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# Computational design of protein self-assembly

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Protein self-assembly is extensively used in nature to build functional biomolecules and provides a general approach to design molecular complexes with many intriguing applications. Although computational design of protein–protein interfaces remains difficult, much progress has recently been made in *de novo* design of protein assemblies with cyclic, helical, cubic, internal and lattice symmetries. Here, we discuss some of the underlying biophysical principles of self-assembly that influence the design problem and highlight methodological advances that have made self-assembly design a fruitful area of protein design.

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## Introduction

Much of the molecular complexity of life is formed by self-assembly of protein monomers into higher-order oligomers. Self-assembly is thus a powerful design template to create complex molecular assemblies from a limited number of building blocks. In the simplest case a single type of building block is sufficient to generate homomeric structures with complex morphologies such as rings, filaments or containers. These assemblies enable functions such as multivalent binding, ultrasensitive regulation and compartmentalization and are therefore ubiquitous in biology [1]. If controlled, the functions encoded by self-assembly in natural systems could be replicated or extended to novel applications in biotechnology, biomedicine and material science [2].

The quaternary structure of self-assembling proteins is stabilized by protein–protein interfaces. Accurate design of protein interfaces is therefore required to control self-assembly. Beyond the design of the simplest α-helical assemblies, computational methods are necessary to explore the vast space of protein interface sequences. Computational methods are also necessary to find suitable building blocks and binding geometries when designing complexes of novel components and assembly structures. Until recently, *de novo* protein interface design was primarily directed towards heterodimers but in the past few years tremendous progress in design of higher-order protein assemblies has been made. The advancements can be attributed to new design methodologies, but the biophysical properties of self-assembling proteins and peptides also make them particularly amenable to design.

We begin our review with a description of the biophysical properties of self-assembling homomeric systems and their implications for design. We then review the recent advances in designing homomeric self-assembly using computational methods. Finally, we describe some of the future challenges in the field.

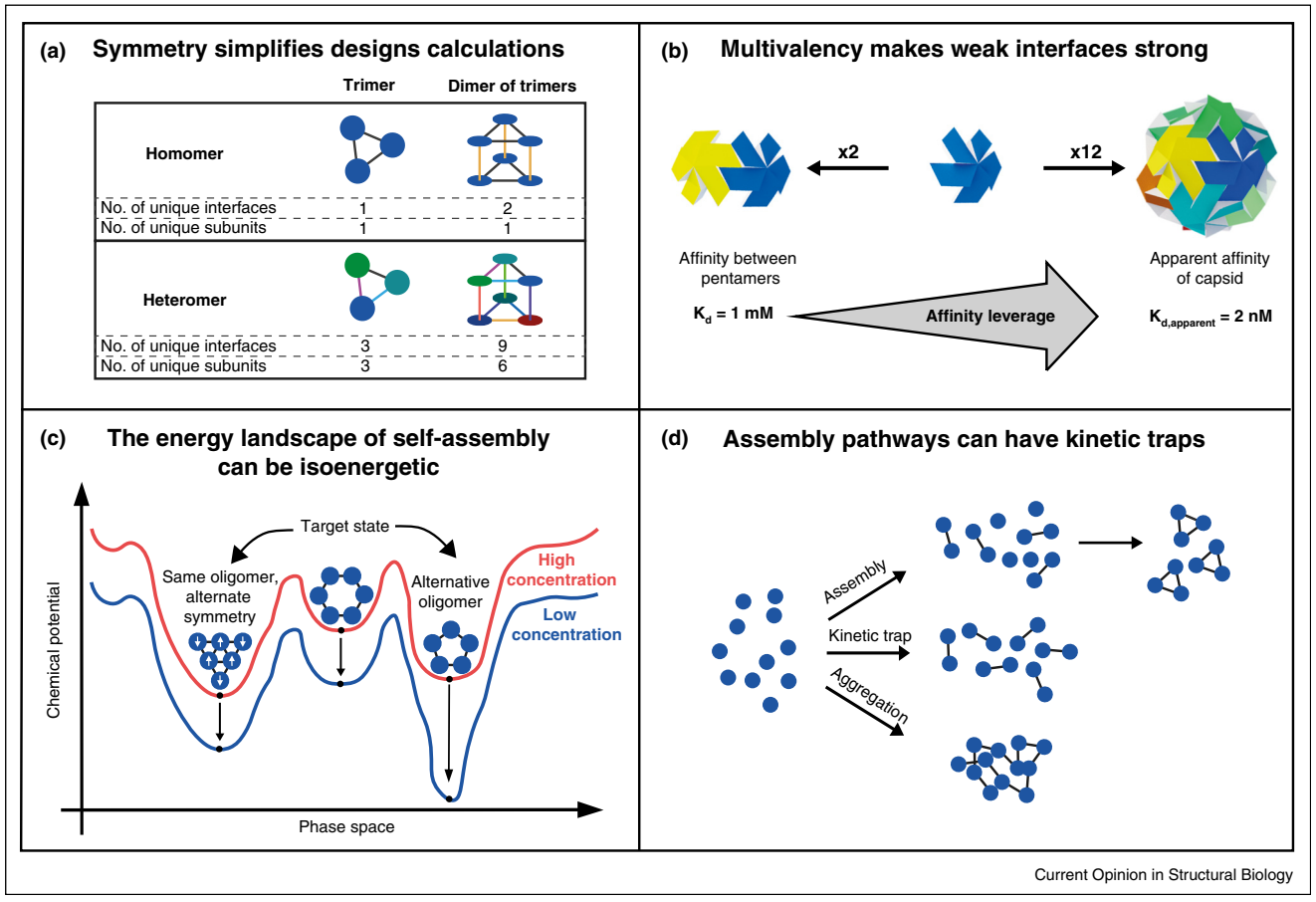
## Biophysical properties of protein self-assembly

Design of protein complexes is simplified by fundamental physical properties of self-assembling systems, which may partly explain why this has been such a fruitful direction in protein design. In this section we briefly consider properties that simplify and set unique challenges in design of protein assemblies.

The vast majority of all homomers have nearly perfect structural symmetry [1]. This simplifies modeling and design calculations as we can assume that homomeric complexes are made up of building blocks with identical structure and interactions (Figure 1a) [3]. Furthermore, symmetry also strongly limits the number of ways that protein subunits may associate in three dimensions. Because interfaces are replicated by symmetry, fewer independent residues must be designed in homomers compared to heteromers or asymmetric homomers. Limiting the number of residue changes in the protein building block is crucial, as the underling assumptions for design (e.g. rigid backbone) are more likely to fail, when more residues are changed.

A second property that favors design of self-assembling structures is avidity. Avidity yields stable complexes from building blocks with weak interfaces. This is for instance illustrated in homomeric icosahedral protein capsids, which can be thought of as assemblies of multivalent cyclic symmetric building blocks. Here the avidity lets the capsids assemble with an apparent stability that is six orders of magnitude higher than the affinity between the individual cyclic symmetric building blocks (Figure 1b)

**Figure 1**




Biophysical principles of protein self-assembly. Biophysical principles works both for and against design of self-assembling protein complexes. **(a)** Symmetry simplifies the design calculations by limiting the number of subunits and interfaces to be considered in design calculations. **(b)** Multivalency yields stable assembly formation from weak protein–protein interfaces. The apparent disassociation constant is defined as the concentration where the building block and the capsid concentration are equal. **(c)** Design of oligomerization specificity is complicated by isoenergetic energy landscapes between different symmetries and oligomers. Further complicating design is that the relative stability of different oligomers is concentration dependent. Finally **(d)** assembly of oligomers is complicated by the possible formation of kinetic traps and aggregates during assembly.

[6,7]. As it remains challenging to design high affinity interfaces by computation alone, the affinity leverage provided by avidity provides a key benefit in the design of higher-order symmetric homomers.

Not all biophysical properties of self-assembling systems help in design. For instance, while it has been argued that the folding energy landscape of oligomeric systems is favorable due to symmetry and avidity [4], one must also consider the complete self-assembly landscape, which includes structures of alternative oligomerization state. These alternative states are often separated by small energy gaps [7–9] that are on the order of the accuracy of the potential energy functions used in design, and this makes it hard to ensure specificity in the oligomerization state (Figure 1c). Furthermore the use of traditional energy functions might not be sufficient, as the relative stability between oligomerization states is concentration dependent and additional entropic terms, such as loss of rotational and translation degrees of freedom, should be taken into account [5].

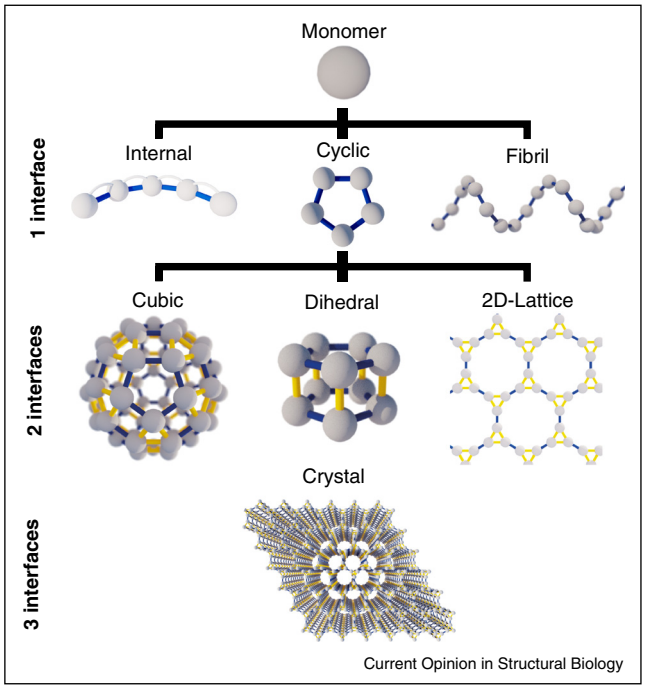
Another factor that complicates self-assembly design is that the target-state might be under kinetic [6], rather than thermodynamic, control. Indeed compelling evidence suggests that natural assemblies have been evolved to avoid kinetic traps, aggregation of intermediates and off-pathway states. For instance, in virus capsid assembly, differentiated interface strengths lead to assembly without kinetic traps [7] and the presence of weak interfaces allows error-correction during capsid formation [8]. Kinetic traps can even occur in very simple systems like

homotrimers [9] (Figure 1d). Thus, to increase the success-rate of design it may be necessary to explicitly optimize the assembly pathway.

## The hierarchy of self-assembly morphologies

Three major classes of symmetries have been designed so far: Internally symmetric repeat proteins and systems with open or closed symmetries. These categories can further be divided into subgroups depending on the minimal number of interfaces that need to be designed (Figure 2). This provides a hierarchy of self-assembly morphologies that increase in complexity of design as one descends in the hierarchy. Other factors influencing design complexity are the number of rotational and translational degree of freedoms allowed by the desired symmetry, the number of competing assembly states with similar stabilities, and complexity of the association pathway. In the following we discuss the different symmetry subgroups that have been designed and discuss methods applied to reduce design complexity.

**Figure 2**




Hierarchy of self-assembly. Self-assembling complexes can be categorized in groups according to the minimal number of different interfaces that has to be present to assemble. Simple symmetrical proteins (internal, cyclic and fibrils) can be generated from monomers by design of a single interface. With design of a second interface, more complex symmetries (cubic, dihedral and 2D-lattices) can be generated from cyclic symmetrical building blocks. Protein crystals can be generated from monomers with design of three interfaces. Examples from each symmetry category are shown.

## Cyclic symmetries provide the basic building block for self-assembling systems

As illustrated in Figure 2, the cyclic symmetries are the building blocks for all higher order symmetries. For *de novo* design of higher order symmetries it is therefore crucial to master the design of such structures.

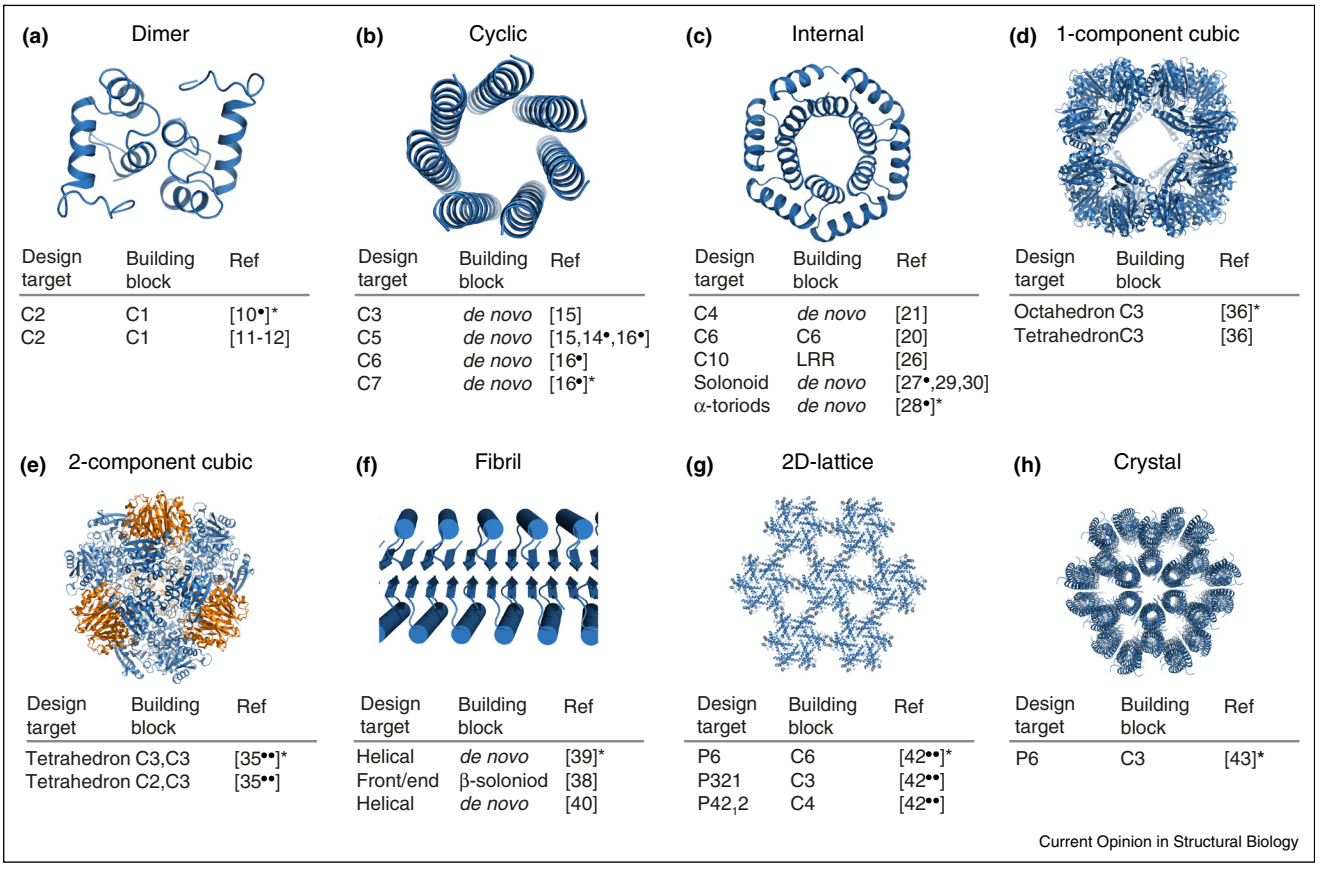
Dimers stand out among the cyclic oligomers by only having one isologous interface in which identical interaction surfaces are contributed from each subunit. Canonical interaction motifs such as association *via* edge β-strands and formation of helical bundles upon dimerization have been used as elements to simplify the design process of homodimers (Figure 3a) [10•,11]. In general, however, *de novo* design of specific high affinity interfaces in homodimers is likely to be as challenging as for heterodimers. The current accuracy of computational dimer design seems sufficient to generate interfaces with micromolar to high nanomolar affinity [10•,11–13]. Because of the affinity leverage endowed by avidity, this is probably sufficient to design higher-order protein assemblies with substantial structural stability.

Higher-order cyclical oligomers have in contrast to dimers two heterologous interfaces per subunit, which must be geometrically compatible within the symmetry, and are thus more challenging to design. So far, the only higher-order cyclic oligomers that have been designed are α-helical bundles, which have simple folds and stereotypical interaction motifs (Figure 3b). Although only representing a small fraction of the fold space available to protein structures, α-helices can self-assemble into a range of geometries, which can be generated with the parametric equations developed by Crick [2,4,18] or by simultaneous folding and docking of helices [14•]. Computational design of backbones generated with such sampling strategies has resulted in α-helical barrels with five [15,16•], six [16•] and seven [16•] helices.

A challenge when designing higher-order cyclical oligomers is to ensure oligomerization specificity. As the number of subunits increases, the difference in contact angle between subunits gets progressively smaller. This results in competing oligomeric states with similar stabilities, as observed for designed α-helical assemblies. For instance, although an impressive fraction of pentameric, hexameric and heptameric α-helical barrels designed by Thomson *et al.* were found in the intended oligomeric states, many adopted alternative or multiple states [16•]. Accordingly, we have shown that it is not sufficient only to optimize for the target sequence for an α-helical barrel but that undesired configurations have to be designed against [14•].

On the other hand, the isoenergetic assembly landscape of α-helices makes them excellent starting points for design of conformational switches. We discovered that

**Figure 3**




*De novo* designed self-assembling complexes. Most of the major symmetry classes of self-assembling proteins have been designed (a–h). One example of a designed protein within each category is shown (marked by *). Where applicable, the point group, plane group or space group is specified for the design target together with the building block used for the design calculation. LRR (Leucine Rich Repeat).

a designed higher-order coiled-coil functioned as a pH dependent oligomerization switch [14•]. Zhang *et al.* also demonstrated that it is possible to design a pentameric sequence that can self-assemble into another oligomerization state upon pH switching, but in the membrane environment [17].

## Internal symmetry covalently links building blocks into stable structures

To overcome difficulties in design of stable higher-order assemblies, a successful strategy has been to covalently fuse subunits into repeat proteins. From a structural point of view, one can think of repeat proteins with multiple identical repeats as internally symmetric protein assemblies with additional stabilization due to covalent coupling of repeats. Assemblies of repeats can associate to form closed ring structures with rotational symmetries or open linear structures with helical curvature. Computational design, sometimes in conjunction with ancient sequence reconstruction, has been used to engineer rotationally symmetric β-trefoils [18,19], β-propeller [20] and TIM-barrels [21]. Collectively, this work has provided strong support for a model in which repeat proteins can evolve from single repeats through gene duplication and fusion [22,23]. Rotationally symmetric repeat proteins are scaffolds for binding and catalysis in nature, and can serve as excellent starting points for engineering of new functions. An example of this is the design of a three-fold symmetric β-propeller to host a metal binding site to generate nanocrystals of cadmium chloride [24].

Linear repeat proteins have less geometric constraints than their cyclic symmetric variants and form structures with a wide range of supramolecular shapes. Linear repeat proteins are extensively used for protein interactions in nature and have been used as scaffolds for engineered interactions [25]. Because of their simple folds, hydrophobic interfaces and front-to-end assembly, linear repeat proteins are highly amenable to computational design. Controlling the supramolecular geometry of linear repeat proteins is of interest both for generation of protein binders with shape-matched interaction surfaces and

for molecular scaffolding applications. Because of the modular nature of repeat proteins, two approaches can be used to control their supramolecular shape: Individual repeats with slightly different structures can be mixed and matched to generate assemblies with predefined geometry [26]. Alternatively, the complete repeat protein can be designed *de novo* assuming identical structure of each repeats [27•,28•,29,30]. A combination of repeats with different conformations can be used to create structures with non-uniform curvature [29]. These approaches make it possible to generate repeat proteins with geometrical shapes not found in nature such as ring-forming leucine-rich repeat (LRR) proteins [26] and α-solenoid repeat proteins with left-handed architectures (Figure 3c) [28•].

## Cubic symmetries can be built from oligomeric building blocks

Starting from cyclic symmetric oligomers, larger protein complexes with closed symmetry can also be designed. Hollow cage structures have been generated with two different approaches: fusion of symmetric building blocks [31–34] or computational design of *de novo* interfaces [35••,36]. In the fusion approach, subunits from two preformed cyclic symmetrical proteins are joined with a linker that is compatible with the target symmetry. This approach does not require interface design, so the structural specificity between subunits is mainly encoded in the linker. Alternatively, specificity can be encoded by computational design of protein–protein interfaces [37]. The first generation of computationally designed cages was homomeric. King *et al.* used C3 symmetric building blocks to generate protein cages with tetrahedral and octahedral symmetry (Figure 3d) [36]. The use of trimeric building block meant that the complete assembly could be designed by only introducing a single new interface [37]. More recently, accurate design of tetrahedral cages comprised of two different oligomers was demonstrated (Figure 3e) [35••]. This two-component approach likewise only requires design of a single interface, but greatly expands the number of building block combinations.

## Open-ended assemblies: building mesoscale structures with nanometer precision

Open-ended assembly provides an approach to link protein–protein interactions at the nanoscale with material properties at the mesoscale. This category of self-assembly can be divided into three classes: fibrils, 2D lattices and 3D crystals.

The simplest approach to engineer fibrils is to redesign a monomer to have a single self-compatible interface generating front-to-end assembly, as have been demonstrated with computationally designed antifreeze amyloid fibers [38]. Many natural protein fibers have more complex topologies with more than a single type of interface. Design of such systems can be simplified if one of the interfaces has a stereotypical interaction motif. For example, amyloid-like fibers often associate through steric zippers between adjoining peptides. Using this motif, we designed an amyloid-like fiber with a novel β-α-β fold (Figure 3f) [39]. Fibrillar assembly can also be triggered by binding to an external surface where protein–surface interactions can cooperate with protein–protein interactions to stabilize the assembly. As an example, Grigoryan *et al.* designed an α-helical peptide that self-assembled as hexamers onto the surface of a single-walled carbon nanotube [40].

Symmetric 2D lattices can also be constructed from cyclic building blocks, which means that the strategies used to simplify design of cubic symmetries can be also applied here. By fusing subunits of cyclic symmetric oligomers, it has been possible to design lattices without engineering of completely novel interfaces [41]. Recent results presented by Gonen *et al.* demonstrate that 2D lattices can also be generated by *de novo* interface design between cyclic symmetric building blocks in an approach using 2D symmetric docking and interface design (Figure 3g) [42••]. The benefit of accurately controlling the interface geometry by the *de novo* interface design approach is that greater structural order can be achieved compared to the fusion strategy. An extension of 2D lattice design is the design of 3D protein crystals, which requires at least three interfaces to enable formation from monomeric building blocks. One design in this category has been presented, again using cyclic building blocks to reduce the degrees of freedom and the number of interfaces that need to be designed (Figure 3h) [43].

Open-ended assembly is often associated with complex assembly pathways and kinetics. This can lead to tradeoffs between structural stability, specificity and assembly efficiency in design. This was considered in the design of peptides binding to nanotubes where weaker protein–nanotube interactions were chosen to avoid kinetic traps [40] and in the design of the 3D crystal where weaker polar contacts were used to direct self-assembly [43].

## Conclusion

Although the protein design field is still far from reproducing the functionality of natural systems, much headway has been made during the past few years. Most of the major classes of symmetries found in natural homomeric systems have now been computationally designed. The recent advancements have been driven by a reduction of design complexity by development of symmetry-aware algorithms, by the use of preformed oligomeric building blocks as starting points, and for the case of cyclic α-helical assemblies, parametric equations to explore backbone degrees of freedom. More fundamentally, the higher-order protein assemblies are also privileged design

targets as avidity yields highly stable complexes from weak interfaces.

The idea of starting from preformed building blocks has been central to the design of higher-order symmetries. Nonetheless, relying on preformed oligomers makes it difficult to find building blocks that can tightly assemble to any desired geometry. Looking forward, developing strategies to design multiple interfaces into monomers, possibly *de novo* designed, could greatly expand the building block repertoire. The design of multiple interfaces at once is likely to have an initially low success rate. One strategy to improve the success rate could be to devise strategies to control not only structure but also the assembly pathways in self-assembling protein complexes. Another strategy to improve success rate could be to develop and improve methods to predict and encode oligomerization specificity.

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