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REVIEW

Peptide and Protein Building Blocks for Synthetic Biology: From Programming Biomolecules to Self-Organized Biomolecular Systems

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ABSTRACT There are several approaches to creating synthetic-biological systems. Here, we describe a molecular-design approach. First, we lay out a possible synthetic-biology space, which we define with a plot of complexity of components versus divergence from nature. In this scheme, there are basic units, which range from natural amino acids to totally synthetic small molecules. These are linked together to form programmable tectons, for example, amphipathic α-helices. In turn, tectons can interact to give self-assembled units, which can combine and organize further to produce functional assemblies and systems. To illustrate one path through this vast landscape, we focus on protein engineering and design. We describe how, for certain protein-folding motifs, polypeptide chains can be instructed to fold. These folds can be combined to give structured complexes, and function can be incorporated through computational design. Finally, we describe how protein-based systems may be encapsulated to control and investigate their functions.

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In its broadest sense, synthetic biology is our attempt to understand nature through mimicry. This approach provides us with the opportunity both to develop novel biological systems and new functions, and to perform a rigorous test of our understanding of biology and how its various components assemble, interact, and function.

Given the elegance of structure and function in nature, developing synthetic-biological systems presents an enormous challenge. Nature employs a variety of basic molecular units (nucleic acids, amino acids, sugars, and lipids). These assemble into intricate and, often, hybrid molecular systems and machines. In turn, these are acted upon through evolution to generate new and improved functions. This path is mirrored in synthetic biology, where scientists can access a multilayered hierarchy of natural and synthetic components at various points in an attempt to piece them together into organized functional systems.

Given the large variety of potential (bio)chemical, structural, and functional starting points for synthetic biology, we place this Review within the wider field by highlighting some, but by no means all, of the possible and recently explored pathways through what we term *synthetic-biology space*. We then provide detailed strategies from work on peptide and protein folding, design, and assembly that can produce a selection of potential building blocks for synthetic biology. We begin by discussing the recent progress in the field of protein design that makes this route to synthetic biology possible. Second, we highlight the specific case of amphipathic α-helix-based tectons in design and give examples of how these may be used to create structures of increasing size and complexity. Finally, we suggest how such

components might be brought together to make functional hybrid systems comprising two or more paths through synthetic-biology space.

Approaches to Synthetic Biology. In Figure 1 we resolve potential components for synthetic biology according to their level of complexity (*i.e.*, the hierarchy illustrated on the *y*-axis) and the degree of divergence from the natural entities being mimicked (*i.e.*, an arbitrary measure of how "synthetic" they are on the *x*-axis). The basic units of natural biomolecules and therefore of biomolecular systems and cells are illustrated at the bottom left of this plot. At present, our abilities to synthesize, design, and engineer these different molecules are varied. For instance, oligonucleotides can be made rapidly, reliably, and cheaply and, if required, produced and amplified in bacteria using recombinant DNA technologies (Figure 1, path 1). By contrast, although encouraging headway is being made, equivalent syntheses and production of oligosaccharides are not yet available (*1*). As a result, synthetic systems based on DNA and RNA are more advanced than corresponding ones based on carbohydrates (*2*). Therefore, one challenge in synthetic biology is to increase the repertoire of chemistries that can be used efficiently and reliably in construction.

Moving up the complexity axis, we must assemble these biomolecules into *tectons*. We have adopted the term tecton from supramolecular chemistry, where it is used to describe programmed molecular components and nanometer-scale building blocks (*3*). For the nucleic-acid-based paths, a tecton would be a short oligonucleotide containing the information required for further assembly into double-stranded helices or other secondary structures (Figure 1, path 1). Similarly, in the parallel polypeptide pathway (path 8), a tecton could be programmed stretches of amphipathic α-helices or β-strands.

Combinations of tectons lead to the next level of hierarchy along the *y*-axis of Figure 1, self-assembled units. For oligonucleotides, base pairing between tectons leads to double-helix-based structures, which can be used as the basis to program the assembly of discrete nanostructures and extended materials (Figure 1, path 2) (*2, 4, 5*). Indeed, in terms of the underlying topic of this Re-

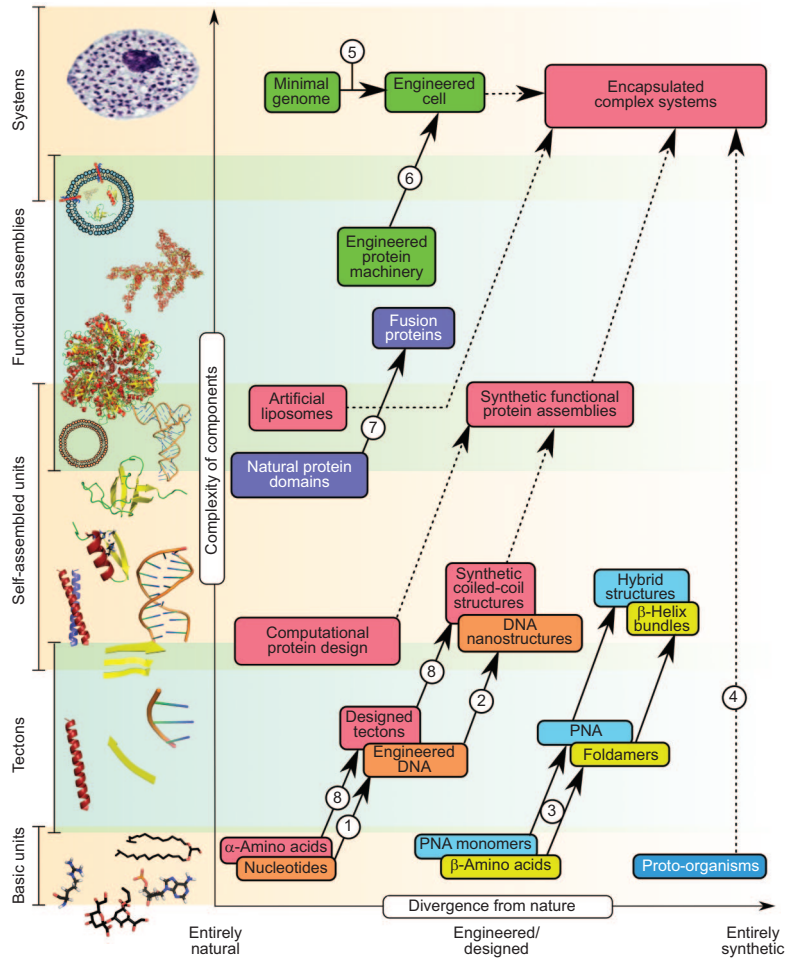

Figure 1. An outline of synthetic-biology space. A few of the routes currently being explored are mapped according to the position in the natural hierarchy from which the work stems and the level of divergence from nature that is being attempted. Path 1: the construction of engineered DNA, which allows manipulation at every level of the natural hierarchy. Path 2: the use of engineered DNA to produce novel nanostructures. Path 3: the development of nonstandard amino acids and base pairs, which can then be assembled into foldamers and DNA analogs. Path 4: the creation of alternative genetic systems. Path 5: producing minimal genomes (synthetic chromosomes) and transplanting them into prokaryotic hosts. Path 6: adding new functions to living organisms by manipulating cell machinery. Path 7: the fusion of proteins to produce assemblies with novel functions. Path 8: the use of peptide synthesis to create programmable building blocks that can assemble further into functional protein components. In combination with novel enzymes and an encapsulation mechanism, a complete path from basic natural building units all the way to synthetic, functional cell-like entities may be charted. Definitions for the terms used for the various components on the *y* axes are given in the text and in the Keywords. The axes are simply provided as a qualitative guide; no linear or other relationship is implied.

view—creating biomolecular assemblies and systems *de novo*—nucleic-acid-based assembly leads the way. For example, practitioners in this area of biomolecular design have demonstrated the construction of defined nanoscale objects and assemblies, extended crystalline lattices, and molecular machines. This area has been reviewed comprehensively recently (*2, 4, 5*). For amphipathic polypeptide tectons, these may combine through their hydrophobic faces (and, for β-strands, backbone hydrogen bonding) to form helical bundles and various β-sheets (*6*).

In these respects, our definition of tecton describes something more specific than a simple element of polypeptide secondary structure. Tecton implies that the polymer has been programmed to assemble locally (into a secondary structure) and that it has additional features to direct further assembly to prescribed higher-order structures. Similarly, though the definitions of self-assembled units and functional assemblies include the tertiary and quaternary structures of proteins, they are intended to imply more than just this. In these cases, they are meant to encompass not only natural structures but also newly designed assemblies, for instance, protein-like fibers made from novel tectons, and hybrid assemblies and materials.

Other starting points for synthetic biology that increasingly diverge from these natural units are also possible (Figure 1, path 3). For example, peptide nucleic acids (PNAs) (*7*), which combine the base pairing of natural nucleic acids with polyamide backbones to make self-assembling polymers, might be considered to fall midway along the *x*-axis. β-Amino-acid-based foldamers, which are receiving increased attention because of their well-defined secondary structures (which therefore are potential tectons) and resistance to proteolysis (*8–10*), would lie slightly further along the axis.

Further from natural basic units, at the right-hand side of Figure 1, several adventurous studies aim to produce completely novel building blocks (path 4). The principles of replication in these systems are analogous to those found in natural systems but on a much more modest scale (*11*). An excellent example is the so-called Protocell project, which aims to produce a minimal self-replicating system from an entirely artificial set of basic units in a manner that closely mimics nature. In this system, a complex series of photocatalyzed reactions is used to metabolize a simple "food" molecule into new encapsulation and genetic material, to the point where division of the capsule occurs and the cycle restarts. Thus, these reactions are able to act in concert to produce a minimal self-reproducing machine enclosed in a lipid membrane (*12, 13*).

Of course, it is also possible to enter synthetic biology further up the complexity scale in Figure 1. At the cellular level, whole chromosomes can now be made and transplanted into hosts (path 5) (*14, 15*); we call these *genome-engineering approaches*. Further down this scale, functional assemblies can be engineered and introduced into cells to provide organisms with new functions and pathways (path 6) (*16*). A further layer down in the hierarchy is path 7, where fusing native or mutated proteins together can create synthetic functional assembles (*17*). Indeed, many such functional units are being formulated and cloned from biology by paring down large, multidomain proteins to their functional components through the BioBrick project (www.biobricks.org). Collectively, we refer to these as *biomolecular-engineering approaches* in synthetic biology.

Another pathway and the topic of the remainder of this Review might be termed the *molecular-design approach* (path 8). Here we discuss approach with an emphasis on work with polypeptides and proteins.

Why Polypeptides? We describe the use of *de novo* designed peptides and engineered proteins as possible, indeed major, components in synthetic biology (Box 1). In natural biology—aside from harboring, transferring, and translating genetic information, providing the universal currency of biological energy, and encapsulation—proteins do pretty much everything required. Moreover, peptides and proteins can now be made reliably, quickly, and cheaply, either synthetically (*18*) or recombinantly. Protein modification, and in particular conjugation of other biomolecules and functional groups, is more taxing, but issues in this area will likely be resolved by synthetic and/or recombinant approaches. Indeed, advances in synthetic peptide chemistry, notably the so-called chemical ligation methods, are having a big impact here (*18*). The remaining and key obstacle is in linking polypeptide sequence to 3D structure; that is, the informational aspect of the protein-

KEYWORDS
Synthetic biology: The attempt to understand nature through mimicry, and to create new functional bio-inspired systems.
Basic units: Small natural or synthetic molecular building blocks.
Tectons: Molecular components usually built *via* the polymerization of basic units and programmed to fold and/or assemble further to prescribed 3D structures.
Self-assembled units: Combinations of tectons that adopt defined reproducible structures.

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Box 1. Why peptides and proteins?

1. Polypeptide chains, that is, peptides and proteins, are readily accessible through synthesis from their constituent amino acids or heterologous expression in bacterial hosts using recombinant DNA technology.

2. All of the information required for the folding and function of a polypeptide is usually encoded within its sequence.

3. Unfolded polypeptide chains, correctly instructed through point 2, usually fold spontaneously and efficiently in milliseconds to form well-defined 3D structures.

4. The folding process is usually faithful (reproducible and robust) in that one polypeptide sequence usually leads to one stable 3D structure.

5. The folded structures show considerable organization, including secondary structural elements such as the α-helix and β-strand (referred to here as tectons), which indicates underlying sequence-to-structure relationships (or rules) for folding. This naturally limits the possible number of so-called protein folds (*21, 22*). Nonetheless, various possibilities exist, which give many different structural scaffolds to display potentially many different biological functions.

6. The folded structures often self-assemble further to form higher-order complexes and nanomachines that are the functional entities of cells and tissues.

7. As hinted at in point 4, many different protein structures and functions exist that can be imported into or provide inspiration for synthetic biology.

The above list does have caveats, hence the qualified language: protein function is often supplemented by nonproteinaceous cofactors, and folding in biology is often steered and aided by chaperone proteins, adding another tier of complexity. Nonetheless, we see the inclusion of cofactors and chaperones as later events, if required at all, in the development of synthetic biomolecular systems through the molecular-design approach.

folding problem. However, for certain peptide- and protein-folding motifs, we do have good "rules" that relate covalent and 3D structure, and these allow increasingly ambitious protein-design targets to be tackled. The breadth and potential of peptide and protein science in cell biology and bionanotechnology are captured wonderfully in recent academic texts and popular-science books (*19, 20*). Here, we focus on specific challenges, solutions, and aspirations in the design and engineering of self-assembling peptides and functional proteins and how these might apply to synthetic biology.

Rational Peptide and Protein Design. Peptide and protein design is a maturing field, which has delivered rules and computer algorithms that allow the successful design of new protein structures and assemblies and the incorporation of new activities into natural protein scaffolds. The question is, can the design field take the next step from producing a basis set of components to constructing self-organized, dynamic, and functional biomolecular systems?

Unlike with nucleic acids, we cannot read or write down polypeptide sequences straightforwardly to predict their 3D structures and functions or to design new proteins from scratch. However, we note that much insight has been gained through homology between protein sequences; evolution navigates workable pathways through protein-sequence-and-structure space, which in principle allows proteins to be related and common structures and functions to be uncovered. This enormous wealth of information is being captured in various databases such as CATH, SCOP, PFAM, and others (*21, 23, 24*). We can use the information from these databases to obtain consensus sequences for a variety of simple protein folding motifs, for example, zinc fingers, collagens, and coiled coils (Figure 2). Certain zinc-finger motifs share a clear signature, a consensus sequence, that directs folding to a common structure, although in this case, folding of the polypeptide chain is dominated by it binding zinc. Colla-

KEYWORDS

Functional assemblies: Higher-order associations of self-assembled units that confer complex structures or functions.

Systems: Collections of functional assemblies that can perform multiple/complex chemical reactions. These are usually encapsulated, but they do not necessarily need to be.

Synthetic-biology space: A hierarchy of the above components.

Path: A route through synthetic-biology space, for example, from a basic unit, or some other "sensible" starting point, toward an organized and/or functional system.

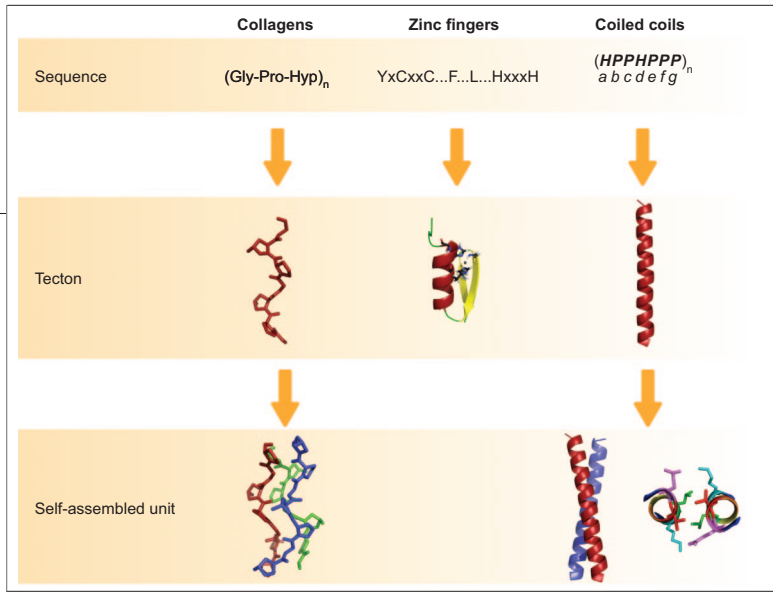

Figure 2. Sequence-to-structure relationships for selected protein folding motifs. The consensus sequences and folding hierarchies for three straightforward protein folds. Collagens: the consensus sequence of (Gly-Pro-Hyp) folds into a polyproline II helix, which then supercoils into the collagen triple helix. Zinc fingers: here the consensus sequence contains the metal-binding amino acids that direct and indeed dictate chain folding by coordinating zinc. Coiled coils: heptad repeats of hydrophobic and polar residues leads to the formation of amphipathic α-helices. The hydrophobic faces of two or more such helices then bundle to form coiled coils. The interaction is mediated through the "core" amino acids (*a*, *d*, *e*, *g*), which are highlighted in sticks, leaving the exposed amino acids (*b*, *c*, *f*) available for functionalization.

gen chains have a tripeptide repeat, Gly-Xaa-Yaa (where Xaa is usually proline and Yaa is usually hydroxyproline), which adopts a polyproline II conformation, and three such chains wrap around one another to form the collagen triple helix.

Other examples include the work of Regan and of Plückthun (*25–29*), who demonstrate the considerable possibilities for making repeat proteins—tetratricopeptide repeat (TPR) and ankyrins, respectively—based on consensus sequences and the cloning and expression of tandem repeats. This has been used to create stable constructs for fundamental protein folding studies, as potential new biomaterials (*27*), and as scaffolds that can be decorated through mutagenesis to give libraries from which new functional binding entities can be selected to rival antibodies (*29*).

In another approach to protein design, Baker and colleagues (*30–32*) describe the design of a new protein structure using their Rosetta Design algorithm. This not only demonstrates the state of the art in computer-assisted globular protein design but also shows that nature's protein-structure space, or at least our current view of it, can be successfully supplemented. In a series of elegant examples, Hellinga's group shows that natural proteins, for instance, the periplasmic binding protein (PBP) from *Escherichia coli*, can be used as chassis for the display of novel functions (*33*). This is done by assessing sites on the protein's surface that might be suitable for harboring the target function and then computationally searching many different side-chain combinations at the site ahead of producing the proteins for experimental validation. Successful functions targeted thus far include various metal-binding sensors (*34*) and an enzyme with triose phosphate isomerase activity (*35*). Along with others, Ambroggio and Kuhlman (*36–41*) challenge the dogma of "one sequence, one fold" to show that peptides can be designed containing two overlapping protein-folding motifs. This allows the peptides to switch their conformational state in response to external stimuli. Finally, a collaboration between Bennett and DeGrado (*42*) has led to the computational design of peptides that target transmembrane helices. In all of these cases, the computational designs have been made and verified either with complete structure determinations or with impressive arrays of biophysical and biochemical assays. This small number of examples gives insight into some of the exciting possibilities in the area of computer-aided peptide and protein design and engineering. Doubtless, these methods will be key to building new structural and functional components in synthetic biology.

The α-Helical Coiled Coil: A Basis for More-Complex Designs. The α-helical coiled coil makes an excellent starting point for the design of peptidic tectons and self-assembled units for several reasons. First and foremost, despite a similar underlying repeat pattern in their sequences, coiled-coil motifs show a surprising richness in structure and function (*43, 44*). Second, there is considerably more subtlety in their sequence-to-structure relationships than for some of the other aforementioned motifs. Third, protein-design rules elucidated thus far have led to a variety of successful coiled-coil designs, though as with most protein-design efforts, the routes to these successes have not always been straightforward and direct. Finally, coiled-coil structures can be functionalized *via* "spare", solvent-exposed amino acids.

The central tenets of coiled-coil sequences, folding, and assembly are illustrated in Figure 2. In essence, most coiled coils are based on the heptad sequence repeat, which has seven residues often labeled **abcdefg**. The first and fourth positions (**a** and **d**) are usually occupied by hydrophobic (**H**) amino acids. With the remaining sites largely polar (**P**), the resulting pattern, **HPPHPPP**, sets up the potential for an amphipathic α-helix. In water, two or more such helices can then combine to bury their hydrophobic faces. Because the

averaged spacing between **H** residues (3.5) and the helical repeat of the α-helix (3.6) do not match, the hydrophobic seams wind slowly around the faces of each helix, and in order to pack, the helices must do so at an angle (Figure 2). The result is a rope-like assembly that measures ~1 nm per heptad, which provides a useful metric for design in bionanotechnology and synthetic biology. Coiled-coil regions in proteins can span tens to hundreds of amino acids and, hence, lengths in the nano-to-submicrometer range.

This all seems very straightforward. The catch is that the hydrophobic effect that drives this process is not at all specific. As a result, two or more helices can come together to form stable bundles. Indeed, a wide variety of coiled-coil architectures (number of helices in the bundle) and topologies (relative helix orientation) are seen in nature (*45–47*). This gives a range of what we term "classical" and "complex" coiled-coil assemblies (Figure 3). To make use of coiled-coil helices as tectons, we must be able to control both their oligomerization state and partner specificity to make discrete self-assembled units.

Various groups have contributed to understanding the oligomer-state problem (*48*). The most influential work comes from Harbury and colleagues (*49, 50*), who engineered multiple changes at the **a** and **d** sites of an otherwise natural leucine-zipper sequence. The resulting Harbury rules are summarized in Box 2. Essentially, different combinations of the side chains isoleucine (I) and leucine (L) at the **a** and **d** positions in heptad-repeat sequences lead to different oligomer states, for example, dimer, trimer, and tetramer. These rules are all the more powerful because Harbury crystallized all three forms and derived stereochemical explanations for oligomer-state selection. In nature, dimeric and trimeric coiled coils predominate, and interestingly, the rules are largely seen in natural sequences (*51*). The natural sequences are understandably more diverse and provide additional rules. For example, supplementing the occasional *a* site with asparagine, a destabilizing polar substitution, further specifies dimer (*52–54*). Interestingly, this particular rule can be used to very good effect to specify and stabilize the association of transmembrane helices (*55, 56*). There are almost certainly more hidden rules in natural sequences that specify the degree of association in these structures.

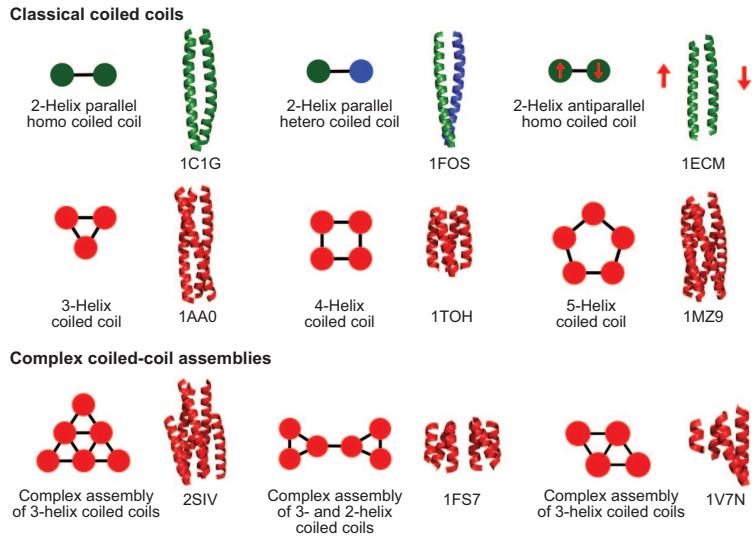

Figure 3. Selected coiled-coil architectures and topologies. The first row shows some of the topologies, for example, parallel, antiparallel, and heterotypic, for two-helix coiled coils. The second row gives other architectures for classical coiled coils, for example, trimer, tetramer, and pentamer. The third row shows some representative complex coiled-coil assemblies. For each case, a schematic cross-section and a ribbon picture (PyMOL (*121*)) of the coiled-coil structure is given. The Protein Data Bank identifier for each protein is given: 1C1G (*122*), 1FOS (*123*), 1ECM (*124*), 1AA0 (*125*), 1TOH (*126*), 1MZ9 (*127*), 2SIV (*128*), 1FS7 (*129*), and 1V7N (*130*).

In addition to oligomer-state specification, there is the problem of how different coiled-coil chains are brought together to specify heterotypic assemblies. Again, certain combinations of residues at **a** (and possibly **d**) contribute to this specificity (*57–62*). However, in the design arena attention has focused on using oppositely charged residues at **e** and **g**

Box 2. Simple parallel coiled-coil designs

Oligomer state	**Sequence** gabcdef
2[a]	$(KIAALEQ)_{n \geq 3}$
3	$(KIAAIEQ)_{n \geq 3}$
4	$(KLAAIEQ)_{n \geq 3}$
Heterodimeric pair[a]	$(KIAALKQ)_{n \geq 3}(EIAALEQ)_{n \geq 3}$

[a]Dimers can be specified further by introducing asparagine at **a** at a rate of ~1 every 4 heptads.

in the complementary chains as these straddle the hydrophobic core and can form inter-helix salt bridges to further cement coiled-coil interfaces (Figure 2).

An important aspect of this work is the use of negative-design principles to avoid unwanted peptide combinations and alternative coiled-coil topologies (*63*). This concept of negative design is key in successful protein designs, because even if they do fold, there is considerable potential for designed polypeptides to form molten-globule ensembles (*64*) or to find alternative free-energy minima (*e.g.*, the fact that the simple **HPPHPPP** coiled-coil pattern is compatible with many different 3D arrangements of helical tectons).

Finally, the design rules outlined above center on the **a**, **d**, **e**, and **g** sites of the heptad repeat, which stands to reason because these lie at the heart of the coiled-coil structure (Figure 2). For relatively simple designs, the remaining sites are usually kept polar and helix-favoring, for example, combinations of alanine, glutamine, glutamate, and lysine (*48, 63, 65*). In this way, designed sequences for simple, parallel homodimers, trimers, and heterodimers can simply be written down (Box 2, *54*). In addition, the **b**, **c**, and **f** sites can be used to guide higher-order coiled-coil structures, as seen in nature (*46, 66*), or developed through designs (*67–70*). Furthermore, these sites can be used to "decorate" existing designed coiled-coil scaffolds, such as designed, coiled-coil-based fibers (*71*). Through this potential for functionalization, one can envisage moving up the complexity scale of Figure 1 to build bioinspired multicomponent systems. Some of the possibilities in this area are outlined below.

Hubs and Spacers. The wide variety of coiled-coil structures (Figure 3) presents both challenges and opportunities in developing self-organizing biomolecular systems. The challenge is that we still have to distill rules that distinguish the various structures. As highlighted above, such rules are likely superimposed on the basic heptad repeats of the natural proteins. Thus, it should be possible to use bioinformatics further to compare sequences of the different natural coiled-coil structures to garner such rules (*48, 72*). The opportunity for synthetic biology is that different coiled coils could be used as hubs and spacers to bring together bioactive components with defined stoichiometries and orientations and at set distances with nanometer precision.

In addition to straightforward structures, other more-complex coiled-coil systems have been developed that might provide hubs for synthetic biomolecular systems and a future synthetic biology. For example, building on from early designs of heterodimeric coiled coils (*65, 73–77*), Alber et al. (*63*) present the ABC trimer in which three different peptide chains are brought together to form a parallel heterotrimer of defined chirality. The design was developed using a computer algorithm employing positive- and negative-design principles to select the three peptides from >16 million combinations of 256 possible starting sequences. The selected peptides differed in charge arrangements at 12 **g** and **e** sites of an otherwise standard 4-heptad trimeric coiled-coil design (Box 2). Biophysical characterization of the peptides culminated in a crystal structure confirming the design (*78*), which provides a firm footing for future hub-based protein engineering.

Inspired by work using DNA-based linkers (*79*), two groups have designed nanoscale linkers based on ternary (*80*) and binary (*81*) coiled-coil assemblies; these have been used to control the aggregation of nanoparticles at set nanometer spacings. Others demonstrate that coiled coils can be used as hubs to bring together and so increase the efficacy of antibodies (*82*).

Fibers and Tracks. Biology makes considerable use of protein-based fibrous materials. For example, in the eukaryotic cytoskeleton, intermediate filaments (which are coiled-coil based) and actin fibers provide strength and shape to what would otherwise be ill-defined and unruly cellular entities, the controlled and localized assembly and disassembly of actin fibers underpins the main mechanism for cell locomotion, and microtubules provide tracks for various motor proteins to ferry efficiently protein and vesicular cargoes through cells. Outside the cell, the main protein-based scaffold is collagen, which provides strength and structure to all eukaryotic extracellular matrices, thus strengthening, binding, and defining organs and tissues such as skin.

In this decade, considerable progress has been made in designing fibrous biomaterials from self-assembling peptide-based tectons. For instance, several groups have used the approach of "sticky-ended" coiled coils and, more recently, collagen peptides to assemble fibrils and stiff rods that span the nanometer to micrometer regimes (*71*). The structural organization within some of these fibers is now being established (*69*). This knowledge, combined with using simple,

chemically accessible tectons, has led to the engineering of a variety of morphologies, the incorporation of various functions, and the tuning of properties such as stability and pH response (*83, 84*). For example, peptides have been engineered to render kinked, branched, and linked fibers (*85–87*), they have been decorated with binding peptides and proteins and hence nanoparticles (*88*), and their assembly has been controlled and even made switchable through rational redesign of the tectons (*84, 89*). The recent development of self-assembling peptides for the construction of collagen-like structures is chemically more demanding and has required further innovation in peptide chemistry and persistence (*90, 91*). These advances are particularly important as collagen-based fibers also have potential applications in the development of fibrous structures that mimic the ECM for 3D cell culture and tissue engineering (*92*).

There is another solution to making peptide- and protein-based fibers, namely, to use amyloid-like structures, which essentially comprise β-strand tectons in our scheme. Indeed, this is a large and active research field (*93–96*), and the fibers produced have potential applications in areas as diverse as molecular electronics (*96–98*) through tissue engineering (*99–103*). One feature of these systems is that they tend to gel, particularly at high peptide concentrations, which does lead to broad potential applications in wound healing and regenerative medicine, cosmetics, and personal health. However, this property may present difficulties for incorporating amyloid-based assemblies in synthetic biology systems.

Moving more to the right-hand side of our scheme of Figure 1, one of the most notable examples of semisynthetic biocompatible fibers comes from Stupp et al. (*104*). These workers have developed peptide amphiphiles in which polar peptides (usually cell-binding RGD-based sequences) are linked to alkyl chains *via* a cysteine-rich linker. These assemble into cylindrical micelles in which the alkyl chains are sequestered in the core, around which the cysteine side chains form a crossed-linked corona, leaving the polar peptides exposed on the surface. Various chemistries can been appended to this framework, and the fibers have been used as scaffolds in a number of successful tissue-engineering applications (*105*).

Many challenges lie ahead in this area of peptide-based and synthetic fibrous materials, notably, the decoration of the structures with functional entities and the issues of recombinant production and biocompatibility, if these materials are to find their way into regular medical use.

Controls, Switches, and Self-Replication. In addition to being able to control higher-order assemblies by using binary and other multicomponent designs (*89*), an ability to control these by changing conditions will be key to developing synthetic-biology systems that can sense and transduce signals from their environment. Work in this direction is progressing for the aforementioned fibrous systems (*84*). As hinted at above, other peptide designs are now being explored in which two folding motifs are superimposed within one polypeptide sequence. This has afforded coiled-coil-based conformational switches that respond to heat (*36, 37*), disulphide-bond reduction (*38*), and metal binding (*39, 40*). This area has recently been reviewed (*41*). Finally, a key theme for synthetic biology will be the development of self-replicating peptides. Notably, the groups of Chmielewski and Ghadiri have developed self-replicating peptides based on coiled coils (*106–108*).

Encapsulating Complexity. One possible framework for developing the above concepts and tackling the challenges outlined would be to consider the design and engineering of self-organizing, encapsulated systems from self-assembling components. These would be multicomponent and compartmentalized. They would be nonreplicating systems, further distinguishing this approach from genome- and biomolecular-engineering approaches to synthetic biology. Such a challenge will necessarily draw on expertise in design, engineering, and characterization of peptide, DNA, and membrane systems and the modeling of complex systems. Target functions for such encapsulated systems could include the ability to sense and transduce signals from their environment and the ability to generate new materials, biofuels, or drug molecules in a controlled manner.

The Need for Compartmentalization in Biology and Synthetic Biology. Compartmentalization is a key feature of all biological systems from viruses through bacteria to yeasts and higher organisms. Such subdivision allows multiple and different chemical reactions and higher-level functions to be conducted efficiently, that is, simultaneously and without entanglement. Indeed, along with the abilities to metabolize, replicate, and evolve (*109*), one might add compartmentalization to the list of defining features of biological systems. This

comes at a price, however, as the barriers defining the compartments have to be bridged to allow nutrients in, waste and defense molecules out, and signals across. As a result, a myriad of biomolecules, including peptides, proteins, carbohydrates, lipids, and hybrids of these, have evolved to provide these functions at or within the barriers. Indeed, from genome sequences it is now clear that integral-membrane and membrane-associated proteins comprise about one-third of most proteomes.

Clearly, synthetic biology must learn from this and adopt strategies to develop and use compartmentalization. For the genome- and biomolecular-engineering approaches, the strategy is clear and pragmatic: use a natural cell, usually from a bacterium, as a host. The host provides the compartment(s), raw materials, and additional infrastructure to allow the production, expression, and reproduction of the introduced synthetic elements. In this way, orthogonal functions can then be added, or existing cellular functions rewired through protein engineering and the introduction of new DNA (*16, 110*). In the molecular-design approach, however, there is a greater choice of encapsulation methods.

Methods of Encapsulation. One can imagine many mechanisms to achieve encapsulation of (bio)molecular systems; however, nature almost exclusively uses lipids as its primary basic unit for encapsulation and compartmentalization. Lipids assemble into higher-order structures, specifically bilayers, in an aqueous environment. Although lipid bilayers are typically only of the order of a few nanometers thick, their nonpolar cores present near impermeable barriers to the passage of polar or charged species; in nature, as noted above, these barriers are bridged effectively by integral-membrane proteins.

It is now routine to isolate or synthesize natural lipids and combine them in the correct proportions to form small aqueous volumes encapsulated by a lipid bilayer analogous to natural cell membranes to give liposomes or vesicles. It is also possible to encapsulate reagents within these and carry out reactions and gene expression (*111, 112*). In order to progress from simple nanoscale chemistry to true synthetic biology, ways must be found to transport reagents selectively across membranes. Again, the trick would be to learn from biology and create or borrow molecules to give selective permeability, and many groups are investigating this problem.

Although the majority of natural encapsulation is accomplished with the use of lipid membranes, plants and bacteria also have a second layer of encapsulation, provided by a matted sheet of fibrous polysaccharide material. A synthetic analog to this polymeric cell wall is provided by the relatively recent advent of polymersomes (*113*). These polymeric encapsulating layers can be formed *via* a variety of methods, but all are distinct from the lipid bilayer paradigm and analogous to the cell wall *via* the extensive covalent cross-linking within the membrane. Polymersomes are generally endowed with increased stability relative to their lipid counterparts and also less permeable, though again, selective permeability can be introduced (*114–116*).

Engineers of synthetic-biological systems are not necessarily restricted to working exclusively in the aqueous environment occupied by natural systems (*117*), which enables a second, entirely separate mode of encapsulation: using emulsions. For instance, Bayley and colleagues (*118*) have used water-in-oil emulsions to trap and study biomolecules, such as protein ion channels. By bringing together two lipid-stabilized water droplets in the emulsion, a lipid bilayer is formed between the droplets. Membrane-active molecules—in Bayley's example, ion channels formed by the protein hemolysin—can then be introduced into the bilayer through the aqueous phase and their activities recorded *via* electrodes embedded within droplets of the network. Bayley has also described how these emulsions may be manipulated with careful control of the contents of the various droplets to form a number of novel, functional systems, such as photovoltaic devices based on ion currents generated *via* light-driven proton pumps inside the droplets. Griffiths *et al*. take the use of emulsions further from examples of encapsulation in nature with the encapsulation of enzyme systems generated through *in vitro* translation in the aqueous phase of a water-in-perfluorinated hydrocarbon emulsion. Impressively, such systems allow the evolution of enzyme function in a microfluidics format (*119, 120*). The ability to introduce evolution into such systems is a key step toward true synthetic biology systems (*109*).

Conclusion. Synthetic biology is an emerging and exciting area for research. It encompasses many potential approaches in the bid to create complex, functional, bioinspired systems. At one extreme, there is the syn-

thesis of whole chromosomes, which may be transplanted into host cells to create new minimal living organisms. At an intermediate level, functional groups or cascades of natural biomolecules (nucleic acids and proteins) can be engineered into cells to elicit new phenotypes and functions. At another level down in this hierarchy, raw but nonetheless natural building blocks (nucleotides and amino acids) can be used to engineer polymers. These can be programmed to create self-assembling and functional biomolecular components, which in turn can be combined to create functional systems. Digressing further from natural systems, semi- and totally synthetic approaches to synthetic biology can be used to create interacting components and, hence, functional systems. The ambition and success of this approach rest only on our imagination and ability to synthesize new molecules. This Review has been concerned with the third approach in synthetic biology. Specifically, we have attempted to illustrate one possible route through the vast potential synthetic-biology space with examples in peptide and protein designs. Of course, this is not the sole approach, and polypeptides are not the only (bio)molecular building blocks. Nonetheless, by choosing this focus, we hope to have conveyed some sense of the order, potential hierarchy, and schemes needed to approach and deliver successful synthetic-biology systems. We anticipate that our improving ability to relate protein sequence to structure and function and, with this, improvements in generating new and engineered proteins will feed the growing effort in peptide- and protein-based synthetic biology.

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