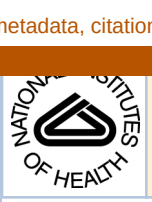

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Strategies to control the binding mode of de novo designed protein interactions

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Abstract

There has been significant recent progress in the computational design of protein interactions including the creation of novel heterodimers, homodimers, nanohedra, fibril caps and a protein crystal. Essential to these successes has been the use of innovative strategies for finding binding modes that are achievable, i.e. identifying binding partners and docked conformations that can be successfully stabilized via sequence optimization and backbone refinement. In many cases this has involved the use of structural motifs commonly found at naturally occurring interfaces including alpha helices inserted into hydrophobic grooves, beta-strand pairing, metal binding, established helix packing motifs, and the use of symmetry to form cooperative interactions. Future challenges include the creation of hydrogen bond networks and antibody-like interactions based on the redesign of protein surface loops.

Introduction

Engineering of protein-protein interactions can generate pair-wise complexes, multi-component complexes, and interaction networks with extensive structural and functional diversity. Practical applications for designed interactions are numerous and include the creation of competitive inhibitors, affinity reagents, biosensors, scaffolding molecules and nanomaterials [2-20].

Directed evolution methods and selection strategies such as phage display are effective for generating new target-binding interactions using antibody scaffolds [21] and non-antibody scaffolds [22], and binders identified with these approaches have shown promise in clinical trials [23]. Computational interface design is an alternative approach that is currently not as robust as experimental selection and screening, but offers precise control of binding location and binding mode and can be applied to design goals that are more difficult for selection technologies such as homomeric assemblies, arrays, fibril caps, and multi-specificity.

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Methods in computational protein design have been used to redesign the affinity and specificity of naturally occurring interactions [24-27] as well as create new interactions from scratch (Figure 1, Table I). This review focuses on novel interfaces that have been designed in the last two years. In most cases, new interactions have been created by mutating the amino acids on the surfaces of naturally occurring proteins so that favorable interactions occur upon formation of the target complex. In these simulations there are three possible degrees of freedom, the relative rigid-body orientation of the proteins, the backbone conformation of residues at the interface, and the amino acid sequence of the interacting residues [28]. Sequence optimization is typically performed with rotamer-based side-chain sampling methods such as simulated annealing or dead end elimination [29,30]. In most studies, backbone sampling has not been performed or only small perturbations have been allowed. Accurate remodeling of the protein backbone is a challenging goal in its own right. As a more conservative approach for allowing alternative backbones at the interface, several methods computationally consider a large set of proteins as the template for interface design.

Despite continued improvements in conformational searching and energy evaluation, interface design remains an imposing challenge due to limitations in these two areas [31]. In particular, the design of interfaces that make use of cooperative hydrogen bonding networks has been especially challenging. As a result, successful designs have relied on carefully chosen strategies in which a desired high-probability binding mode limits the conformational search and is somewhat robust to inaccuracies in energy calculations. Here we review the emerging strategies for designing a desired binding mode (Figure 2).

Helix in a known binding groove

An alpha helix docked in a hydrophobic groove is a favorable scenario for interface design [12,32,33]. Overall shape complementarity of a helix for a concave groove guides the binding orientation and avoids the error-prone challenge of designing polar contacts from scratch.

As a prominent example, broad inhibition of influenza viral fusion [6] was accomplished starting with computational methods to bind the hydrophobic groove in the conserved stem region rather than the variable head domain of hemagglutinin [32]. The first design step positioned disembodied hydrophobic side chains at the target stem to serve as hotspots, and the second design step docked protein scaffolds to connect these disembodied hotspot side chains. By building outwards from native-like and de novo hydrophobic side-chain hotspots, two moderate-affinity binders for hemagglutinin were produced ($K_d > 5\ \mu M$), and the crystal structure of an affinity-matured variant demonstrated high accuracy in the intended binding location and orientation [32].

Secondly, the C-terminal coil region of the GoLoco peptide binds a hydrophobic groove in the heterotrimeric G-protein alpha subunit ($G\alpha_{i1}$), and this peptide coil was redesigned to form a helix and bind the same hydrophobic groove. Rather than start from side-chain hotspots, this study used simultaneous backbone design by fragment insertion and sequence design of the GoLoco peptide. Binding of the redesigned helix was ~2-fold weaker than the native coil, but the crystal structure demonstrated accurate backbone and side-chain positions [33].

High-order symmetry (O, T, C6, D2)

High-order symmetry helps specify a binding orientation because off-target binding modes are unlikely to be compatible with cooperative symmetric assembly. Also, high-order symmetry enables design of large complexes using a small number of designed contacts. The first approach to design a large nanocage used native dimers and trimers fused by a

rigid helix [34,35]. Building on this result, instead of using a rigid linker, a designed interface was used as the rigid connection between native oligomers. In this study, the outer surfaces of native trimers were redesigned to self-associate and accurately form a 24-mer octahedron and a 12-mer tetrahedron. These large nanohedra resulted from one designed protein interface with mostly hydrophobic interactions [17].

Hydrophobic shape complementarity in a symmetric system was also used to design a hexameric channel. Classical coiled-coils feature core hydrophobic side-chain interdigitation and peripheral electrostatic complementarity, but surprisingly, a six-membered classical coiled-coil had not been previously observed. Inspired by an approach of hydrophobic expansion [36], a designed coiled-coil tetramer was mutated (peripheral lysines to alanines) to modulate the symmetric hydrophobic complementarity and produced a hexameric channel-forming assembly [37].

Strand pairing

Hydrophobic contacts will usually need help from polar contacts to specify a desired binding mode. Strand pairing is an effective strategy to generate new polar contacts – it provides cooperative formation of multiple hydrogen bonds to specify binding orientation, and these backbone-backbone hydrogen bonds avoid the entropy loss of side-chain hydrogen bonds. The following two studies combine strand pairing with hydrophobic side-chain interdigitation.

Amyloid fibrils are associated with human disease and can form via repeating strand-strand interactions. To cap and inhibit amyloid fibril formation, short peptides were designed to form beta-strand hydrogen bonds and interdigitating hydrophobic side-chain interactions at the fibril end. A designed D-peptide inhibited fibril formation by the tau peptide of Alzheimer's disease, and a designed noncanonical L-peptide inhibited fibril formation by a PAP peptide fragment associated with HIV transmission. Strand pairing was a central design element, and the specific side-chain interactions were critical for binding in both cases [7].

Secondly, a monomeric protein with a surface-exposed beta strand was redesigned to form a symmetric beta-strand-mediated homodimer. To supplement the strand pair, side chains were designed to form mostly polar or mostly hydrophobic interactions. The successful design (K_d = 1 μM) had hydrophobic side-chain interactions. A crystal structure revealed highly accurate positioning of the strand-pair atoms and highly accurate binding orientation, with mostly accurate positioning of the hydrophobic side chains. Thus, the strand-strand polar contacts were primarily responsible for the high accuracy of the overall binding orientation, and the interdigitating hydrophobic side chains were critical for binding [38].

Metal binding

Metal binding is another strategy for generating new polar contacts – metal coordination geometry is well-defined and coordination bonds are stronger than hydrogen bonds. Metal binding sites and a small hydrophobic protein interface were designed together to achieve high affinity and a predetermined binding orientation. In the absence of metal, an unanticipated binding orientation was observed, highlighting the importance of polar contacts to govern the binding mode. Addition of zinc improved affinity by >100-fold and recovered the intended binding orientation [39]. As evidenced in this study and other zinc-binding studies [20,40], a three-His one-Asp/Glu motif is favored over a four-His zinc coordination motif.

As another example of metal cofactor-mediated protein assembly, multi-heme arrays in a helix bundle were designed with the goal of artificial electron transport through a membrane. A previous method for designing water-soluble multi-porphyrin binding helical

bundles [41] was extended to design porphyrin proteins in a membrane (PRIME) [42]. In this study, two Fe^{III} diphenyl porphyrins were coordinated by a bis-histidine motif with second-shell threonine hydrogen bonds from helices arranged in a tetrameric bundle with D2 symmetry. This approach combined classic coiled-coil side-chain motifs, metal coordination, and symmetry to govern complex formation. Similar methods have also been used to achieve selective binding of an artificial porphyrin chromophore [43].

Super-helix and helix glycine motifs

Two types of helical interaction motifs were used to design a predetermined protein crystal lattice in the P6 space group. Crystal lattices in the rarely observed P6 space group feature planar layering. Contacts between layers were formed by a stacking helix-to-helix end-to-end interaction – this pseudocontiguous “super helix” crystal contact required N-terminal acetyl and C-terminal amide groups and was derived from the previously observed P321 crystal lattice of the original three-helix bundle design [44]. Contacts across a layer were formed by GxxxG helical glycine interactions for helix association with tight packing and a predictable crossing angle [45]. This is the first example of a de novo designed protein crystal.

Hexameric helical assemblies coating carbon nanotube surfaces were also designed using a superhelical motif. The second major feature was the use of repeating alanine or glycine residues to interact with the repeating depressions in 6-membered carbon ring centers of the nanotube surface. The peptide was labeled with gold particles to generate TEM images to confirm a patterned array of protein coating the nanotube. Nanotube binding is not amenable to crystallization, but a crystal structure of a tetrameric form of the designed protein showed repeating hydrophobic and polar side-chain interactions between adjacent helices [16].

Thirdly, non-natural beta peptides were designed to form beta helices that bind an alpha helical transmembrane domain. The designed beta helices featured outward-facing apolar residues with lysine and tryptophan residues near the lipid headgroup region. A GxxGxxG motif in the designed beta-helix was used to target a GxxxG motif in the target transmembrane helix [46].

Electrostatic patterning of coiled coils

Electrostatic patterning in coiled-coils was used in some of the studies already discussed [37,45], but this feature is most prominent in a designed interaction that inhibits a bZIP transcription factor called BZLF1 [8]. The BZLF1 transcription factor homodimerizes (A:A) to bind DNA, so the designed competitive interaction (A:B) inhibits transcription. Given the structural similarity of the inhibitor and target, inhibitor designs are likely to homodimerize (B:B) and lose potency, so self-association of these inhibitory peptides was explicitly disfavored. The method combined experimental data from known bZIP coiled-coils, physics-based calculations, and machine learning to generate a primary sequence scoring function for rapid design with multi-specificity [47]. The best sequences for BZLF1 binding featured charge-charge repulsions that disfavored B:B homodimerization and charge-charge attractions that favored A:B complex formation [8].

Hydrogen bond from an aromatic sidechain

Although it is a challenging design goal, side-chain hydrogen bonds can be used as hotspots. In two-sided design of a de novo binding pair, tyrosine and tryptophan residues were intended to simultaneously form aromatic/hydrophobic hotspots and a hydrogen bond [48]. One designed pair bound tightly (K_d = 120 nM). Structure determination of an affinity-matured variant revealed that the actual binding orientation was flipped 180° compared to the model, and that the tryptophan hotspot did not form. However, the tyrosine hotspot at the

center of the interface did form and was the pivot point between the actual and intended binding orientation, suggesting that the intended tyrosine interaction motif was a strong hotspot.

Grafting: scaffold redesign to accommodate known interface contacts

The hotspot side chain design strategy [32,48-50] is similar in spirit to a side-chain-only grafting strategy previously used to generate a new binding pair [51]. A more recent strategy grafted backbone and side-chain epitopes from HIV for possible applications in vaccine development. Grafting of 6- to 11-residue linear epitopes required aggressive computational backbone sampling and resulted in successful binders [3]. Additionally, a discontinuous epitope – two segments from HIV gp120 – were grafted onto an unrelated scaffold, and computationally-guided directed evolution generated a high-affinity binder that demonstrated close mimicry in one epitope and partial mimicry in the second epitope [4]. Interestingly, grafting studies [3,4,52] take atomic-level binding motifs directly from native interactions but are still susceptible to atomic-level inaccuracies, highlighting the challenge of accurately designing new interactions from scratch.

Conclusions

There are many routes to engineering new protein complexes, including redesign of native interfaces, directed evolution, genetic fusion, and empirical approaches (Table I). This short review emphasizes computer-based de novo interface design with a desired orientation. In the past two years, the field of protein interface design has advanced significantly. However, despite many new successful designs, a low success rate [1] burdens the experimental stage and calls for high-probability binding modes that can overcome current limitations in the conformational search and energy evaluation. So far, these strategies and binding modes include: helices in hydrophobic grooves, side chain interdigitation, high-order symmetry, strand pairing, metal binding, glycine-mediated helix crossing, coiled-coil electrostatic patterning, hydrogen-bonding aromatic sidechains, grafting of native interface fragments, and fusion of native proteins. Interfaces containing loops are common in nature [53] and directed evolution studies [54], but they have not yet been designed computationally due to many challenges: loop conformations are challenging to predict, loop flexibility results in entropic penalty upon binding, loops require backbone and side-chain polar atoms to have their hydrogen bonding potential satisfied, and this design goal would require simultaneous design of the scaffold backbone and interface contacts. Additionally, a hydrogen bond network has not yet been a major component of a de novo designed interaction, in part due to side-chain flexibility and the penalty for desolvating side-chain polar atoms. Designing these and other challenging types of interfaces will require continued improvements in conformational searching, energy evaluation, and experimental throughput, as well as continued innovation of strategies to control the binding mode.

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Inset

Currently, the major challenge in protein interface design is generating polar contacts [1]. While successful de novo interface designs to-date are predominantly hydrophobic, polar contacts are often required for monomer solubility and binding specificity. Successful strategies for designing hydrophobic and polar contacts have been:

Hydrophobic binding strategies:

- Helices in binding grooves
- High-order symmetry/avidity
- Side chain interdigitation
- Helix crossing at glycine residues

Polar binding strategies:

- Strand pairing
- Helix stacking
- Metal binding
- Electrostatic patterning (coiled-coils)
- Tyrosine hotspots
- Grafted backbone and side-chain motifs

Highlights

- Many computationally designed protein-protein interactions were reported over the past two years.
- Recent design goals include heterodimers, homodimers, homooligomers, fibril caps, and arrays.
- Diverse polar and nonpolar interaction motifs can control the binding orientation.
- Crystal structures show accurately designed interactions compared to atomic level predictions.
- Loops and hydrogen bond networks are outstanding interface design challenges.

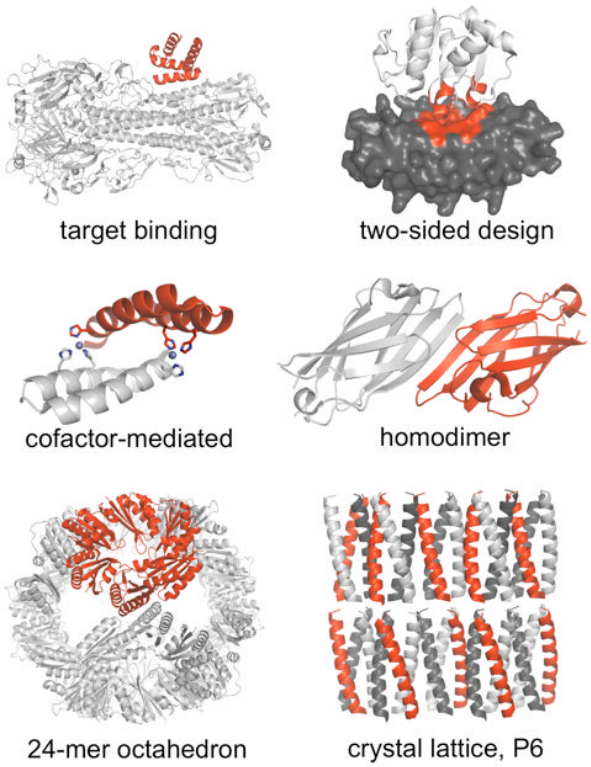

Figure 1. Types of protein complexes designed using computational methods

Target binding [32]: a helical scaffold (red) redesigned to bind the stem region of influenza hemagglutinin (white). **Two-sided design** [48]: redesigned scaffolds were ankyrin repeat protein (gray) and a coenzyme A binding protein (PH1109, white), interface contacts in red. **Cofactor-mediated binding** [39]: A helical hairpin designed for zinc-mediated homodimerization. Histidine residues (sticks) coordinate zinc (spheres). **Homodimer** [38]: the γ-adaptin appendage domain – a monomer with an exposed beta strand – redesigned to allow intermolecular beta-sheet formation. **Nanohedra** [17]: a native trimer (red) redesigned to form an octamer of trimers, a 24-mer octahedron. **P6 crystal lattice** [45]: a previously designed coiled-coil homotrimer modified to form a predetermined crystal lattice in a rare space group, P6.

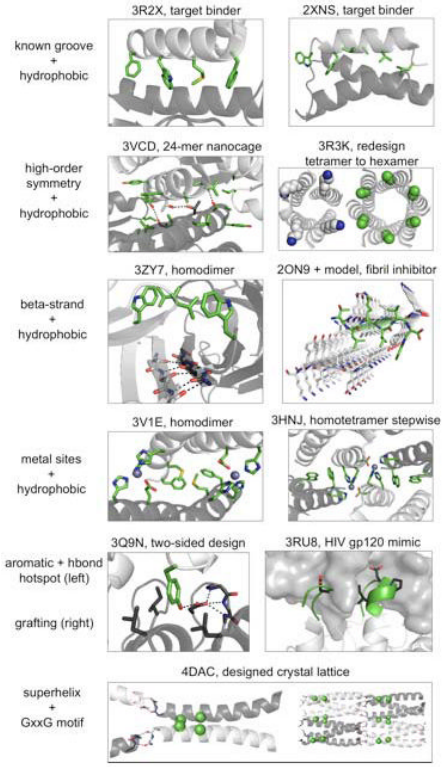

Figure 2. Types of interaction motifs used in de novo designed protein interactions

Known groove: influenza hemagglutinin binding (left), $G\alpha_{i1}$ binding (right). **High-order symmetry**: a trimer redesigned to form a 24-mer octahedron (left), a coiled-coil tetramer mutated to form a coiled-coil hexamer (right). **Beta-strand**: a monomer redesigned to form a strand-mediated homodimer (left), amyloid fibril formation inhibited with a designed binding peptide (right). **Metal coordination**: a monomer redesigned to form a zinc-mediated homodimer (left), a stepwise process converted a crystal-contact tetramer to a solution-phase tetramer by placing histidines at crystal contacts. Subsequently, the zinc-mediated tetramer was improved by computational design (right). **Aromatic and hbond hotspot**: tyrosine was used to form a hydrophobic and hydrogen bonding hotspot interaction with a preordered aspartate side chain in a hydrophobic pocket. **Grafting**: discontinuous side-chain and backbone interaction motifs from a known antibody-antigen pair were grafted onto an unrelated scaffold. **Superhelix and glycine crossing**: a previously designed and crystallized coiled-coil trimer was redesigned to form superhelix stacking interactions and helical glycine crossing interactions, generating the intended honeycomb-like P6 crystal lattice.

Table I
Summary of recent computer-based designed interactions

Goal	Strategy	System	PDB codes	Ref
Interface de novo design				
Target binding	Known hydrophobic groove, hotspots	Hemaglutinin	3R2X	[32]
Target binding	Known hydrophobic groove	GoLoco peptide, $G\alpha_{il}$	2XNS	[33]
Target binding	Known hydrophobic groove	PAK1		[12]
Target binding, multi-specificity	Dataset-driven scoring of coiled-coils	bZIP coiled-coil		[8]
Fibril inhibition	Strand pairing and hydrophobic sidechains	Tau and PAP fibrils		[7]
Two-sided design	Aromatic sidechain hydrogen bond	Ankyrin and PH1109	3Q9U, 3Q9N, 3QA9	[48]
Homodimer	Strand pairing and hydrophobic sidechains	Native monomer	3ZY7	[38]
Homodimer	Metal binding and hydrophobic sidechains	Native monomer	3V1A, 3V1B, 3V1C, 3V1D, 3V1E, 3V1F	[39]
Heterotetramer	Nonnatural zinc chromophore	Heterotetramer coiled-coil		[43]
Transmembrane targeting	GxxGxxG motif in a beta helix	Beta-peptide		[46]
Transmembrane	Porphyrin binding	Four-helix bundle		[42]
Octahedron, 24-mer	Hydrophobic sidechains, hydroxyl hbonds	Native trimer	3VCD, 4DDF	[17]
Tetrahedron, 12-mer	Hydrophobic sidechains	Native trimer	4EGG, 4DCL	[17]
Nanotube coating	Helix Ala/Gly interaction with carbon ring	Coiled-coil hexamer	3S0R	[16]
Array, P6 crystal	Superhelix stacking, GxxG helix	Previous trimer lattice	3V86, 4DAC	[45]
Scaffold design for new binding complexes				
Target binding	Grafting	HIV epitopes 2F5 and 4E10	3RPT, 3RU8 and 3RI0, 3RHU, 3RFN	[3]
Target binding	Grafting	MMP-14 and TIMP-2	not released	[52]
Target binding	PAK1 biosensor	Autoinhib. helical domain		[13]
Nanohedron	Genetic fusion, rigid helix linker	Native dimer, trimer, helix	3VDX, 4D9J	[35,55]
Arrays, 1D and 2D	Genetic fusion, rotational symmetry		(TEM, AFM data)	[56]
Interface redesign				
Specificity	Second-site suppressor mutations	GoLoco peptide and Gai, UbcH7 and E6AP		[57] [14]
Specificity	Flexible backbone design to replace Phe	GTPase/GEF (1KI1)	3QBV	[14]
Multi-specificity	Structure-based modeling and library screening	Bcl-x(L)		[58]
Affinity	Noncanonical amino acid at a native interface	Calpain, calpastatin		[59]
Affinity	Sequence design with backbone perturbation	PDZ peptide and CAL		[2]
Affinity	Hydrophobic interface design	Cytochrome cb562	3HNI, 3HNJ, 3HNL	[60]
Affinity	Two hydrophobic mutations	PCNA and polymerase		[9]
Homologous target	Computationally-directed library	Ubc12 and E6AP		[61]
Hexamer	Augmentation of coiled-coil tetramer	GCN4-p1 starting tetramer	3R4A, 3R4H, 3R3K, 3R46, 3R48	[37]
Metal switching	Replace native interactions with metal coordination residues	Ferritin cage	4DYX, 4DYY, 4DYZ, 4DZ0	[62]