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Targeting recognition surfaces on natural proteins with peptidic foldamers

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Molecules intended to antagonize protein–protein interactions or augment polypeptide-based signaling must bind tightly to large and specific surfaces on target proteins. Some types of unnatural oligomers with discrete folding propensities ('foldamers') have recently been shown to display this capability. This review covers important recent advances among several classes of foldamers, including α-peptides with secondary structures stabilized by covalent bonds, D-α-peptides, α/β-peptides and oligo-oxopiperazines. Recent advances in this area have involved enhancing membrane permeability to provide access to intracellular protein targets, improving pharmacokinetics and duration of action *in vivo*, and developing strategies appropriate for targeting large and irregularly-shaped protein surfaces.

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Introduction

Inhibiting specific protein–protein interactions or augmenting polypeptide-based signaling can be effective for the treatment of diverse human diseases. Poly-L-α-peptides, including engineered antibodies, polypeptide hormones and hormone analogues, have been very successful in this regard, but such molecules can be challenging to produce, susceptible to proteolytic degradation *in vivo* and subject to adverse immunological responses. These realities have inspired exploration of oligomers based on unnatural amide-based backbones that display discrete conformational propensities, collectively designated 'foldamers', as an alternative source of ligands specific for large surfaces on target proteins. Significant progress toward these goals has been reported in the past few years and serves as the focus of this brief review.

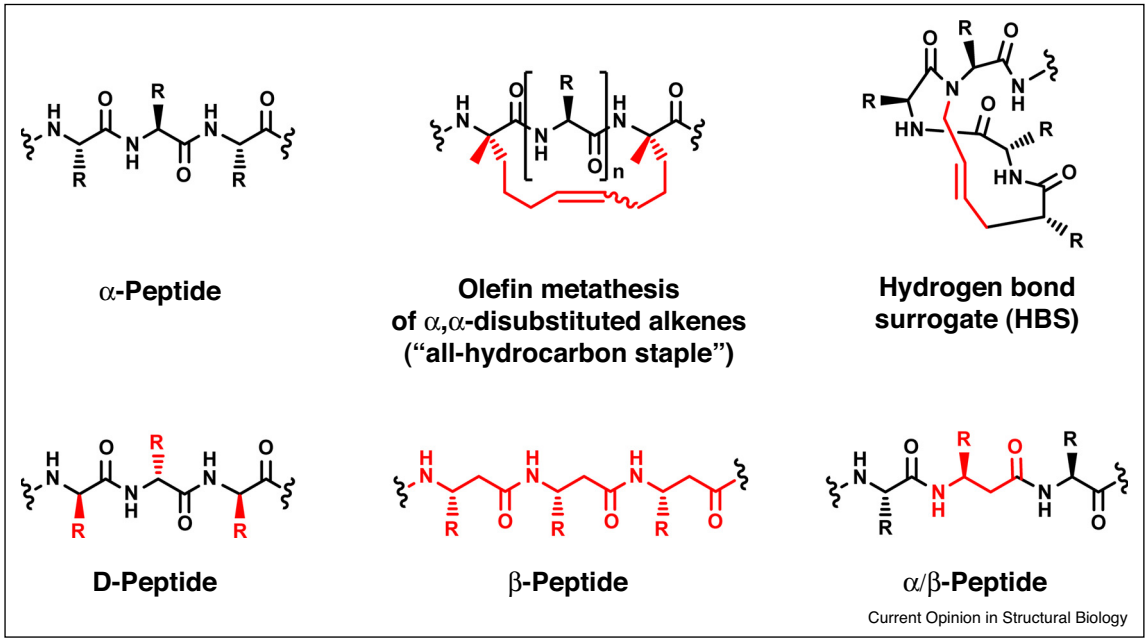
α-Peptides with buttressed secondary structures

Individual α-helices play important roles in many protein–protein interfaces [1,2], but isolated α-helices are generally not very stable conformationally. Strategies to fortify short α-helical conformations via side chain crosslinking have therefore received considerable attention. Although the α-amino acid-derived backbone is retained, this work seems pertinent to our consideration of 'foldamer' approaches. Many crosslinking chemistries have been evaluated [3]; perhaps most popular now is crosslinking of unnatural alkene side chains via olefin metathesis, as first described by Blackwell and Grubbs [4]. Schafmeister and Verdine subsequently employed α,α-disubstituted alkene residues and purely hydrocarbon crosslinkers (Figure 1) [5]. These 'stapled' helical α-peptides have been designed to target a variety of protein–protein interactions, and some cross cell membranes to reach intracellular targets [6–8]. In a recent example, a hydrocarbon-crosslinked peptide derived from B cell lymphoma 9 (BCL9), designed to disrupt the BCL9-β-catenin complex (a transcriptional regulator in the Wnt signaling pathway), suppressed tumor growth in two Wnt-driven mouse models of cancer [9••]. Peptides with more than one crosslink generally provide modest benefits relative to those with a single crosslink [10,11]. Recently, Grossmann *et al.* showed that hydrocarbon crosslinking can stabilize an irregular (non-helical) secondary structure, which opens new possibilities for application of this design strategy [12••].

At present, it is not clear how a hydrocarbon crosslink can confer membrane permeability. The high hydrophobicity of such appendages presumably favors peptide-membrane association. Such peptides may enter cells through passive diffusion or endocytosis [6,13•]. However, the incorporation of a hydrocarbon crosslink does not guarantee membrane permeability [7,14,15••,16••]. Furthermore, some of these peptides display problematic properties including low solubility and inactivation by serum [7,8,17,18•]. The crosslink can make direct contacts with a target protein, which may alter recognition properties and selectivity relative to the non-stapled prototype peptide [19,20].

Arora *et al.* have pioneered an alternative crosslinking strategy for α-helix stabilization, the hydrogen bond surrogate (HBS) approach (Figure 1). Here, an *i*,*i* + 4 backbone hydrogen bond is replaced by a carbon–carbon bond generated via olefin metathesis [21,22], leading to substantially enhanced helix stability and less exposure of the crosslinker to the environment relative to side chain-to-side chain

Figure 1



Structures identifying some of the helix-stabilization and foldamer approaches described in the text. Structural elements that deviate from traditional peptides are highlighted in red.

crosslinks [22,23]. Relative to side chain crosslinking, the HBS approach has the advantage that side chain functionality is not altered. Recent applications of the HBS approach include a design based on hypoxia-inducible factor 1α (HIF-1α), a protein that affects expression of certain oncogenes [24]. One such HBS peptide bound co-activator protein p300 and blocked its interaction with HIF-1α, inhibiting transcription of target genes. A related HBS peptide targeting p300 was shown to suppress tumor growth *in vivo* [25••], which represents a very significant advance. A different HBS design, derived from the guanine nucleotide exchange factor Sos, inhibited the Ras-Sos interaction in living cells [26], which shows that the HBS strategy has generality in terms of achieving cell-permeability.

D-Peptides

Peptides comprised of D-α-amino acid residues (Figure 1) are dramatically less susceptible to proteolytic degradation relative to L-peptides [27,28]. However, rational design of D-peptides to target a particular protein surface is challenging [29,30]. Mirror-image phage display, a clever strategy developed by Kim *et al.* [31], enables the discovery of D-peptides that target a specific protein surface [32]. Phage-based peptide libraries are screened for binding to the enantiomer of the protein target (a D-protein); the enantiomer of an L-peptide identified in this way must bind to the native L-protein. D-Peptide ligands for gp41 [33–35], MDM2 [36,37], PD-L1 [38••], and vascular endothelial growth factor (VEGF) (Figure 2a) [39••] have been described. An anti-VEGF D-peptide displayed a longer *in vivo* half-life and lower immunogenicity in mice relative to the enantiomer [40•]. A potential limitation of mirror-image phage display is the requirement for chemical synthesis of the D-protein target, which becomes increasingly difficult as size increases. D-Peptides are competent as haptens, that is, antibodies against D-peptides can be raised if T cell help is provided [41•]; thus, it remains unclear whether long-term administration of D-peptides or D-proteins could provoke an immune response. This concern applies to all oligomers with unnatural backbones.

β-Peptides

Some of the earliest research on peptidic foldamers focused on oligomers of β-amino acids, or 'β-peptides' (Figure 1) [42–44]. Although β-peptides can adopt specific helical conformations, and they are highly resistant to protease degradation, this foldamer class has not proven to be generally effective in terms of α-helix mimicry [44,45]. Helical conformations available to β-peptides, such as the 12-helix or 14-helix, do not recapitulate the three-dimensional side chain display patterns observed for α-helices. This mismatch was recently highlighted in the context of efforts to mimic the type 2 diabetes drug exenatide, a potent α-peptide agonist of the glucagon-like peptide-1 receptor (GLP-1R) [46]. A long α-helix formed in the C-terminal portion of exenatide makes extensive contacts with the extracellular domain of GLP-1R [47]. Replacement of this α-helix-forming segment with a β-peptide segment caused a dramatic loss in agonist potency [48].