fusion proteins.

The last example relies on molecular recognition of the same peptides as those in the cell-labeling example; in this case, however, they are not used to monitor a system but rather to effect large changes in a system. The peptides were connected by means of a short flexible spacer to a membrane-anchoring phospholipids. Structurally the lipopeptides are simplified versions of SNARE proteins, which are involved in lipid membrane fusion in vivo (see Figure 16 and Section 2.3). The lipopeptides were incorporated into liposomes, and by the formation of heterocoiled coils between liposomes the SNARE proteins caused liposome fusion that displayed the key characteristics of native membrane fusion (Figure 26).

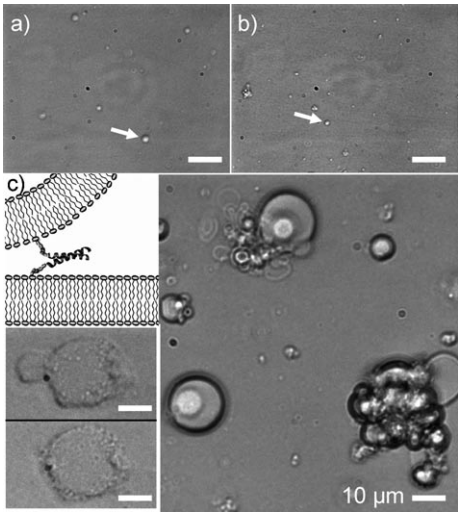

Figure 26. Simplified versions of SNARE proteins are embedded in liposomes and the formation of heterocoiled coils triggers liposome fusion. a,b) Optical microscopic images of two batches of large liposomes, each functionalized with one type of lipopeptide. Examples of liposomes are indicated by arrows. c) Upon mixing, giant liposomes are observed. Top inset: Two lipid membranes are connected through coiled-coil complexation. Bottom inset: Cell-sized liposomes are fused. Scale bars: 10 μm.[159]

Because this synthetic system is simpler than natural systems, long coiled coils are not necessary for the peptides to fulfill their function: the SNARE complex (four helices, six or seven heptad repeats each) and the lipopeptide complex (two helices, three heptads each) both induce fusion in similar manners. This fusion system extends the realm of synthetic biology, allowing one to understand an aspect of nature—liposome fusion in eukaryotic cells—through mimicry and also leading to new functions, such as the directed delivery of encapsulated reagents to cells or liposomes.[159]

4. Summary and Outlook

By combining the basic units of coiled coils—amino acids—in different sequences an amazing variety of coiled-coil units, assemblies, and systems are possible. Changing just two amino acids in a sequence can alter factors such as the binding strength of the coiled coil or the size of the hierarchical aggregate by many orders of magnitude. Owing to this extreme variability, coiled coils have developed over billions of years to perform a vast range of functions in every living cell. Coiled coils control the binding of cellular components, they form structural edifices of varying dimensions, and they have dynamic functions such as levers, force transducers, hinges, and clamps.

These many functions are fertile ground for the creation of synthetic biology systems, with the important benefit that the rules for mapping amino acid sequences to coiled-coil assembly are relatively well understood. There have been many investigations of coiled coils as units in which the binding specificity and stability have been studied. Based on and building upon this knowledge, the self-assembly of these units into higher order structures has been probed—assemblies composed wholly of coiled coils as well as those composed of coiled-coil hybrids. Also their dependence on environmental conditions has been examined. An area that has yet to be explored to any great extent is the combination of coiled coils or coiled-coil hybrids with other self-assembled structures in order to compose functional systems.

Although intricate and with a wealth of function, self-assembly as observed in nature is not always the best solution to a particular problem. By reverse-engineering nature we can develop methods for the construction of structures with a wider range of functions than those found in biology. We can construct coiled-coil hybrids that are unavailable in nature and investigate self-assembly by pathways that are not possible in natural processes. We can use the assembly of coiled coils in nature, as developed slowly over billions of years, as a "launch pad" to new areas of synthetic biology.

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