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5.05 Foldamers in Medicinal Chemistry

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Abbreviations

Aβ Amyloid-β
ABSM Amyloid-β-sheet mimics
AMP Antimicrobial peptides
CPFs cell-penetrating foldamers
GB1 B1 domain of streptococcal protein G
GLP-1 Glucagon-like peptide-1
GPCR G-protein-coupled receptor
HBS Hydrogen-bond surrogate
IAPP Islet amyloid polypeptide
i.v. Intravenous
MHC Major histocompatibility complex
MPTP 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD Parkinson's disease
PPI Protein–protein interaction
PTH Parathyroid hormone
SPS Solid-phase synthesis
TCR T cell receptor
VEGF Vascular endothelial growth factor
VIP Vasoactive intestinal peptide

5.05.1 Introduction

Foldamer chemistry has initiated a profound change in biopolymer mimicry over the last 20 years, by showing that isolated and stable secondary structure elements can be created from a variety of oligomeric backbones either close to (e.g., aliphatic β- and γ-peptides,[1] oligomers of *N*-alkyl glycines (peptoids)[2]) or beyond (e.g., *m*-phenylene ethynylene oligomers,[3] aromatic oligoamides[4])

natural biopolymers. In biopolymers, folding and function are intimately linked; this is the reason why sequence-based biopolymer mimicry with foldamers can provide unique and useful tools to study biology complementary to more conventional chemical approaches, and may lead to downstream applications such as novel diagnostic/therapeutic agents. If the diversity of monomer units in biopolymers, that is, amino acids and nucleotides, has been limited through evolutionary pressure, this is not the case in foldamer chemistry where researcher creativity allows an entirely new chemical space to be explored beyond Lipinski's criteria.[5] The identification of foldamers that recognize specific biopolymer targets is certainly facilitated by the control exerted over the monomer sequence and secondary structure in many synthetic folded oligomers as well as by the diversity of the building block repertoire (substitution patterns, side chains, backbone isosteres) which has considerably expanded in two decades. However, the task remains extremely challenging at the current level of knowledge, and still too few foldamer-based approaches to faithfully mimic protein secondary structural elements engaged in binding events are routinely available. Highly modular chemistries, synthesis of higher order foldamer architectures, access to structures of complexes between the target biomolecules and designed foldamers at atomic resolution, as well as computational design approaches are needed to facilitate the process leading to the discovery of potent foldamer-based modulators of biomolecular interactions. Substantial progress has been achieved in these directions and will be covered in the following sections. The next frontier for foldamers in medicinal chemistry certainly lies in in vivo applications. The improved resistance to proteolysis demonstrated for a number of foldamer backbones is a significant advantage that can translate into increased duration of action. Current efforts to meet this challenge focus on the cellular uptake properties of foldamers and approaches to improve their cell permeability when designed to engage intracellular targets of therapeutic relevance, as well as on their pharmacokinetic (PK) properties and bioavailability.

5.05.2 Foldamers: Exploring a New Chemical Space to Design Original Pharmaceutics

Today, foldamers represent a rapidly growing ensemble of molecules in terms of diversity and chemical complexity that is populating a new and largely empty area of chemical space highly complementary to small molecules and biologics. Foldamers can be classified according to the chemical nature of their backbone, which can be either fully aliphatic (e.g., α-, β-, or γ-peptides, peptoids, peptidomimetic oligoureas), based on aromatic units (e.g., oligoaryl- or heteroarylamides), or result from the combination of the two (Fig. 1). Helices are by far the most widely found structural motif among foldamers, even though more and more examples of β-strands or β-sheets are now found in the literature. Whereas many foldamer backbones exhibit robust and highly predictable conformations, others do not show a strong conformational preference on their own but are induced to fold upon binding to their target. Mimics of secondary structure elements based on rigid and extended backbones represent another class of conceptually different molecules that do not fold like the natural secondary structure elements they intend to mimic, but are rather minimal scaffolds designed to project the right functional groups with the proper orientation to achieve molecular recognition processes. This section briefly reviews foldamer systems developed in the context of medicinal chemistry applications with a particular focus on those foldamers which have been studied in aqueous solutions which is particularly relevant for most biological studies.

5.05.2.1 Artificial Helical Scaffolds

A large number of biological processes, from cellular communication to programmed cell death, involve interactions between proteins and another biopolymer (e.g., proteins, nucleic acids, lipids, and carbohydrates). α-Helical domains, the largest class of secondary structural elements in proteins, play a major role in these interactions (roughly 62% of protein–protein complexes in the Protein Data Bank possess α-helical interfaces according to a recent statistical analysis[6]); hence, artificial helical scaffolds, intended to mimic side-chain display of α-helices present at such interfaces, are of important pharmaceutical interest.[7]

5.05.2.1.1 Biotic helices

Helically folded aliphatic oligomers containing proteinogenic side chains are classified as biotic helices. They usually differ by the number of methylene units and saturated/unsaturated bonds in the backbone, as well as by the nature of the bonds connecting each unit (amide, urea, hydrazide, …) and the side-chain substitution patterns (Fig. 2). The helical conformation is generally characterized by a specific hydrogen-bond network, handedness, and respective orientation of backbone H-bond donor and acceptor groups, which determine the resulting macrodipole. In proteins, helices are right-handed with 10 or 13 atoms in hydrogen-bonded rings (3_{10}-helices or α-helices, respectively).

5.05.2.1.1.1 β-Peptides

In β-peptides, the backbone of each amino acid residue is homologated by one additional methylene unit compared to α-peptides, with the side chain (in the case of mono-substituted β-amino acid residues) lying next to the carboxylate or the amine functional group (β^2- or β^3-peptides, respectively). β-Peptides can form 14-, 12-, 10-, 8-, and 10/12-helices depending on the substitution pattern and stereochemistry of the side chains along the backbone, as well as on the use of cyclic or acyclic

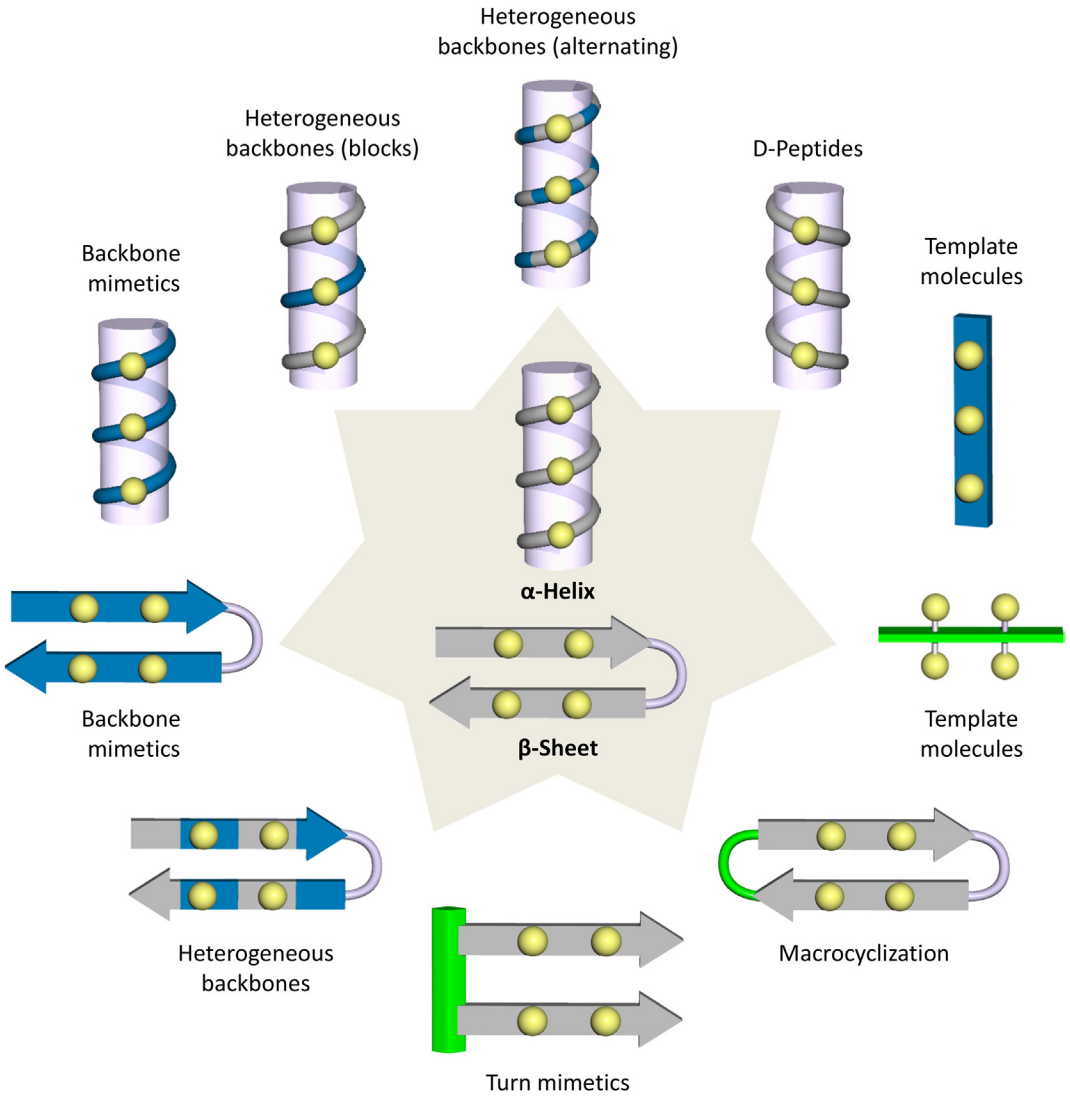


Figure 1 Molecular mimicry and structural diversity of foldamers.

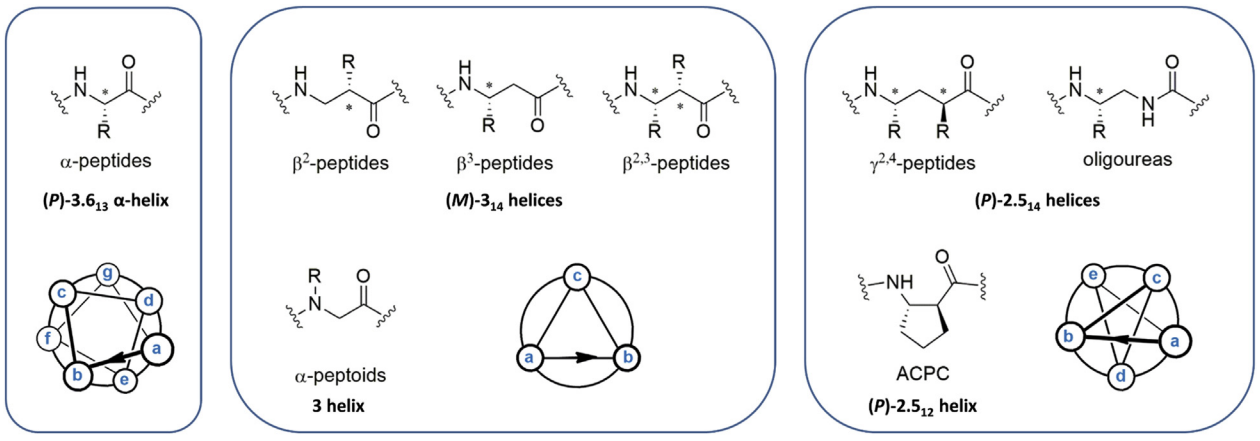


Figure 2 Examples of aliphatic oligoamide helical foldamer structures and their helical wheel representations.

monomers.[1c,8,9] Among all described β-peptide helical conformations, the 14-helix has been the best studied. This helix is stabilized by 14-atom H-bonded pseudorings between the N–H (i) and C═O ($i+2$) with approximately 3 residues per turn.

Following the pioneering work of Seebach,[10] β^3-substituted residues have been widely used due to their straightforward synthesis, mainly *via* homologation of natural α-amino acids, and their high propensity to form a 14-helix conformation. Concurrently, Appella et al. have shown that the formation of the 14-helix is even more favored when one or more constrained "ACHC" (*trans*-2-aminocyclohexanecarboxylic acid) β-residues are incorporated.[11] However if the cyclic β-residue is switched to a five-membered ring cycloalkane ("ACPC" or *trans*-2-aminocyclopentane carboxylic acid) then the more tightly wounded 12-helix is obtained. The use of γ-branched β^3-residues has also been shown to be fully compatible with the formation of the 14-helix.[12] A β-peptide composed of a sequence of alternating β^2- and β^3-residues will however adopt a 10/12 helical conformation,[13] as well as β^3-peptides consisting of residues of alternating chirality.[14]

There are multiple reports of β-peptides with the ability to form stable helical conformations in water.[15] The 14-helix macrodipole having a partial negative charge at the N-terminus and a partial positive charge at the C-terminus, this helix can be stabilized by charged residues interacting favorably with this macrodipole. Electrostatic interactions leading to salt bridges between oppositely charged side chains of the residues have also been used to drive the helix formation.[15c,e,16] Alternatively, constrained cyclic β-amino acid residues,[9,15b,d,f,17] as well as more classical side-chain cross-linking approaches,[18] have been used to increase the helix content of β-peptides in aqueous solutions. These approaches have been exploited in attempts to design helical β-peptides to recognize various protein interfaces.[19]

5.05.2.1.1.2 γ-Peptides

γ-Amino acids are residues containing three backbone carbon atoms between the amino and carbonyl groups. The resulting γ-peptides have been less studied than the corresponding β-peptides, one reason being that the additional homologation reduces the number of potential hydrogen bonds and increases the flexibility of the whole chain. However, it has been shown simultaneously by Seebach and Hanessian in 1998 that γ-peptides obtained by double homologation of natural α-amino acids adopt a helical conformation in solution, stabilized by a 14-membered hydrogen-bond ring, which possesses the same screw sense and polarity as the α-helix.[20] Disubstituted $\gamma^{2,4}$-peptides,[20a] as well as trisubstituted $\gamma^{2,3,4}$-peptides,[21] have also been investigated as they could reduce the number of accessible conformations and more effectively stabilize a folded conformation. When the 2,4- and 2,3,4-substituents are chosen with the appropriate relative configuration(s), a more stable 14-helix-type conformation is formed compared to the corresponding γ^4-peptides. Comparatively, pure γ-peptide helices have not received as much attention as their β-peptide counterparts and relatively few studies have investigated their possible use in the context of biological applications.[22]

5.05.2.1.1.3 Oligoureas

Aliphatic *N*, *N*′-linked oligoureas are aza-analogs of γ-peptides, where the $^{\alpha}CH_2$ has been substituted by a NH.[23] This additional nitrogen atom acts as a rigidifying element, since it allows the formation of a three-centered H-bond network. The oligoureas adopt a stable 2.5 helical fold, similar to the γ^4-peptide 14-helix, but with the presence of both 12- and 14-membered H-bond rings (12/14-helix).[23c,24] Detailed NMR and CD studies[25] as well as X-ray diffraction analyses[26] have been conducted by the Guichard group on these types of foldamers to understand their conformational behavior. Only four to five urea residues are needed to initiate helical folding in low-polarity solvents,[27] and this helicity has proven to be largely unaffected by the nature of the side chains used, which makes these foldamers highly robust and tunable.

Water-soluble oligoureas have also demonstrated that their ability to form a stable helix is maintained in aqueous solution, even if the folding propensity is weaker.[28] Their design has been mainly inspired by the sequences of bioactive peptides, and these foldamers showed interesting activities as mimics of host defense or cell-penetrating peptides (CPPs),[22,28,29] but it remains to be seen whether homooligoureas or related oligourea/peptide chimeras[30] could also serve as scaffolds for the design of inhibitors of protein–protein interactions (PPIs).

5.05.2.1.1.4 Peptoids

Peptoids (poly-*N*-substituted glycines) are analogous to α-peptides but their side chains are shifted from the α-carbon to the nitrogen of the amide function. The propensity of peptoids to form stable helices, despite the lack of hydrogen-bond network, has been reported by the Zuckermann group shortly after the discovery of β-peptides.[2a,31] The absence of a chiral center at the α-carbon in peptoid backbones can be compensated and helicity controlled by the use of chiral branched side chains at selected positions along the sequence. In the absence of an H-bond donor group, the folding behavior of this type of foldamer is largely governed by *cis*–*trans* isomerization of tertiary amides, and the resulting conformation shares similarities to polyproline-type-I helix. A 36-mer water-soluble oligopeptoid with the $[Nsce Nsce Nspe]_{12}$ sequence (where *N*sce is (*S*)-*N*-(1-carboxyethyl)glycine and *N*spe is (*S*)-*N*-(1-phenylethyl)glycine) synthesized by the Zuckermann group was shown to adopt a very stable right-handed helix with *cis*-amide bonds.[32] The resistance of peptoid secondary structure to solvent environment and thermal perturbations has proven that this type of helical structure is stabilized by steric factors.

5.05.2.1.1.5 Heterogeneous backbone: peptide-foldamer chimeras

Heterogeneous backbone foldamers are oligomers that contain at least two different types of monomers. They have specific folding patterns which depend on backbone composition as well as side-chain sequence, two variables that can be independently altered.[33]

In particular, the combination of unnatural monomers (including abiotic ones[34]) with natural α-amino acids has proven extremely useful to create unnatural backbones with new folding patterns and molecular recognition properties and numerous examples have been reported. The introduction of unnatural-residues in the sequence brings an additional level of conformational control to form specific secondary structures and new ways to arrange side chains in space, whereas α-amino acid residues provide side-chain diversity at low cost, thus limiting the number of noncanonical building blocks to prepare. Two different strategies have been envisioned: either discrete replacements of α-amino acids along the sequence, usually following a regular pattern of alterations,[33a] or the "block" strategy where distinct blocks, each of them containing a specific backbone, have been combined together (i.e., block co-foldamers).[30a,35]

α/β-Peptides with mixed α- and β-residues in various patterns are among the best studied chimeric oligomers. The first studies have been conducted independently by Zerbe and Reiser, and by the Gellman group, on 1:1 alternating α/β-peptides (i.e., αβαβαβ repeated pattern).[36] Both groups have shown that these homochiral heterogeneous backbones can adopt helical conformations and that preorganized (cyclic) subunits are needed for helix stability in polar solvents. Many other patterns (e.g., 2:1 and 1:2 α/β patterns) have since been explored and found to lead to helical structures,[33a,37] some of which being quite close to α-helices.[38] The Gellman group has since demonstrated the utility of this approach for mimicking α-helices, extending it to biologically relevant natural sequences.[37d] α/β-peptides containing around 25%–33% of β-residues evenly dispersed along the backbone can provide effective α-helix mimics with binding affinities to protein surfaces similar to those of cognate α-peptide ligands, despite the additional atoms in the backbone (see section "**Design of Bioactive Foldamers**"). Compared to a systematic 1:1 α → β replacement, the ααβααβ pattern causes minimal distortion to the α-helix geometry (see **Fig. 3**); the sequestration of all β-residues on one face of the helix allows key α-residues on the opposite face to be conserved for the interaction.

Although less mature, other related approaches like those based on the incorporation of γ-residues of various types are also worth being mentioned. A number of α/γ-peptide scaffolds with the propensity to adopt helical structures have been reported.[39] β/γ-Peptides with alternating β- and γ-amino acid residues are also of special interest in the context of α-helix mimicry because the β/γ-dipeptide subunit matches the number of atoms of an α-tripeptide segment and because appropriately preorganized residues can promote the formation of a helical conformation with strong relationship to the α-helix.[40] It is interesting to note that β/γ peptide segments have been inserted in long α-helical segments forming coiled coils.[35c,41] Gellman et al. have also reported α/β/γ heterogeneous sequences with an αγααβα pattern with the propensity to form stable helices in water.[42] Surprisingly they showed that γ^4-residues displayed actually a high helical propensity, and that the best sequence for α-helix mimicry combined cyclic β-residues with acyclic γ^4-residues to release constraint in the backbone.

5.05.2.1.1.6 D-Peptides

Nature relies on a specific stereochemistry to build proteinogenic architectures as only the L-configuration of α-amino acids is used during protein biosynthesis. Occasionally, diastereomeric proteins containing discrete L → D residue replacements have been prepared to evaluate how a single D-amino acid insertion can affect local and global folding behavior, and in some cases this approach led to increased protein stability.[43] Although homochirality in these polymers is essential for proper folding and adoption of specific and functional shapes, mixing L- and D-residues in polypeptide sequences can be used to expand the scope of accessible folded patterns.[44] This is the case of gramicidin-A, a hydrophobic 15-mer membrane active peptide with an alternate L,D sequence which folds into a mixed helix, also named β-helix because of its similarity to β-sheet-type structures (pairs of φ and ψ angles lie in the allowed β-sheet region of the Ramachandran map for L and D residues).[45] β-Helices formed by D,L-peptides can be either right- or left-handed depending on parameters such as sequence, length, and environment. Various periodicities and hence pore sizes have been predicted and found experimentally. β-Helices such as gramicidin-A also exist either as single helices stabilized by parallel H-bonds or as parallel (or antiparallel) double helices.[46] As a proof of principle toward the development of ligands for biological targets, Kulp and Clark engineered a hydrophilic D,L-peptide sequence that is able to fold into a β-helix in polar media.[47] They have

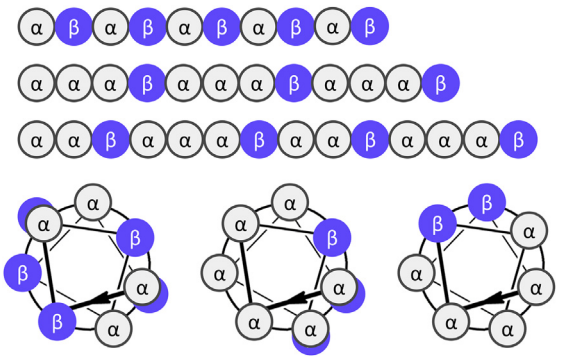


Figure 3 α/β-Peptides with αβ, αααβ, and ααβαααβ backbone patterns and helical wheel diagram showing the distribution of β-residues *(blue disk)* around the helix surface for each of these patterns.

shown that, upon switching solvent from aqueous buffer to methanol, the heterochiral peptide folds from β-hairpin-like secondary structure to a β-helical supersecondary structure.

All D-peptides do not naturally occur in nature. They however represent an interesting class of peptidomimetics for therapeutic applications, as they could mimic natural bioactive peptides while being resistant to enzymatic degradation, and less immunogenic than their corresponding L-enantiomers. In this context, mirror image phage display (see also sections "**Designability, synthetic accessibility, and diversity enhancement to improve biopolymer mimicry**," "**α-Helix Mimicry for Modulating Protein–Protein Interactions**," and "**Foldamers That Target Intracellular PPIs**) is a remarkable approach to identify D-amino acid peptide and D-mini protein sequences that specifically bind to a selected protein target.[48]

5.05.2.1.2 Abiotic helices

Abiotic foldamers typically contain aromatic rings in the backbone, which distinguish them from the aliphatic backbone of proteins and related biotic foldamers.[49] Although the folding propensity of these aromatic oligomers relies on noncovalent interactions (π–π stacking, H-bonding), the restricted rotation around aromatic units and their strong geometric constraints are essential features that make the secondary structure of these foldamers highly stable and predictable.

Hamilton and coworkers originally described oligoamide aromatic foldamers obtained by combining *ortho*-aminobenzoic acid (i.e., anthranilic acid, **Fig. 4A**) and pyridine-2,6-dicarboxylic acid monomers (**Fig. 4B**).[4a,50] They have demonstrated that the corresponding heteropentamer adopts a helical conformation, similar to that of helicenes, stabilized by an intramolecular H-bond pattern between nearest neighbor groups. This structure resembles the helicates reported by Lehn, in which oligobipyridines undergo spontaneous self-organization into a double-helical structure upon metal binding.[51]

Since this early work, several oligoaryl- (such as 4,6-dimethoxy-3-amino-benzoic acid oligomers, **Fig. 4C**) or heteroaryl-amides (such as oligoquinolines, **Fig. 4D**) have been described to promote a helical conformation, as well as oligoaromatics linked with hydrazone or urea moieties. The curvature of the resulting helices can be modulated by several factors: the substitution pattern on the aromatic ring (i.e., *ortho*, *meta*, or *para*); the introduction of hydrogen-bond donor/acceptor groups to rigidify the backbone; and the type of aromatic ring (benzene, pyridine, pyrimidine, naphthalene, …).[3,49b,c,52] The handedness of the helix can also be promoted by the choice of a stereo-controlled monomer leading to chiral induction.[53]

Making aromatic foldamers water soluble and biologically active is certainly more challenging than for their aliphatic counterparts, because of their inherent hydrophobicity, chemical divergence from biomacromolecules, and synthetic challenges. Nevertheless, there are a number of examples in the literature. Linear aromatic oligomers with amphipathic character and exhibiting potent antimicrobial activities have been reported by the groups of DeGrado and Tew.[54] Huc and coworkers designed the first fully water-soluble helical quinoline oligoamides by attaching several ammonium side chains along the backbone.[55] They actually demonstrated that the helix conformational stability of these molecules is enhanced in polar media compared to organic solvents.[56]

Heterogeneous backbones have also been explored with abiotic foldamers to form hybrid scaffolds, which were expected to display a unique folding behavior due to the absence of common features between the aliphatic and aromatic building blocks. If the number of aromatic units along the sequence is properly controlled, then the folding properties of the abiotic part may be kept while benefiting from the wide diversity of side chains of commercially available amino acids. Water-soluble α-amino

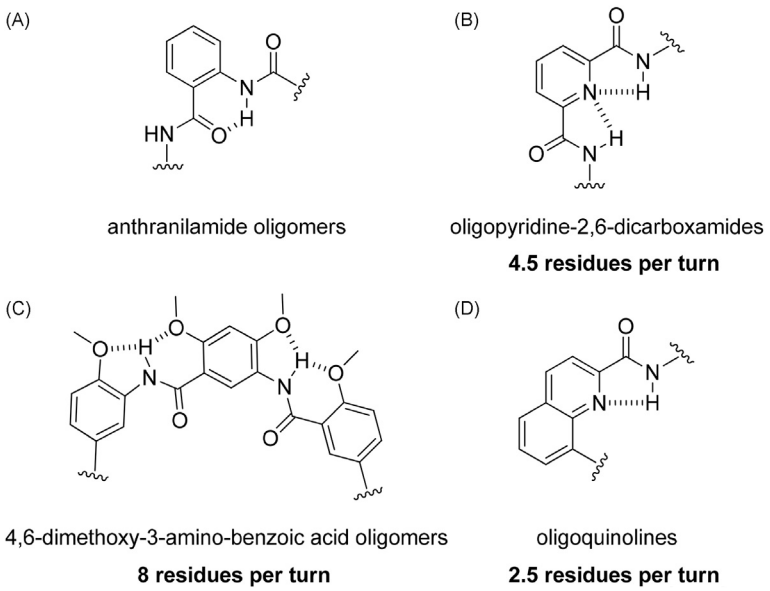


Figure 4 Examples of aromatic oligomer structures and their intramolecular hydrogen-bond network.

acid/quinoline oligoamides have thus been designed in order to form a linear array of proteinogenic side chains on one face of the helix.[57] To perform the synthesis of such hybrid sequences, a powerful solid-phase synthesis (SPS) strategy has been developed *via* the in situ formation of amino acid chlorides.

5.05.2.1.3 Helix mimetics based on nonhelical scaffolds

To interact with the specific secondary structure elements of a protein target, alternative structure-based approaches have been developed. They require only the knowledge of the effective functional group arrangement at the surface of the protein and are based on well-defined preorganized minimalist scaffolds which are further decorated by side-chain functional groups in an appropriate spatial distribution, in order to interact favorably with the target (Fig. 5). In particular, it has been postulated that a linear array of appropriately spaced side chains on a nonhelical scaffold can efficiently mimic the projection of native α-helix residues.[58] These topographical mimetics may be synthetically more accessible than conventional helices. However, to be practically useful, such peptidomimetic frameworks need to be chemically modular to allow appendage of proteinogenic side chains. High rigidity of the scaffold is only desirable if a perfect overlap with the critical functional groups of the α-helix can be achieved; otherwise some conformational flexibility may be necessary as long as the relevant conformations may be sampled with accessible thermodynamic and kinetic barriers.[59]

The Hamilton group designed the first true α-helix mimetics by using a terphenyl scaffold and reported structural and functional analogs of a defined region of a kinase α-helix.[60] Because the key side chains involved in biological interactions (such as PPIs or membrane disruption) are usually located along the same face of the α-helix, only residues *i*, $i+3/4$, and $i+7$ have to be considered in terms of functionality. Orner et al. found that 3,2′,2″-tris-substituted terphenyl derivatives in a staggered conformation allow the right arrangement of substituents to project critical functional groups in similar places as in the α-helix. Several potent inhibitors of PPIs have then been developed with functionalized terphenyl **1**,[61] terephthalamide **2**,[62] and tris-pyridylamide[63] scaffolds. Later they designed benzoylurea oligomers **3** as an alternative to oligophenylenes to mimic up to eight turns of an α-helix, and to solve synthesis as well as water solubility issues when extended scaffolds are needed.[64] Other groups have also developed efficient nonhelical mimetics of biologically active α-helices, based on aromatic backbones (such as oligo-benzamides,[65] pyridazines,[66] -pyridyles,[67] or -piperidine–piperidinones[68]). Tošovská et al., on the other hand, have reported the oligooxopiperazine scaffold as chiral nonaromatic topographical helix mimetic.[69] They have shown that oligooxopiperazine derivatives could mimic the orientation of three critical binding residues of the α-helical domain at the interface of hypoxia-inducible factor 1α and the cysteine/histidine-rich 1 (CH1) domain of p300 (see section "**p300/HIF-1α interaction**").

A next important challenge was the design of amphipathic α-helix mimetics based on nonhelical scaffolds with both improved affinity to the target and selectivity. Toward this goal, both faces of the helix have to be considered, and strategies to mimic residues *i* and $i+7$ on one face, and residues $i+2$ and $i+5$ on the other, have been explored.[70]

5.05.2.1.4 β-Strand and β-sheet peptidomimetics

In comparison to helices, there are far fewer examples of foldamers designed to mimic β-sheet-type secondary structures; this is mainly due to the large surface of interaction needed to stabilize such structures and also to the fact that β-sheet structures tend to aggregate and precipitate making their characterization more difficult. α-Peptidic β-sheets are composed of extended β-strand regions that are aligned laterally and interact with one another through hydrogen bonds. Each β-strand is made from a linear array of α-amino acids with side chains pointing out alternately above and below the backbone plane. When β-strands are aligned in the same direction, they form parallel β-sheets stabilized by 12-membered hydrogen-bond rings. Conversely, antiparallel β-sheets with 10- and 14-membered hydrogen-bond rings are obtained when β-strands are aligned in opposite directions. Most of the β-sheets in

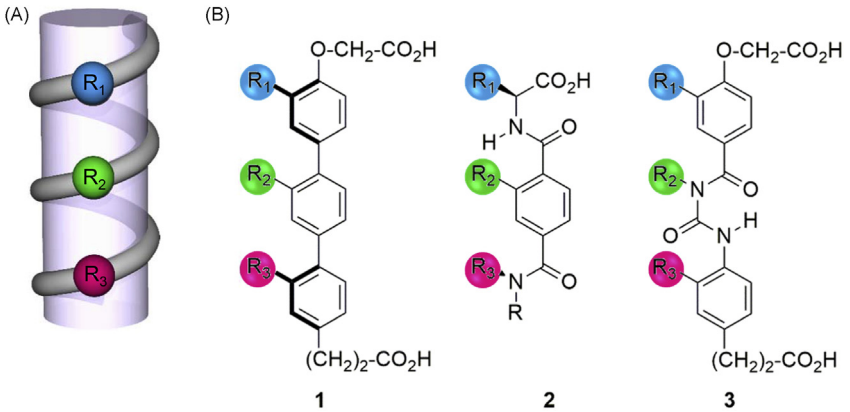


Figure 5 (A) Schematic representation of an α-helix (spheres represent positions *i*, $i+4$, and $i+7$ of the side chains). (B) Examples of helix mimetics based on nonhelical scaffolds.

proteins are not flat but rather twisted in a right-handed fashion. They also tend to self-assemble through hydrophobic interactions with other secondary structural elements to create compact tertiary or quaternary structures.[71]

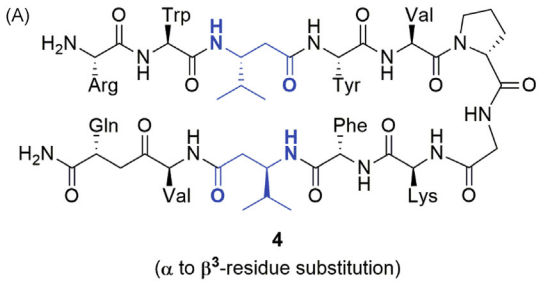
Early examples of de novo design of foldamers with sheet-like and hairpin-type structures in organic solvents have been reported for β-peptides and γ-peptides by the groups of Gellman and Seebach.[72] Other designs with β- and γ-peptides have been proposed[73] but very few structures have been studied in an aqueous environment. The more top-down approach whereby α-residues in the structure of an α-peptidic β-sheet are replaced by their β- (or γ-) residue counterparts has been explored.[74] Starting from a prototype water-soluble hairpin sequence, Horne and coworkers have shown that mixed α/β-peptides (e.g., **4** and **5**) containing a single α → β mutation at cross-strand positions can adopt a two-stranded hairpin fold in aqueous solution (**Fig. 6A**).[75] The main difficulties when designing heterogeneous α/β-peptide sheet-type structures reside in the difference of directionality of interstrand hydrogen-bond pattern imposed by β-residues as well as by altered display of side chains relative to the natural α-peptide. Later the Horne group has shown that alternate β-residue replacement strategies (αα → β or αα → ββ) could be used to maintain the folding pattern and side-chain display of the original α-peptide hairpin, but were associated with an energetic penalty resulting from additional backbone conformational freedom.[76] Martinek et al. were the first to report the effects of a systematic α- to β^3-residue substitution on a multiple stranded water-soluble bioactive β-sheet (e.g., **6-11**, **Fig. 6B**).[77] They showed a higher detrimental effect on folding when the substitutions occurred in the hydrophobic core region, mainly due to geometrical constraints preventing good packing, rather than to the destabilization of the hydrogen-bond network. Although these foldamers displayed a decreased folding propensity compared to their natural counterparts, they could still exhibit interesting biological properties. Alternatively, the introduction of constrained γ-amino acid residues (e.g., *m*-aminobenzoic acid and (1*R*,3*S*)-3-aminocyclohexanecarboxylic acid) in each strand of a protein-derived hairpin was found to be an effective approach to stabilize native-like hairpin folding in aqueous solution and to maintain original display of natural side chains.[78]

Successful design of conformationally stable water-soluble β-strand and β-sheet peptidomimetics may also integrate additional elements that can further promote β-sheet secondary structures including nonnatural turn segments,[79] preorganized H-bond templates[80] (e.g., Hao unit invented by Nowick),[81] macrocyclization,[82] and combinations thereof[83] (**Fig. 7**).

Alternative approaches include those based on short topographical mimetics of extended peptide structures, such as the oligopyrrolinones developed by the groups of Smith and Hirschmann (**Fig. 8A**),[84] the pyrrolinone–pyrrolidine oligomers invented by Burgess and coworkers (**Fig. 8B**),[85] or the 2,2-disubstituted indolin-3-ones (**Fig. 8C**),[86] 1,3-phenyl-linked hydantoin oligomers (**Fig. 8D**),[87] and aryl imidazolidinones[88] developed by the Hamilton group (**Fig. 8E**). These "minimalist mimetics" have been shown to be interesting nonpeptidic molecular scaffolds that mimic only the desired side-chain functionality (*i*, *i*+2, and *i*+4) of a β-strand, but water-soluble examples for functional studies in aqueous media are still lacking.

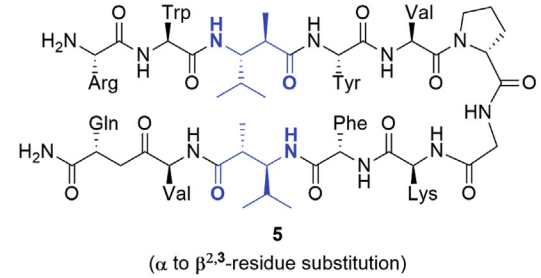
5.05.2.1.5 Folding upon binding

In Nature, numerous recognition events are triggered by conformational switches that are most of the time reversible, allowing alternation between an "on" and an "off" state in response to an external stimulus. Based on this principle, chemists have sought to



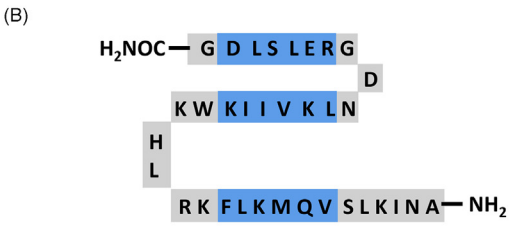
4

(α to β^3-residue substitution)



5

(α to $\beta^{2,3}$-residue substitution)



anginex	ANIKLSVQMKLFKRHLKWKIIVKLNDGRELSLDG-NH_2
6	ANIKLS**β³V**QMKLFKRHLKW**KIIVK****β³L**NDG**β³R**ELSLDG-NH_2
7	ANIKLSV**β³Q**MKLFKRHLKWKIIV**β³K**LNDGR**β³E**LSLDG-NH_2
8	ANIKLSVQ **β³ M**KLFKRHLKWKII **β³ V**KLNDGRE **β³ L**SLDG-NH_2
9	ANIKLSVQM **β³ K**LFKRHLKWKI **β³ I**VKLNDGREL **β³ S**LDG-NH_2
10	ANIKLSVQMK **β³ L**FKRHLKWK **β³ I**IVKLNDGRELS **β³ L**DG-NH_2
11	ANIKLSVQMKL **β³ F**KRHLKW **β³ K**IIVKLNDGRELSL **β³ D**G-NH_2

Figure 6 (A) Sequence of α/β-peptide analogs of a hairpin model α-peptide; design of **4** involves (αα → β^3)-residue substitution, while design of **5** involves (αα → $\beta^{2,3}$)-residue substitution. (B) The three-stranded alignment of the anginex peptide with a systematic α- to β^3-residue substitution in-registry (**6**–**11**) in the sheet forming regions *(blue frame)*.

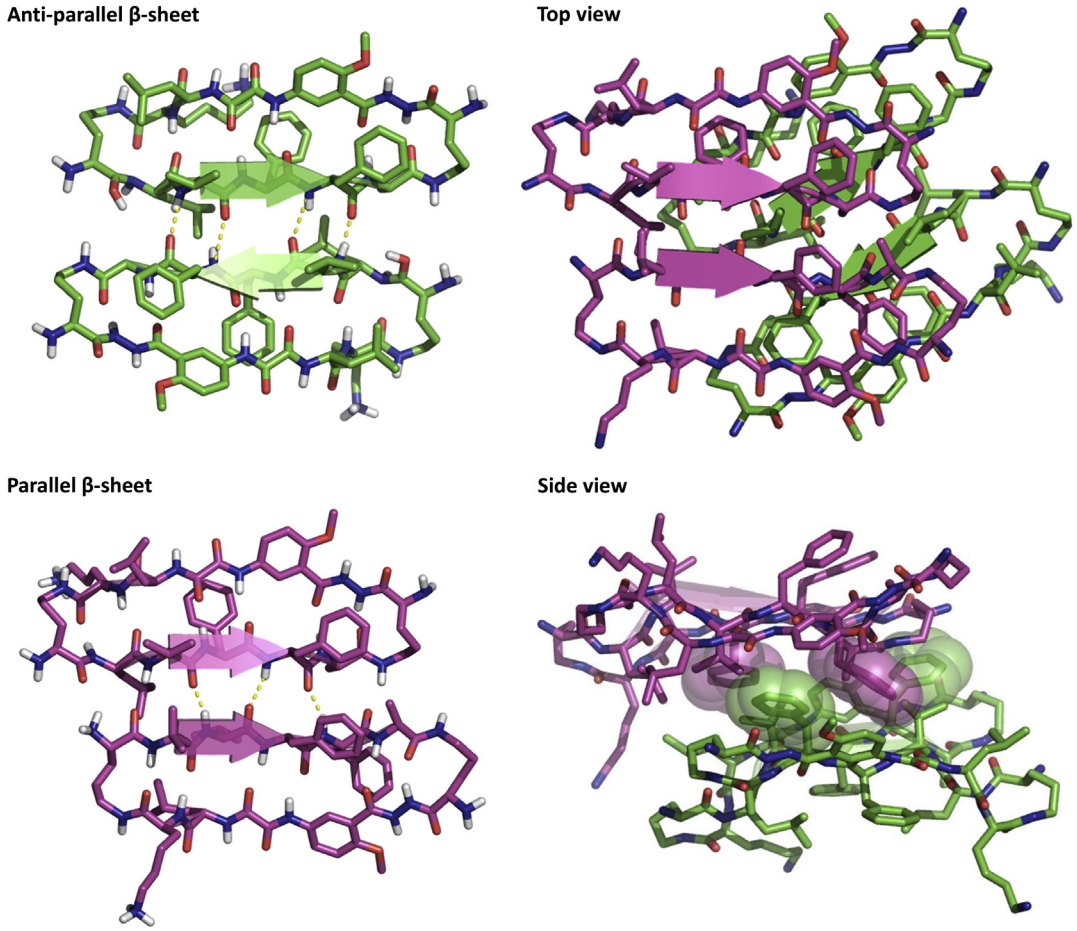


Figure 7 Crystal structure of a macrocyclic peptide designed by Nowick and mimicking amyloid β-sheets (PDB ID: 3Q9H). The *magenta* and *green* structures correspond, respectively, to the parallel and antiparallel β-sheet dimers. The top view and side view show packing of the two dimers (spheres emphasize hydrophobic contacts between them).

design molecules able to predictably change conformation under specific conditions. The stimuli can be from various sources, such as light excitation, redox process, protonation state, ion binding, or interaction with a biopolymer.[89] In the field of foldamers, several classes of oligomeric backbones do not show strong conformational preference when they stand alone in solution, but can fold into a well-defined secondary structure upon binding to a complementary target. Many abiotic systems have been reported

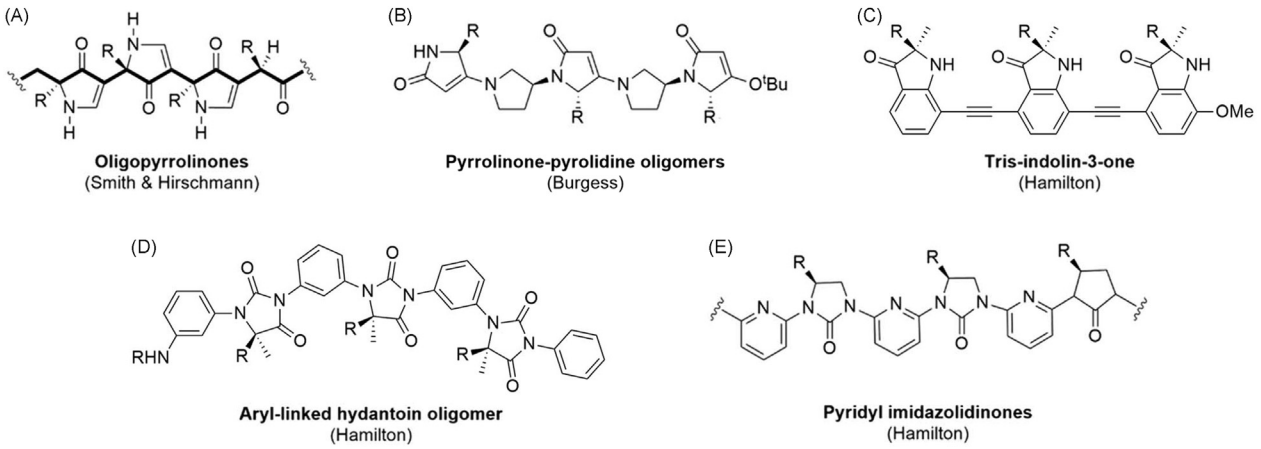


Figure 8 Examples of topographical mimetics of extended peptide structures.

in which folding and guest recognition (e.g., ions) are coupled processes (mainly in organic solvent). There are few cases of biologically relevant synthetic oligomers that express similar properties in an aqueous environment, that is, folding induced by recognition of a biological target.

The pyrrole–imidazole (Py–Im) oligomers developed by Dervan and coworkers are an important class of DNA-binding polyamides, whose design was based on the natural product distamycin A.[90] These oligomers adopt a hairpin-shaped structure only when bound to the minor groove of DNA. Their sequence is encoded by antiparallel side-by-side pairings of heterocyclic amino acids that distinguish the edges of the four Watson–Crick base pairs according to the following "pairing rules": Im (*N*-methylimidazole)/Py (*N*-methylpyrrole) codes for G–C base pair, while Py/Im codes for C–G; Hp (hydroxypyrrole)/Py codes for T–A, while Py/Hp codes for A–T; and Py/Py recognizes both T–A and A–T base pairs.[91] In the past decade, extensive studies have been conducted (on the turn segment, the monomers, the backbone) to improve these recognition properties, the specificity for the targeted DNA and binding affinities. For example, the introduction of flexible β-alanine residues in the aromatic backbone was found to improve binding of polyamides at long binding site by alleviating curvature mismatch,[92] whereas chiral substitutions of the γ-aminobutyric acid turn motif enhanced the properties of polyamide hairpins in terms of specificity and binding orientational preferences.[93] These optimized oligoamides bind DNA in a sequence-specific manner with affinities in the range of transcription factors and display interesting biological activities (see sections "**Nucleic Acid Recognition**" and "**Pharmacokinetics and Tissue Distribution**"). These oligoamides can also selectively distinguish DNA from the corresponding RNA sequences, mainly due to a decrease in polyamide-RNA shape complementarity and a reduced solvation of the wide shallow of double-stranded RNA minor groove.[94] This makes them useful probes for DNA-mediated processes.

Peptide nucleic acids (PNA) which have been developed by Nielsen and coworkers are another family of oligomers that fold upon hybridization to DNA.[95] These mixed oligomers display a pseudopeptide backbone, composed of *N*-(2-aminoethyl)-glycine units, bearing the A, G, C, and T nucleobases as side chains attached *via* a methylene carbonyl spacer. PNAs have been shown to form stable hybrid complexes, based on Watson–Crick base pairs with either DNA or RNA, that are more stable than the cognate nucleic acid–nucleic acid duplexes.[96] Binding events occur through strand invasion, where a PNA oligomer displaces a strand of the oligonucleotide duplex.[95,97] Crystal structures of palindromic six-base pair PNA duplex have been obtained,[98] as well as PNA complexes that formed homo-duplex and -triplex motifs.[99] The latter structures illustrate the conformational flexibility and the high structural adaptability of the PNA backbone, with the ability for a single PNA strand to adopt an extended helical conformation to allow nucleobase stacking and complex hydrogen-bond network formation. Chiral γPNA–DNA duplexes have also been characterized leading to right-handed helices of the P-form.[100] These γ-substituted PNAs are already preorganized into this characteristic helical structure, even in the absence of hybridization, showing thus a stronger helix propensity due to the presence of the substituent at the γ-position which strongly preorganizes the backbone. The helix propagates then from the C- to the N-terminus in a cooperative fashion. PNA foldamers are good candidates for antisense gene therapy[101] as well as applications in molecular biology or diagnostics,[102] but their poor solubility under physiological conditions still prevents the development of PNA-based therapeutics.

A number of foldamer backbones which are known to adopt a well-defined conformation in organic solvent do not necessarily fold into a single, well-characterized structure in aqueous environment. This is the case for **12**, a β^3-pentadecapeptide reported by Seebach and coworkers to bind DNA duplexes (**Fig. 9**).[103] It was designed assuming a 3_{14}-helical conformation by distributing positively charged side chains (i.e., β^3-Lys) that could mediate ionic interactions with the phosphate groups of the oligonucleotide backbone in a nonspecific manner and β^3-Asn residues that could make specific H-bond interactions into the DNA major groove on the recognition face of the helix. In aqueous environment, the β^3-pentadecapeptide **12** does not exhibit the typical CD signature which would indicate helical folding. However, in the presence of DNA duplexes, the characteristic CD signal of a 3_{14}-helix was observed, supporting a stabilization of the helical conformation upon DNA binding. The same group also reported that hairpin-type arrangement of secondary structures of β-peptides could also be initiated and/or enforced through the chelation with Zn^{2+} cation, when Cys or His side chains were introduced at appropriate positions,[104] similar to what is found in Zn finger domains of gene-regulatory proteins.

Altogether, these specific examples and studies conducted on other types of backbones (e.g., peptoids and aromatic-aliphatic oligoamides[105]) where multiple conformations might coexist support the idea that synthetic oligomers with conformational variability are not necessarily less important than the congeners adopting more stable and more defined structures, and also have potential for applications.

5.05.2.1.6 Higher order foldamer structures: toward more sophisticated functions?

A large fraction of foldamer backbones described in the previous sections are intended to mimic individual protein secondary structure elements such as α-helices and β-sheet-type architectures. These foldamers and α-helix mimetics in particular have found widespread use as scaffolds to target PPIs (see section "**α-Helix Mimicry for Modulating Protein–Protein Interactions**"). Whereas some protein interfaces are relatively simple, accommodating individual α-helices as key recognition elements, others are much more complex, being broader and less defined, which suggests a potential for larger foldamer architectures comprising two or more secondary structure elements arranged in space.[106] The de novo design of complex unimolecular folds (supersecondary and tertiary structures) as well as noncovalent assemblies (quaternary structures) constructed from foldamer backbones, with a high level of control over the final structure, is however much more challenging. This is due (i) in part to the difficulty to chemically synthesize long oligomeric sequences required to create tertiary structures, (ii) to the necessity to implement still poorly

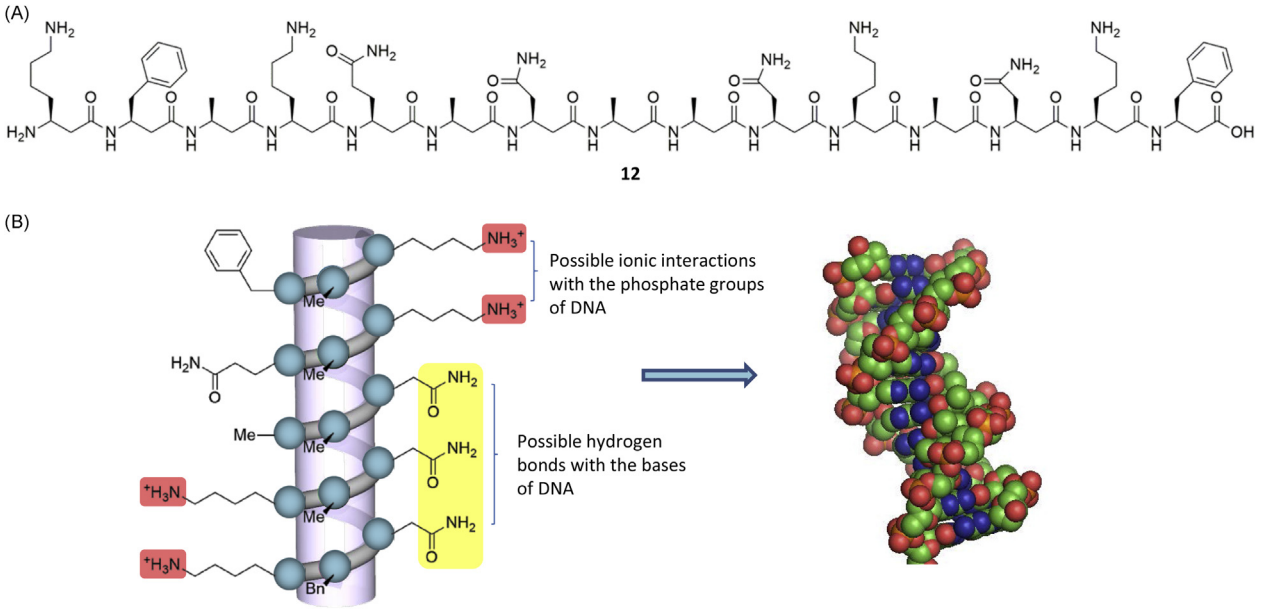


Figure 9 (A) Structure of a β^3-pentadecapeptide **12** designed to bind duplex DNA. (B) Schematic representation of the 3_{14}-helix and a CPK model of a DNA double helix (PDB ID: 1D98).

understood design principles for bringing secondary elements together, and (iii) to the need to characterize assemblies at high resolution to test hypotheses.

The helix-turn-helix (HTH) or helical hairpin motif obtained when two antiparallel α-helices are connected by a short turn/loop segment (typically 2–9 residues) is probably the most simple design beyond individual helices and appears to be a privileged scaffold for targeting large protein interfaces.[106,107] Principles that govern helical hairpin formation in proteins have been analyzed in detail[108] and strategies to stabilize these structures have emerged.[107a] In recent years, there have been several attempts to construct foldamer-based HTH motif supersecondary structures, but few studies have been conducted in aqueous solution.[109] Two β-peptide helical hairpins utilizing D-Pro-Gly and cystine units as interhelical segments have been characterized in solution.[110] Helical aromatic oligoamides and rigid diamine interhelix connectors have also been employed.[111] α/β-Peptide helix-loop-helix tertiary structures whereby α-residues at regular intervals are replaced by β^3-analogs bearing the same side chain in the sequence of a biologically relevant α-peptide have been developed to target specific protein surfaces.

Some progress has been made toward the creation of even more complex fully artificial tertiary structures combining multiple secondary structure elements, but only a few structures have been characterized at atomic resolution. Examples include compact multi-helical structures and multi-stranded artificial β-sheets from peptoid,[112] β-peptide,[113] and aromatic oligoamide[114] backbones. Mirror image phage display has also been applied to select fully artificial, protease-resistant mini D-protein scaffolds that target specific protein surfaces (PDB ID: 5HHC, http://dx.doi.org/10.2210/pdb5hhc/pdb, **Fig. 10A** and section "**Targeting vascular endothelial growth factor signaling**").[48f] Another promising approach involves redesigning biopolymers by mixing natural and nonnatural backbones in a single chain (e.g., protein prosthesis).[115] Such composite foldamer proteins can be obtained by replacing subsets of α-residues by unnatural building blocks in the sequence of a parent protein[38,116] or by swapping an entire protein secondary structure element (such as α-helices) with its foldamer counterpart.[117] Protein backbone engineering, which benefits from current advances in total protein chemical synthesis and semisynthesis, can be used to interrogate protein folding, address the role of individual folded segments, modify physicochemical properties, and replicate or modulate protein function. A noteworthy example is a variant of the semisynthetic hIL-8 proteins in which the C-terminal α-helix was replaced by a designed sequence of 14 β^3-residues and which was shown to display an activity comparable to that of the native hIL-8. However, high-resolution structural information and structure activity relationship studies were not reported in this study. Complementary work from the Gellman and Horne groups has provided details at atomic precision about the impact of β-residue substitutions in helical segments within discrete tertiary structures (e.g., villin headpiece subdomain,[118] B1 domain of protein G from *Streptococcus* bacteria (GB1)[119]) and quaternary structures (e.g., yeast transcriptional activator GCN4-derived peptides[38,116d,120]). In particular, the Horne group has shown that the GB1 tertiary fold can accommodate up to 20% unnatural backbone content (combination of D-amino acid, Aib, *N*-Me-amino acid, and β^3-amino acid replacements) in all different secondary structure elements (helix, loop, sheet, and turn), highlighting the remarkable plasticity of the protein backbone (e.g., PDB ID: 4OZC in **Fig. 10B**). Further comparative analysis of folded structures and thermodynamics of GB1 derivatives according to residue types in the α-helical secondary structure shows several trends: (i) β^3- and β^2-residues have similar helix folding propensities when their side chains are not involved in key

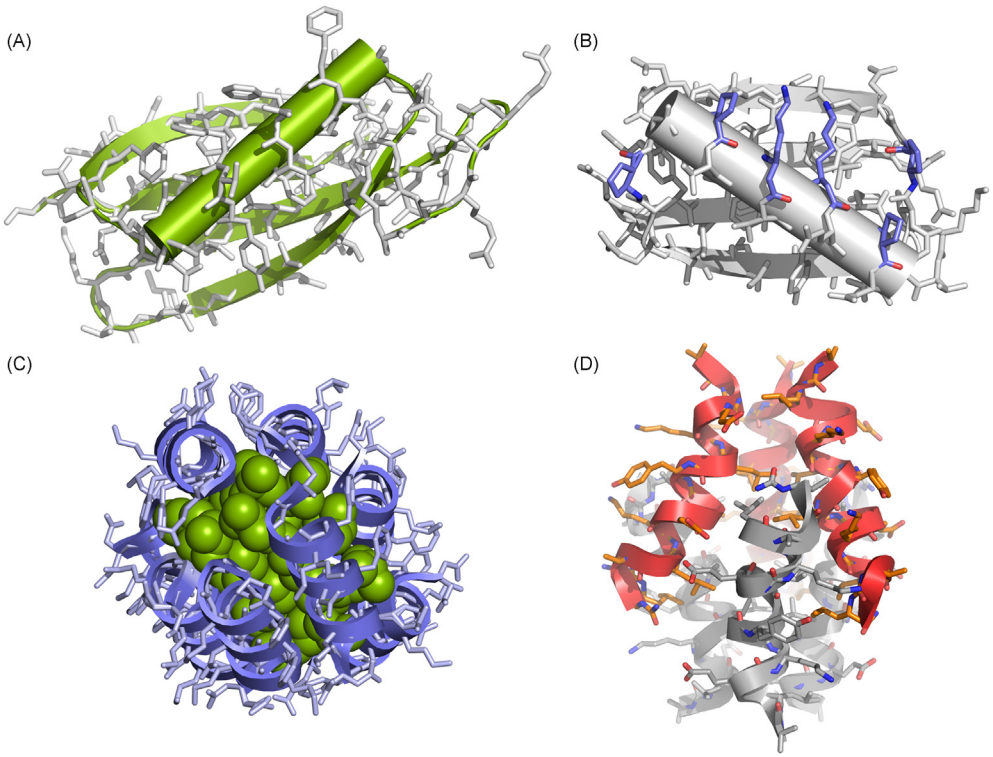


Figure 10 Examples of higher order foldamer architectures mimicking natural proteins. (A) Crystal structure of a protease-resistant mini D-protein scaffold; (B) X-ray structure of a GB1 tertiary-fold analog accommodating six β-amino acid residues in its helical and adjacent loop segments; (C) X-ray structure of a β^3-peptide octameric helix bundle; and (D) X-ray structure of an oligourea six-helix bundle.

interactions, (ii) constrained cyclic β-residues generally lead to a modest increase in folded stability compared to acyclic β-residues, and (iii) Aib residues (and their chiral counterparts) proved better than β-residues at stabilizing tertiary folds.[119b,121]

Effort to assemble foldamers into quaternary structures is also a promising direction to create increasingly diverse foldamer higher order structures including single-strand tertiary folds after covalent linkage of the different elements. Macrocyclic β-sheet peptide mimics (see Fig. 7) have been reported to self-assemble into atomically precise nanosized structures such as fibril-like and annular pore-like assemblies.[71] Foldamer helix bundles of various stoichiometries have been characterized in the crystal state, including β^3-peptide octameric helix bundles (e.g., Fig. 10C), tetrameric bundles formed from α,β-peptide hybrid helices, and six-helix bundles formed from *N*,*N*′-linked oligoureas with proteinogenic side chains (e.g., Fig. 10D).[122] Alternative topologies such as superhelical channels with tunable diameters can also be formed by varying the proportion and distribution of hydrophilic and hydrophobic side chains at the helix surface.[122] Some of these assemblies (e.g., β^3-peptide octameric helix bundles) have demonstrated useful molecular recognition properties including the ability to bind and differentiate biomolecules such as sugars in aqueous solution.[123]

5.05.3 Design of Bioactive Foldamers

Remarkable progress has been accomplished over the past 15 years with the discovery of many potent bioactive foldamers originating from diverse backbones and targeting a whole spectrum of bio(macro)molecules such as membranes, protein surfaces, and nucleic acids. Secondary structure predictability, sequence programmability and modularity, as well as compatibility with SPS methods are major determinants of success. Structural data at high resolution and structure-guided design have also become increasingly important in projects aimed at designing potent inhibitors of PPIs.

5.05.3.1 General Considerations

5.05.3.1.1 Designability, synthetic accessibility, and diversity enhancement to improve biopolymer mimicry

Developing a particular foldamer scaffold (such as those discussed in the previous sections) for medicinal chemistry applications and transforming it into an enabling platform still require multiple challenges to be met. Currently, one of the most challenging tasks resides in reproducing the spatial arrangement of the key side chains found at the surface of the cognate biopolymer epitope to mimic.

Synthetic accessibility and modular chemistries are thus highly needed to introduce multiple levels of diversity (diversity of side chains[124] and substitution patterns,[21b,26c,125] backbone isosteric modifications[126]) and to rapidly access large series of compounds that can be screened against targets of interest. Compared to parent α-amino acids, β- and γ-amino acids as monomeric units are characterized by a much greater chemical diversity and conformational versatility. For example, β-amino acid units have five substitution positions compared to three for their α-counterpart and the number of configurational isomers increases to eight (vs. two for α-amino acids).[1c] Access to this diversity is often needed to exert control over folding and over side-chain display, and to finely tune and improve interactions in terms of affinity and/or specificity of a given foldamer with its target surface (e.g., combination of β^3-amino acid and cyclic β-amino acid residues for the design of effective α-helix mimics with α/β-peptides[127]). Studies aimed at delineating the rules that govern folding of constituent units with new substitution patterns and at characterizing structures of the resulting oligomers at atomic resolution have been conducted systematically on many different systems (e.g., γ-amino acids, oligourea monomeric units). For example, shifting the side chain from one carbon to the other in 1,2-diamino monomer constituents of 2.5-helical oligoureas may be used to subtly vary the relative distribution of side chains at the surface of these peptidomimetics and possibly optimize binding to a given protein surface.[26c] This effort toward expansion of monomer libraries has not been limited to aliphatic units, and in the context of abiotic aromatic oligoamides for example, a complete array of suitably designed monomers varying in size and shape has been employed to produce foldamer receptors with very high selectivity for specific monosaccharides (e.g., fructose).[128] Mixing natural and nonpeptide backbones to further modulate the conformational and functional behavior of natural biopolymers is another promising approach that can lead to functional mimics of bioactive α-peptide sequences (e.g., α/β-peptides[37d], hybrid α-helix mimetic[34c]). To have a practical impact in medicinal chemistry programs, the development of useful monomer libraries must be coupled to efficient and rapid oligomer synthesis schemes. Peptide backbone-modification strategies such as those based on β-amino acid residue replacements have been readily implemented because the necessary α/β-peptides are accessible via effective SPS procedures,[129] and many protected β-amino acids have been reported or are commercially available. The same holds true for peptoids for which synthetic methodology on solid support from appropriate (sub)monomeric precursors is extremely well established.[130] Efficient SPS methodologies have been developed for a growing number of less conventional foldamer backbones such as aliphatic oligoureas,[131] oligomers of 7- or 8-amino-2-quinoline carboxylic acids,[132] hybrid α-amino acid/quinoline oligoamides,[57] 3-*O*-alkylated and *N*-alkylated aromatic oligobenzamide α-helix mimetics,[133] and Py–Im polyamides.[134] In many cases, microwave assistance has been employed to overcome synthetic difficulties inherent to some of these backbones (e.g., poor nucleophilicity of aromatic amines) or simply to accelerate the production of longer foldamer sequences. In a few cases, optimized SPS procedures have been exploited to prepare large foldamer libraries for high-throughput screening. Gellman and coworkers used polystyrene macrobeads and microwave irradiation to generate medium-size one-bead-one-stock solution combinatorial libraries via a split-and-mix approach. Screening the library for inhibitors of the p53–MDM2 interaction however did not permit identification of compounds significantly more potent than a previously reported β-peptide sequence,[19a] presumably because of the limited size of the library. Similarly, high-quality one-bead-one-β-peptide libraries suitable for on-bead screening have been reported by Schepartz and used to identify inhibitors of the p53/MDM2 interaction with IC_{50} values in the low μM range following simple tandem mass spectrometry (MS/MS) decoding method.

Complementary to chemical methods to generate nonnatural oligomers and corresponding libraries, several biotechnological approaches are gaining increasing attention in the context of nonnatural oligomers and foldamers. Mirror image phage display[48a,d] is a very powerful and elegant technique to identify D-peptide ligands against a specific protein target. However, mirror image phage display which requires the mirror image of the target to be chemically synthesized is currently limited to relatively small protein targets. Exciting new approaches with far-reaching development and application potential are currently being explored to exploit the biosynthetic machinery responsible for protein synthesis to incorporate noncanonical backbone-modified residues at one or several desired positions in a peptide chain.[135] Spectacular progress has been made in this direction using in vitro-reconstituted translation systems to produce short polypeptides containing diverse exotic units such as D-amino acids,[136] *N*-methyl-amino acids,[136a] and β-hydroxy acids.[137] Suga and coworkers have used genetic code expansion and reprogramming methodologies to incorporate single and multiple β^3-amino acids in a peptide chain.[136a,138] In 2016, they have shown that 13 β-amino acids are compatible with ribosomal translation with β^3Gly, L-β^3Ala, L-β^3Gln, L- and D-β^3Phg, and L-β^3Met showing the highest incorporation.[138] Double-incorporation experiments using β-amino acids revealed that elongation of peptides with successive β-amino acids is prohibited but that double incorporation of β-amino acid residues is possible if an α-residue is inserted between. The same year, Schepartz reported that β^3-Phe residues can be incorporated into full-length proteins in vivo using *Escherichia coli* strains expressing mutant ribosomes.[139]

5.05.3.1.2 Resistance to proteolysis

One inherent limitation to the use of isolated L-α-peptides in therapeutics is their susceptibility to degradation by circulating enzymes, which can considerably shorten their biological lifetime and duration of action in vivo. In contrast, foldamers built from non-α-amino acid units have a strong potential to resist enzymatic degradation. An increasing number of biotic and abiotic backbones have now been tested and all of them display remarkable in vitro stability against a variety of peptidases from bacterial, fungal, and eukaryotic origins (e.g., leucyl aminopeptidase, proteinase K, trypsin, chymotrypsin, elastase, amidase, β-lactamase, 20S proteasome, pepsin, subtilisin, cathepsin, thermolysin). This is the case for β- and γ-peptides[140] as well as for α-peptoids,[141] β-peptoids, *N*,*N*′-linked oligoureas,[142] aromatic oligoamides,[55a] D-peptides,[143] and sulfono-γ-AApeptides.[144] However, only a few experiments have been conducted with other classes of enzymes involved in drug metabolism such as cytochrome P450 enzymes.

The situation is more complex for hybrid foldamers that combine unnatural monomeric units with proteinogenic α-amino acids. The interest for such hybrid foldamers stems from both the increased chemical and structural diversity brought by the combination of different types of monomeric units and from the possibility for such backbones to more closely mimic targeted protein-binding surfaces. It is well documented that the insertion of a single noncanonical unit (β-amino acid residue, peptide bond isostere) at a scissile bond can also increase stability in the proximity of the modified residue.[145] Early model studies by Seebach and coworkers on β-peptides containing N-terminal and internal β-amino acid residues have shown that the stability of hybrid α/β-peptides to degradation by pronase (a mixture of nonspecific endo- and exo-peptidase) is highly dependent on the location of α-residues in the sequence.[146] When α-amino acids are located at the N-terminus of the β-peptide, cleavage of α/α and some α/β-peptide bonds (depending on the α-residue) by pronase has been observed. However when the natural α-amino acids are embedded into β-peptide sequences (1:1 pattern) or when the N-terminus is capped, no hydrolysis was detected. Proteolytic stability is of course expected to increase with the density of unnatural units in the sequence. To better understand the extent to which the insertion of noncanonical residues (e.g., D-amino acids, *N*-methylated amino acids, β^3-amino acids, tetrasubstituted amino acids) protects adjacent positions and to gain a more comparative view between different types of noncanonical units, Horne and coworkers have systematically studied the effect on resistance to chymotrypsin of single or tandem α-amino acid replacements (varying by their nature and position) within a model peptide sequence containing a chymotrypsin-specific cleavage site. For this specific sequence, it was found that the inversion of the stereogenic center (D-α-residue replacement), or Cα tetrasubstitution (Aib-type residue replacement), is the most effective modification for protection against degradation, whereas α → N-Me-α-residue was the least effective replacement. An intermediate level of protection from degradation was observed for peptides containing α → β^3 replacements. By extending this study to tandem mutants with different spacing, Horne and coworkers found that combined replacements can be synergistic but that the correlation between the density of unnatural units and proteolytic stability is not necessarily simple. In a pioneering work on α-helix mimicry with α/β peptides, Gellman and coworkers further demonstrated that evenly spaced α → β replacements along a bioactive α-peptide sequence certainly represent the most efficient way to achieve maximal protection while keeping chemical alterations of the starting peptide sequence to a minimum. For example, α-helix mimetics with ααβααβ and αααβ patterns described in section "**Heterogeneous backbone: peptide-foldamer chimeras**" (see also sections "**α-Helix Mimicry for Modulating Protein–Protein Interactions**" and "**Ligands of G-Protein-Coupled Receptors: Hormone Like Analogs**) are significantly more resistant (often ≥100 fold) in the presence of proteinase K than the original bioactive α-peptides they intend to mimic.[37d] Results from the Wilson Laboratory suggest that this general approach leading to reduced susceptibility to proteases can be extended to hybrid backbones containing other types of unnatural units such as aromatic units.[34c]

5.05.3.2 Membrane-Active Foldamers as Antimicrobials

There is a growing interest in the development of cationic membrane-active antimicrobial peptides (AMPs) as alternatives to conventional antibiotics for the treatment of bacterial infections.[147] Endogenous AMPs are essential components of the innate immune system of eukaryotic organisms, from plants to mammals, serving as a first line of defense against a broad range of microorganisms, including Gram-negative and Gram-positive bacteria.[148] AMPs form a large and highly diverse family of molecules ranging in size from 15 to >50 amino acid residues; they do share common physicochemical features though. AMPs are largely cationic and display an amphiphilic character, thus allowing them to interact preferentially with the negatively charged bacterial membranes. A number of issues, such as susceptibility to proteolytic degradation and toxicity following systemic application, need to be addressed for AMPs to meet their full potential in the clinic. In addition to this, there is increasing evidence that pathogenic bacteria can develop resistance to endogenous AMPs through a variety of mechanisms often involving biophysical and biochemical changes to the bacterial cell wall.[149] One approach to circumvent some of these limitations involves designing synthetic analogs that can recapitulate the amphiphilic character of AMPs[150] such as membrane-active foldamers.[8,151] Linking structure and function in foldamers is generally facilitated by the dual control over monomer sequence and secondary structure as well as by the diversity of the building block repertoire. A large fraction of foldamer AMP mimics is reported to have been designed to form globally amphiphilic helices by clustering hydrophobic and cationic residues at the surface of the helix (**Fig. 11**). This is the case for antimicrobials based on β-peptide 14-, 12-, and 10/12-helices,[15d,151,152] peptoids,[150a,153] *N*,*N*′-linked aliphatic oligoureas and oligo(urea/amide) hybrids,[22,28] and more recently sulfono-γ-AApeptides.[144,154] For helical scaffolds with three residues per helix turn such as β^3-peptide and peptoids, most antimicrobial sequences reported are based on short triad repeats (hydrophobic–hydrophobic–cationic residues). For helical structures with 2.5 residues per turn (i.e., 12-helical β-peptides and *N*,*N*′-linked oligoureas), cationic residues are arranged to create a polar surface that covers between two-fifths and three-fifths of the helix circumference. These design principles led to potent molecules with broad-spectrum activity against a range of multidrug-resistant Gram-positive and Gram-negative bacterial pathogens including clinically relevant strains (among which *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are the most frequently tested). The reported activities for the most potent derivatives (minimum inhibitory constants (MIC) in the range of 1–10 $\mu g.mL^{-1}$) compare favorably with those of known peptides such as melittin (a honeybee toxin), pexiganan (a potent synthetic analog of natural magainins), and omiganan (a 12-residue-long peptide currently in the clinic for dermatologic applications). Interestingly, cationic foldamer mimics of AMPs with peptoid and oligourea backbones have been shown to be equally active against other Gram-positive infectious bacteria such as *Mycobacterium tuberculosis* and *Bacillus anthracis* (the causative agent of anthrax), respectively.[142,155] Systematic structure–activity relationship studies have been conducted to improve potencies and more

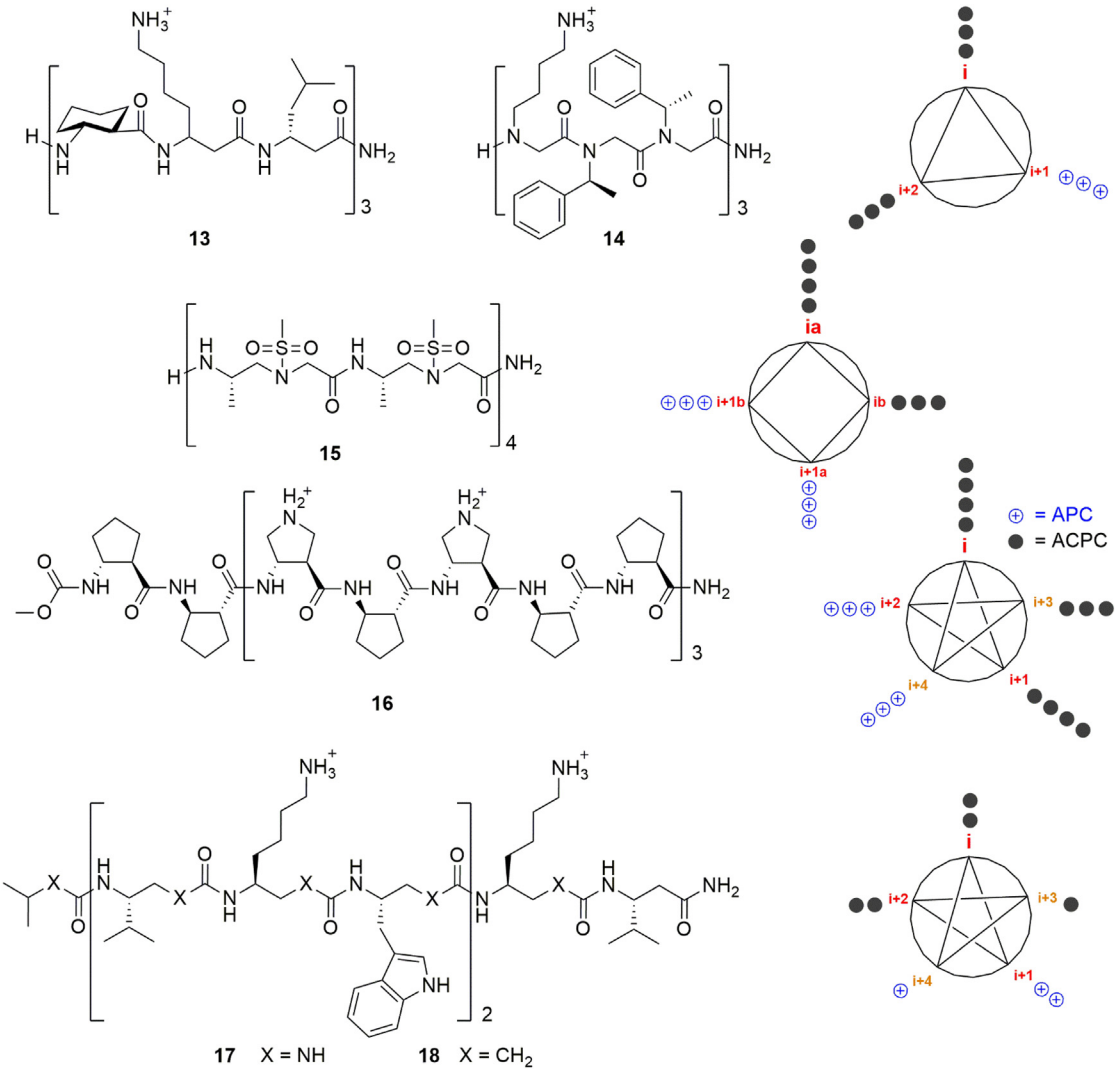


Figure 11 Design of representative amphiphilic helical cationic antimicrobial foldamers assuming idealized helical structures.

importantly reduce hemolytic activity and increase the selectivity of these compounds for bacterial membranes. Representative examples in these series include 3_{14}-helical β-nonapeptides containing *trans*-ACHC residues and β^3-HLys residues (e.g., **13**),[15d] peptoid dodecamers containing α-chiral monomers such as **14**,[153] the sulfono-γ-AApeptide **15**,[144] the 17-mer 12-helical β-peptide **16** composed of hydrophobic ACPC residues and cationic aminopyrrolidine carboxylic acid (APC) residues,[15b] and the 8-mer helical oligourea (e.g., **17**).[28] Surprisingly, the isosteric γ^4-peptide analog of **17** (e.g., **18**), despite close structural similarity and similar side-chain distribution, was found to display only marginal antibacterial activity.[22] The origins of this difference between oligoureas and γ^4-peptides are not yet fully understood but could be related to subtle differences in polarity and geometry between the two helical backbones. Alternatively it has recently been shown that aliphatic oligoureas are ideally preorganized to interact with anionic guests in a site-specific manner at the positive end of the helix dipole.[156] This property may suggest a complementary mechanism to account for the interaction properties of antimicrobial oligourea helices with model phospholipid membranes as well as bacterial membranes. There are other cases of unexpected and complex relationships between structure and function in the design of antibacterial foldamers, particularly among 14/15-helical α,β-peptides where sequences designed to adopt globally amphiphilic helical structure led to moderately active compounds in contrast to the control scrambled sequences.[157]

Concurrently, folded backbones even far remote from natural peptides such as arylamide and arylurea foldamers have been explored as membrane-active antibacterial agents. Beginning with polymers comprising alternating 1,3-phenylenediamine and isophthalic acid units,[54a] the groups of Tew and Degrado have developed a very successful program that led to the discovery of potent antimicrobials with remarkable activities and safety profile.[150b] These oligomers with molecular weights in the range of 600–1000 Da adopt a facially amphiphilic structure with cationic and hydrophobic side chains distributed on opposite faces

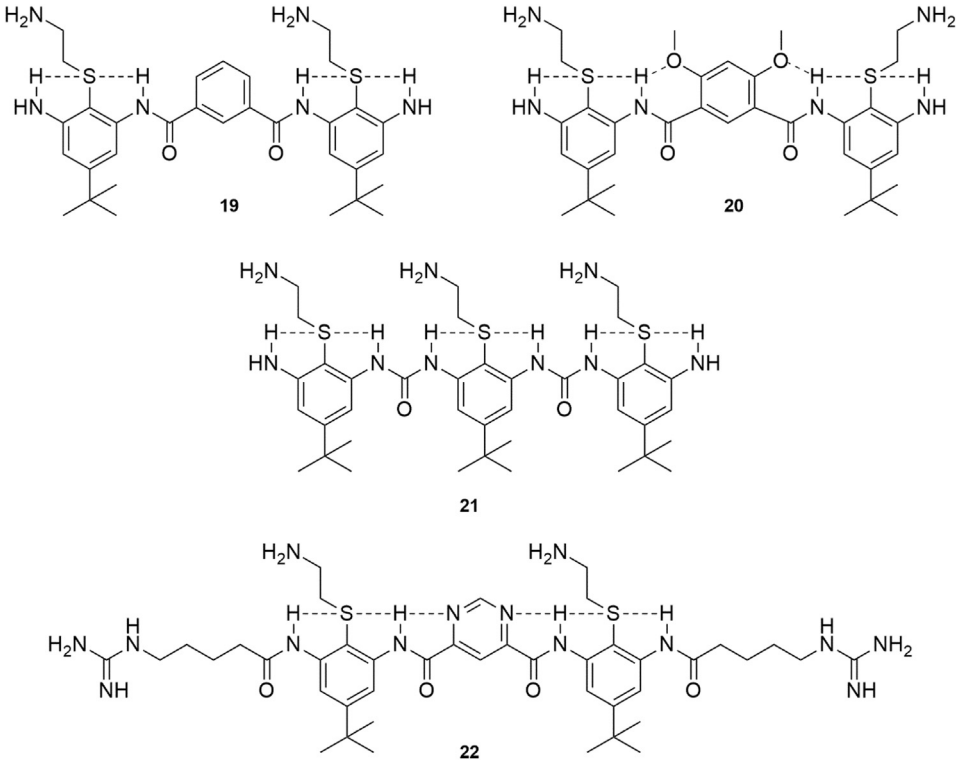


Figure 12 Membrane-active facially amphiphilic arylamide and arylurea foldamers.

of the molecule (Fig. 12). The conformation of these molecules is controlled by the formation of intramolecular hydrogen bonds between thioether groups installed on isophthalic units and NH of phenylene diamine units. First-generation oligomers exemplified by **19** were shown to exhibit modest activities against *S. aureus* and *E. coli*. Replacement of the isophthalic unit in **19** by a pyrimidine unit or a 4,6-alkoxyisophthalic acid unit was used to reinforce the H-bond network and further stabilize the planar conformation of the molecules.[158] This additional rigidification of the backbone was accompanied by a significant gain in activity against both Gram-positive and Gram-negative bacteria as shown for **20** (MIC = 0.87 μM against both *S. aureus* and *E. coli* and HC_{50} = 145 μM; HC_{50} = concentration that causes 50% lysis of human red blood cells). Facially amphiphilic urea-linked aryl oligomers (e.g., **21**) were found to be equally potent but exhibited significant hemolytic activity with HC_{50} near their MIC.[54c] The introduction of terminal guanidinium groups and replacement of *tert*-butyl groups by trifluoromethyl groups led to further improvement in potency with molecules such as **22** exhibiting low MIC against a variety of Gram-positive and Gram-negative human clinical isolates and minimal toxicity to mammalian cells.[158] Detailed biophysical studies suggest that molecules like **22** destabilize anionic lipid membranes primarily by altering membrane electrical potential, and by creation of transient defects in the membrane but in a less disruptive manner than typical AMPs.[159]

Despite the fact that most foldamer backbones for which antibacterial activities have been reported are more resistant to proteolytic degradation than cognate α-peptides, relatively few reports have described activities in animal models. In vivo studies which include evaluation of arylamide foldamers in a *S. aureus* thigh infection model[158] and *N*,*N*′-linked oligoureas in inhalational and cutaneous mouse models of *B. anthracis* infection are discussed in section "**In Vivo Studies of Bioactive Foldamers**."

5.05.3.3 α-Helix Mimicry for Modulating PPIs

PPIs mediate numerous biological processes and functions in living organisms, and thus represent promising new targets for the development of new therapeutics. Although the field of PPI inhibitors/modulators has witnessed spectacular progress over the past two decades with the development of various approaches based on small molecules and recombinant proteins, the design of synthetic molecules aimed at interfering with PPIs has remained exceptionally challenging in part because protein–protein interfaces are exceptionally diverse in terms of sizes and shapes.[160] Medium-size molecules such as α-peptides can be used to mimic the secondary structural elements at larger protein–protein interfaces (so-called secondary-structural epitopes) and thus may contribute to fill the gap between small molecules and biologics for the development of novel classes of PPI modulators. This interest in peptides also stems from their growing contribution to the worldwide pharmaceutical market as witnessed by the number of peptide therapeutics in clinical trials and by the unprecedented number of market approvals in 2012.[161] However, as already discussed, isolated linear peptides generally have some limitations such as (i) poorly defined conformations, (ii) susceptibility to

degradation by circulating enzymes, and (iii) with few exceptions lack of cell permeability, which possibly limits their use to extracellular targets. In this context, chemical approaches aimed at reinforcing or mimicking protein secondary structure elements to develop innovative PPI modulators have flourished. Because α-helices are frequently found as recognition elements between proteins[6,106] and perhaps more amenable to chemical modifications, much effort has been devoted to their stabilization and mimicry.[58c] The most popular approaches developed to specifically increase the helical content of short α-peptides include but are not limited to (i) the introduction of constrained α,α-disubstituted amino acids,[162] (ii) side-chain-to-side-chain macrocyclization,[163] and (iii) the introduction of *N*-terminal templates and covalent hydrogen-bond surrogates.[164] These methods have all been applied with some success to create effective modulators of α-helix-mediated PPIs.[165] Note that some of these peptide-based inhibitors exhibit improved properties such as cell permeability expanding the range of targets currently considered suitable for drug development.

Complementary approaches for α-helix mimicry and PPI modulation based on foldamers have only emerged in the last 10 years but already show great promises. Essentially two strategies have developed in parallel: the first one features helicity as a guiding principle focusing on artificial backbones with predictable helical patterns and a high degree of similarity to α-peptides whereas the second one is more minimalist focusing on side chain display and on rigid and extended backbones ("topographical mimics"[69c]) quite remote from natural peptides. Today, significant progress in α-helix mimicry for inhibition of PPIs has been accomplished with different classes of artificial backbones including β-peptides (e.g., 14-helical β-peptides as p53 mimetics[19a,166]), heterogeneous oligoamides containing various combinations of α- and β-amino acid residues (e.g., mixed ααβαααβ and αααβ peptides that recapitulate the binding surface of known α-peptide inhibitors targeting gp41 and BCL-x_L proteins, respectively[37d]) and extended scaffolds (e.g., terphenyl,[61a] aromatic oligoamide,[167] oligopyridine,[168] and oligooxopiperazine[69c] derivatives as inhibitors of BCL-x_L proteins and as p53 mimetics).

5.05.3.3.1 Helices mimicking viral glycoproteins to inhibit viral fusion

Enveloped viruses like HIV-1, influenza, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) coronaviruses, and Ebola viruses share a common fusion mechanism mediated by viral membrane fusion glycoproteins with high helical content.[169] The fusion process leading to infection of cells involves major refolding of the glycoprotein and the formation of a so-called trimer of hairpins (i.e., antiparallel trimer of dimers or six-helix bundle). Following binding to cellular receptors, the glycoprotein undergoes a conformational change leading to exposure of a first heptad repeat (HR1) domain and insertion of a fusion peptide into the cellular membrane. Subsequently, in another major conformational change, a second heptad repeat (HR2) domain folds back onto HR1 to form a stabilized six-helix bundle structure (trimers of dimers) which juxtaposes the virus and cell membranes for membrane fusion. The successful development of enfuvirtide (T-20, Fuzeon), a 36-mer peptide that targets the transiently exposed HR1 domain of gp41 for the treatment of HIV-1 infection,[170] has established the therapeutic value of peptide-based approaches aimed at inhibiting the assembly of this six-helix bundle. It is noteworthy that the strategy has since been adapted to other enveloped viruses such as SARS and MERS coronaviruses,[171] Ebola virus,[172], measles,[173] and RSV,[174] leading to the discovery of potent viral fusion inhibitors. Some drawbacks associated with the use of natural HR2 peptide sequences include rapid in vivo proteolysis (enfuvirtide has a relatively short half-life in the plasma), drug resistance, and cross-reactivity with preexisting antibodies in patients. Various chemical approaches such as macrocyclization (i.e., lactam and hydrocarbon stapling[165c,174]) and backbone modifications have been explored to increase the resistance to proteolysis of peptide fusion inhibitors.

Successful mimicry of helical HR2 peptides for inhibiting the postfusion six-helix bundle structure formation has been achieved by periodic replacement of α-amino acids by protease-resistant β-amino acids in the sequence of **T2635** (**Fig. 13A** and PDB ID: 3F4Y), an optimized analog of T20.[175] Several α/β-peptide analogs with ααβαααβ pattern were designed by introducing β-residues at positions that are not involved in contacts with HR1 trimer surface. The peptides differ in the nature of their β-residues which can be either acyclic β^3-substituted or ring-constrained (ACPC, APC). In a competition fluorescence polarization assay measuring displacement of a fluorescently labeled CHR-peptide from an engineered five-helix bundle protein, GP41–5, compound **23** with both constrained ACPC and β^3-residues in its backbone was found to be much more effective ($K_i = 9$ nM) than **24** which contains exclusively acyclic β^3-residue replacements ($K_i = 3800$ nM), suggesting that some helix preorganization is required. Remarkably, compound **23** was found to be equipotent to the parent HR2-derived α-peptide **T2635** in a cell–cell fusion inhibition assay ($IC_{50} = 5$ nM) and in inhibiting HIV-1 infectivity (X4 and R5 strains, $IC_{50} = 28$–110 nM) and displayed >280-fold improvement in proteolytic stability (proteinase K assay) over **T2635**. Crystal structure of α/β-peptide **24** in complex with HR1 peptide at 2.8 Å resolution (PDB ID: 3F50) confirmed the formation of the expected six-helix bundles and the orientation of β-amino acid residues toward the solvent as predicted (**Fig. 13B**).[127]

The introduction of β^3Glu/β^3Arg pairs with *i*, *i* + 4 spacing within α/β-peptide helix (see compound **26**) was subsequently found to be equally suitable to ring-constrained β-residues for enhancing helical propensity, binding to HR1 trimer, and inhibiting HIV infection in cell-based assays. However, its stability to proteolysis was not significantly enhanced compared to the cognate peptide **25** despite the fact that all sites cleaved by proteinase K but one were suppressed. The substitution of three ring-constrained ACPC residues for β^3-residues near the remaining proteinase K cleavage site in **26** (i.e., **27**) resulted in dramatic (i.e., >1000-fold) improvement in half-life as well as increased binding to HR1 trimer. The main advantages of this approach are that the conversion of a given α-helical peptide into a corresponding analog with improved properties is relatively direct and that it is likely to be general (*vide infra*); yet the number, positions, spacing (ααβαααβ), and nature (β^3- vs. cyclic) of β-amino acid replacements certainly need fine-tuning for optimal bioactivity.

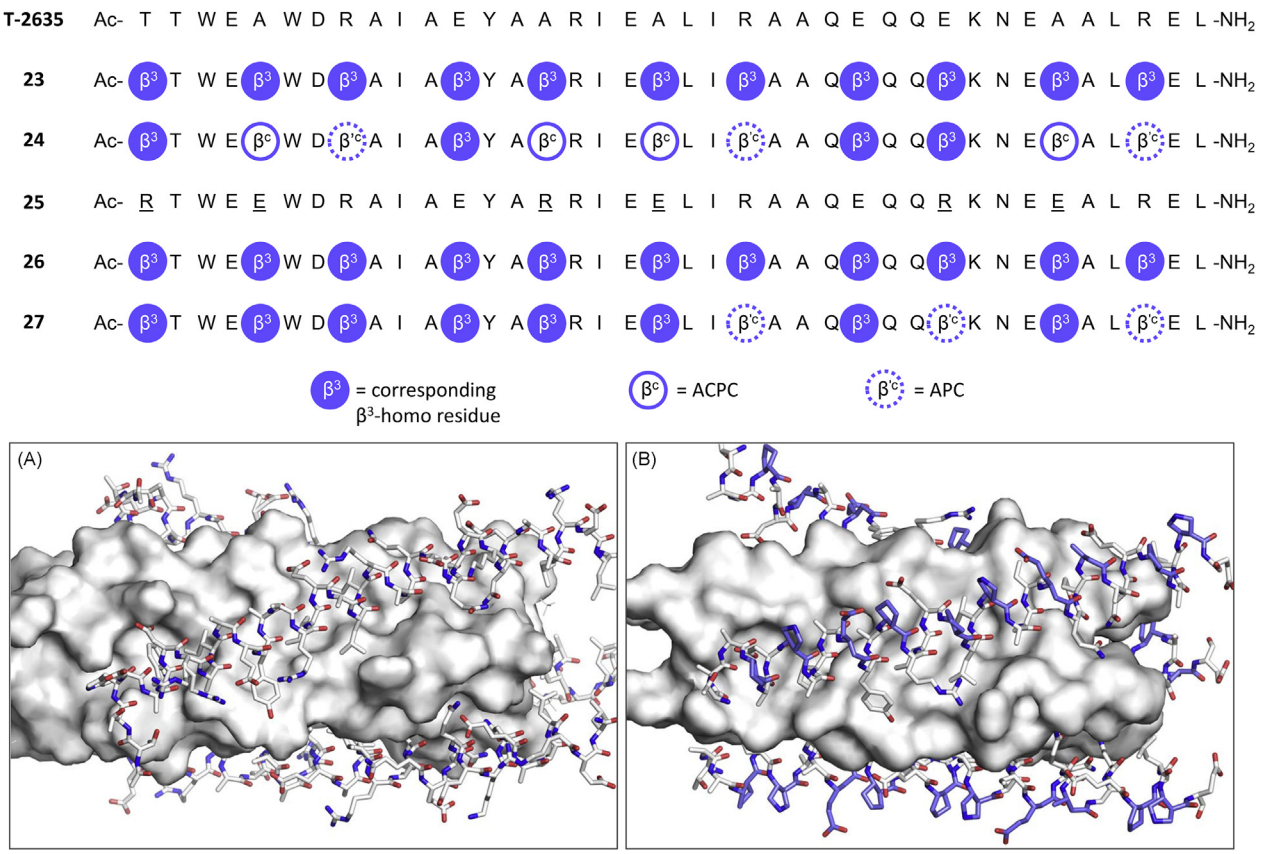


Figure 13 (Top) Sequences of α-peptides **T2635** and **25** derived from the second heptad repeat domain of gp41 and corresponding designed α/β-peptides **23**, **24** and **26**, **27**. β^3- and cyclic β-amino acid residues are shown in *blue disks/circles*, respectively. (Bottom) Crystal structures of **T2635** (A) and α/β-peptide **24** (B) bound to HR1 trimer surface in a six-helix bundle. Carbon atoms of β-residues in **24** are colored in *slate blue*.

Several other foldamer-based strategies aiming at discovering viral fusion inhibitors have been described and are worth being mentioned although the inhibitors reported were not yet as potent as those cited earlier. These studies include (i) the identification of D-peptide inhibitors of HIV-1[48b,176] and Ebola virus[172] using mirror image phage display technique,[48a,c] (ii) the de novo design of β^3-peptide analogs of the hydrophobic pocket-binding domain (628–635),[19c,177] and (iii) the use of the terphenyl scaffolds bearing hydrophobic side chains intended to mimic hydrophobic repeats in HR1 and HR2 peptides.[178]

5.05.3.3.2 Foldamer-based inhibitors of PPI linked to cancer

The ability of foldamer mimics of α-helices to interact with protein surfaces with high specificity has been assessed on several therapeutically important PPIs linked to cancer including p53/MDM2, and p300/HIF-1α (hypoxia-inducible factor 1α), and those involving anti-apoptotic and pro-apoptotic proteins of the BCL-2 family. Whereas most studies have concentrated on single-helix mimetics, some recent developments have explored more complex foldamer architectures such as supersecondary and tertiary structures to target broader protein surfaces.[106,107b,117b] Cellular uptake and engagement of the target inside the cell are additional key issues that have been addressed in some cases and that need to be solved for possible application of these molecules in a cellular context, as most of the targets mentioned in this section are intracellular.

5.05.3.3.2.1 Targeting anti-apoptotic Bcl-2 family members

The Bcl-2 family is a complex network of mutually interacting and structurally related proteins that regulate the fate of a cell by activating or inhibiting apoptosis.[179] The pro-survival members BCL-2, BCL-x_L, MCL-1, BFL-1, and BCL-W which display three to four conserved BCL-2 homology (BH1–4) domains inhibit apoptosis by sequestering the α-helical death inducing BH3 domain of pro-apoptotic family members BAX and BAK in a hydrophobic cleft delineated by domains BH1–3. BH3-only proteins (BID, BIM, BAD, NOXA, PUMA, BIK) which contain just one BH3 domain exert a pro-apoptotic effect either indirectly by binding to pro-survival proteins and preventing BAX and BAK to be sequestered or directly by binding to and activating BAX or BAK to cause mitochondrial outer membrane permeabilization. Because pro-survival BCL-2 proteins are overexpressed in several types of cancers, and thus promote survival of malignant cells, their selective inhibition represents a promising approach for induction of apoptosis in tumor cells and several BH3 mimetics are currently at the clinical trial stage.[180] Structures at atomic resolution of pro-survival

BCL-2 proteins (BCL-x_L, MCL-1) in complex with α-helical BH3 peptides revealed extensive contacts between a stripe of four hydrophobic residues on the BH3 α-helix and a hydrophobic cleft at the surface of the BCL-2 protein-binding partner as well as a significant contribution of polar interactions (a salt bridge between an Asp side chain projecting from another side of the helix and a conserved Arg residue in the BH3-binding cleft).

Various foldamer-based strategies to mimic BH3 α-helices and possibly target pro-survival BCL-2 members have been reported. The main challenges reside in the length of the bioactive BH3 helix to mimic as key hydrophobic residues are distributed over four consecutive helix turns, and in the need to mimic residues on two faces of the α-helix (hydrophobic residues on one face and polar side chains on another face). Early efforts from the Gellman group to reproduce the helical display of BH3 side chains with 12- and 14-β-peptide helices or the 11-helix and 14/15-helix of 1:1 α/β-peptides resulted in only very weak BCL-x_L binders, illustrating the general difficulty to identify an appropriate scaffold for faithful α-helix mimicry. Reducing the foldamer segment to only part of the BH3 helix to generate chimeric (αβ+α) peptides led to significant improvement. (αβ+α) Peptide **29** which combines an *N*-terminal segment made of alternating α- and cyclic β-amino acid (ACPC and APC) residues and a C-terminal α-peptide segment with residues from BAK(73–81) **28** exhibiting tight binding to BCL-x_L (K_i = 2 nM). The crystal structure of **29** in complex with BCL-x_L (PDB ID: 3FDM) revealed that the foldamer retains the general orientation of natural BH3 domains and that the α/β-segment forms a 14/15-helical structure with some cyclic β-amino acid (ACPC) residues making contacts with the protein surface (**Fig. 14**). The more general approach to α-helix mimicry whereby a smaller proportion of regularly spaced α-residues distributed in the target peptide sequence is replaced by the corresponding homologous β-amino acids (25%–33%) to generate the following regular ααβ, αααβ, and ααβαααβ patterns (**Fig. 3**)[37d] has been explored intensively by the Gellman group in the context of BH3 domain (PUMA and BIM) mimetics.

The ααβαααβ and αααβ patterns were found to support effective α-helix mimicry with the identification of potent BIM-derived α/β-peptides like **31** (ααβαααβ pattern) and **32** (αααβ pattern) that largely retain the binding profile of the native BIM BH3 peptide **30**.[181] The crystal structures of **31** and **32** in complex with BCL-x_L (PDB IDs: 4A1U http://dx.doi.org/10.2210/pdb4a1u/pdb and 4A1W http://dx.doi.org/10.2210/pdb4a1w/pdb, respectively) confirm that the two α/β-peptides are close mimics of the natural BIM BH3 domain although they significantly differ by their distribution of β-residues (**Fig. 14**). The β-residues in **31** are aligned along one side of the helix and directed toward the solvent with the four key hydrophobic side chains contributed by α-residues. In contrast, the αααβ pattern in **32** causes the β-residues to spiral around the helix axis with two β^3-residues contributing key hydrophobic contacts with the BH3-binding cleft (e.g., β^3-Ile10 and β^3-Phe14).

The substitution of cyclic constrained β-residues (ACPC, APC, sAPC; **Fig. 15**) for acyclic β^3-residues in **31** and **32** almost systematically improved the binding of BH3-derived α/β-peptides to BCL-x_L and MCL-1.

Interestingly, highly selective ligands (for either BCL-x_L or MCL-1) were obtained from **32** by introducing the $\beta^3 \rightarrow$ β-cyclic residue replacement at either of the two β-residue positions making contact with the protein (e.g., β^3-Phe14 → ACPC causes a >30-fold increase for MCL-1). Furthermore, the global $\beta^3 \rightarrow$ cyclic β-residue replacement in the sequence of **31** resulted in

28		Ac	Gln	Val	Gly	Arg	Gln	Leu	Ala	Ile	Ile	Gly	Asp*	Asp	Ile	Asn	Arg	NH_2		
29		Ac	**APC**	Ala	**ACPC**	Arg	**ACPC**	Leu	**ACPC**	Lys	**β^3hLeu**	Gly	Asp*	Ala	Phe	Asn	Arg	NH_2		
30	Ac	Ile	Trp	Ile^3	Ala	Gln	Glu	Leu^7	Arg	Arg	Ile^{10}	Gly	Asp*	Glu	Phe^{14}	Asn	Ala	Tyr	Tyr	NH_2
31	Ac	Ile	**β^3hTrp**	Ile	Ala	Gln	**β^3hGlu**	Leu	Arg	**β^3hArg**	Ile	Gly	Asp*	**β^3hGlu**	Phe	Asn	**β^3hAla**	Tyr	Tyr	NH_2
32	Ac	Ile	**β^3hTrp**	Ile	Ala	Gln	**β^3hGlu**	Leu	Arg	Arg	**β^3hIle**	Gly	Asp*	Glu	**β^3hPhe**	Asn	Ala	Tyr	**β^3hTyr**	NH_2
33	Ac	Ile	**ACPC**	Ile	Ala	Gln	**sAPC**	Leu	Arg	**APC**	Ile	Gly	Asp*	**sAPC**	Phe	Asn	**ACPC**	Tyr	Tyr	NH_2

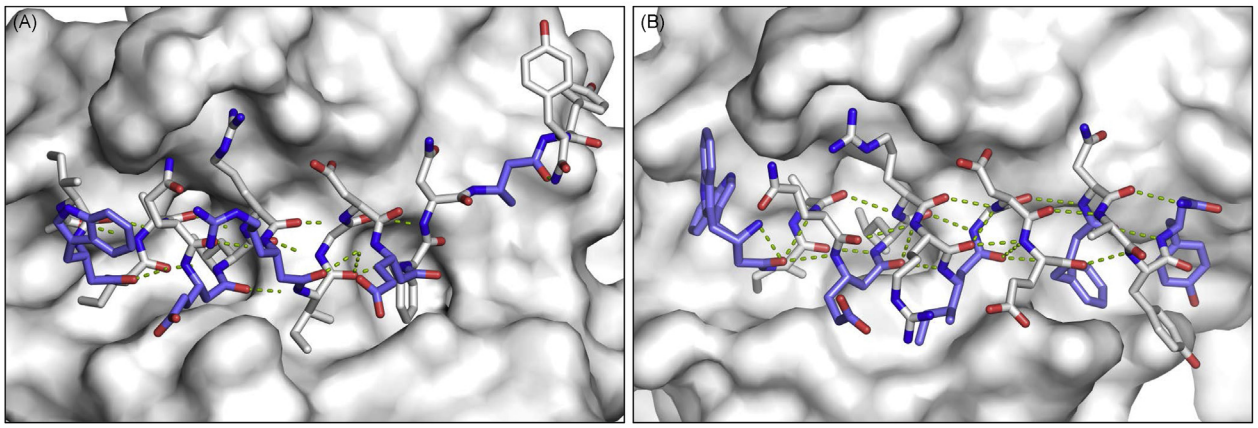


Figure 14 Sequences of BH3-derived α-peptides **28** and **30** and corresponding α/β-peptides **29** and **31**–**33**. The four key hydrophobic residues making contacts with pro-apoptotic proteins are underlined, and the conserved side chain carboxylate forming a salt bridge with one residue of the protein is marked with a star. β-Amino acid residues are shown in *slate blue*. Crystal structures of α/β-peptides **19** (A) and **20** (B) bound to BCL-x_L. Carbon atoms of β-residues are colored in *slate blue*.

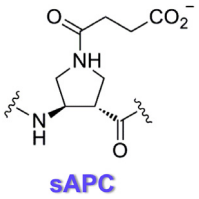


Figure 15 Formula of sAPC residue.

a very potent ligand (**33**) for both BCL-x_L and MCL-1 with enhanced protection against the activity of proteases (half-life 120-fold greater than the cognate α-peptide in the presence of proteinase K). This approach of cyclic β-residue replacement was combined with the introduction of *i*, *i*+4 hydrocarbon staples between α,α-disubstituted pentenyl-containing amino acids to give helix mimics that exhibit remarkable resistance to protease degradation and recapitulate the function of the parent stapled peptide: the ability to enter different types of cells, to engage anti-apoptotic protein targets, and to kill U937 lymphoma cells.[18d]

Smaller nonhelical foldamers with appended side chains mimicking key hydrophobic side chains as well as the conserved carboxylate of BH3 peptides have been designed to target pro-apoptotic BCL-2 family members.[34c,61b,168,182] Molecules based on terphenyl[61b] and triazine–piperazine–triazine[182] scaffolds have been reported to bind BCL-x_L or MCL-1 in fluorescence polarization assays, with K_i in the low μM or sub-μM range for the most potent compounds. It is worth mentioning that some compounds in the terphenyl series show some selectivity with 100-fold lower binding to an unrelated target like MDM2 (section **p53/hDM2 (MDM2) interaction**).

5.05.3.3.2.2 p53/hDM2 (MDM2) interaction

Significant efforts toward α-helix mimicry have concentrated on the p53/*h*DM2 (MDM2 in mouse) interaction, an important target for cancer therapy.[183] The p53 tumor suppressor is a transcription factor that activates cell death in response to various stress conditions, such as DNA damage or hypoxia. The protein p53 is negatively regulated by the double-minute 2 protein (DM2) which is overexpressed in cancer and directly contributes to tumor development and progression. Inhibiting the interaction between p53 and DM2 to restore wild-type p53 activity in tumors that overexpress DM2 is a potential strategy for cancer treatment.[183b] X-ray crystallography of the p53/MDM2 complex revealed that upon binding, the p53 activation domain adopts a helical conformation with three residues on one face of the helix, that is, Phe19, Trp23, and Leu26, forming a hydrophobic patch buried into the binding cavity of MDM2.[184] Since this early report highlighting the structural bases of the p53/MDM2 interaction, very different classes of MDM2/MDMX (an MDM2 homolog that also inactivates p53) antagonists have been developed as potential anticancer agents, ranging from low-molecular-weight compounds to peptides.[183c] In a seminal paper focusing on short peptides encompassing residues 19–26 of p53, researchers at Novartis demonstrated the benefit of stabilizing the α-helical conformation to achieve effective inhibition of the p53/MDM2 interaction.[165a,185] Peptide-based approaches aimed at stabilizing the p53 helix and improving binding to MDM2 have since flourished such as the introduction of α,α-dialkylated residues,[186] side chain cross-links,[187] and hydrogen bond surrogates (HBS)[188] as well as the use of mini-protein scaffolds.[189] The design of artificial oligomeric scaffolds with the ability to reproduce the projection of p53 key hydrophobic side chains has been concurrently developed.

Salt bridge-stabilized 14-helical β-peptides bearing *h*DM2-binding residues on one face of the helix (β^3Leu, β^3Trp or analogs, and β^3Phe in an *i*/*i*+3 relationship) have been investigated as possible p53 mimics.[19a,190] More potent β-peptides with the ability to bind both *h*DM2 and *h*DMX in the nanomolar range were subsequently obtained using computer-aided optimization and replacement of the central indole side chain by other aromatic moieties such as a 3-trifluoromethylphenyl (**34**, K_d = 28.2 nM (*h*DM2), K_d = 518 nM (*h*DMX)) or 3,4-dichlorophenyl (K_d = 27.6 nM (*h*DM2), K_d = 155 nM (*h*DMX)).[191]. Two approaches have been subsequently explored to improve the cell permeability of these MDM2-binding helical β-peptides: cationic–patch insertion and hydrocarbon side chain bridging. Variants of **34** in which a minimal cationic motif was embedded on one face (the salt-bridge face) of the β-peptide 14-helix[192] were shown to bind *h*DM2 equally well and to be taken up by cells with an efficiency that equals that of $(PRR)_3$ a control CPP.[193] This approach could avoid the attachment of an extra (Arg rich) cell-penetrating sequence (see section "**Cell Penetrating Foldamers: Intracellular Drug Delivery and Membrane Disruption**") that would significantly increase the molecular mass of the derivatives. By analogy to stapled peptides that may increase cell uptake, β-peptide analogs containing *i*, *i*+4 diether or hydrocarbon staples have also been prepared. β-Peptides bridged between positions 4 and 7 (e.g., **35**) were found to bind MDM2 slightly better, and most importantly to be taken up by cells more efficiently than the corresponding unbridged peptides.[18c] It is worth mentioning that the foldamer approach is also compatible with other α-helix-stabilizing technologies such as the HBS approach. Arora and coworkers have designed conformationally stable p53 peptide analogs combining beta amino acid replacements (ααααβ backbone pattern) and an HBS cap that are equally potent to the corresponding HBS-terminated α-peptide in binding recombinant MDM2 (e.g., **36**, $K_D = 80 \pm 21$ and 71 ± 16 nM, respectively) (**Fig. 16**).[194]

High-affinity (low nanomolar range), proteolytically stable, helical (left-handed) D-peptide ligands of MDM2 have been identified by mirror image phage display.[143,195] High-resolution structural analysis of one such peptide (i.e., DAsp-DTrp-DTrp-DPro-DLeu-DAla-DPhe-DGlu-DAla-DLeu-DLeu-DArg, **37** K_D = 53 nM for MDM2) in complex with $MDM2_{(25-109)}$ (PDB ID: 3IWY)[195] (**Fig. 17A**) suggested that the aromatic side chain of the DPhe7 residue was not fully filling the corresponding binding

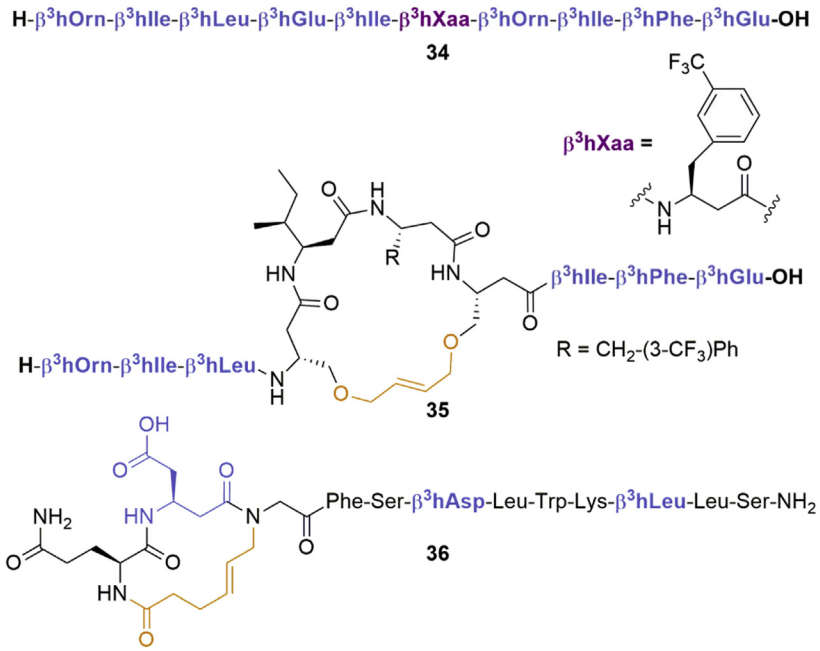


Figure 16 Examples of β-peptide (**34**, **35**) and α/β-peptide (**36**) p53 mimics with various degrees of backbone preorganization (salt bridge, side chain cross-link, HBS).

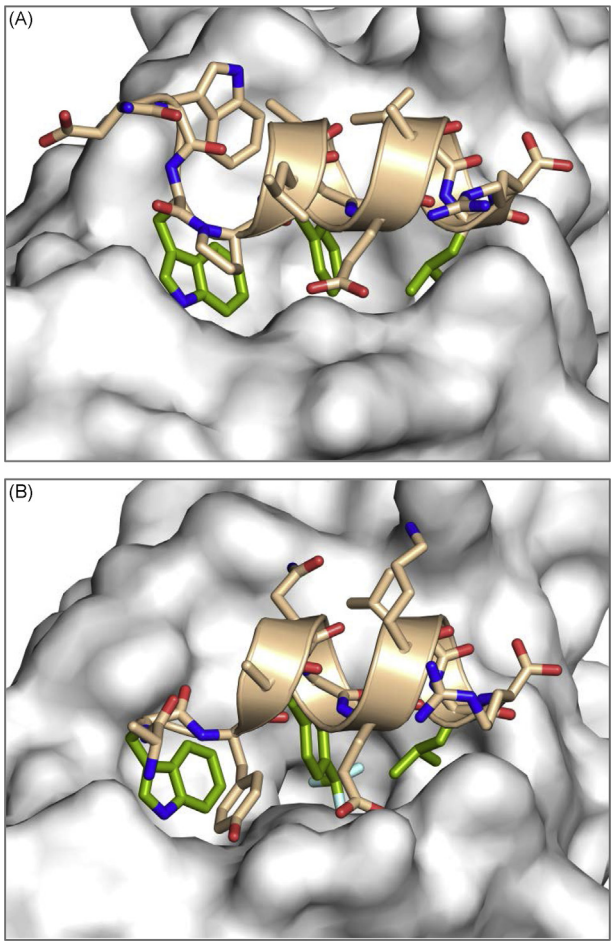


Figure 17 Crystal structures of helical D-peptide ligands of MDM2 identified by mirror image phage display **37** (A) and **38** (B) bound to $MDM2_{(25–109)}$. Key hydrophobic side chains are colored in *green*.

cavity on MDM2, suggesting a rationale to enhance binding of these peptide ligands. Introduction of substituents at the *para* position of DPhe7 in the sequence of a related peptide was found to enhance binding to both MDM2 and MDMX with the para-trifluoromethyl substitution being the most effective (DAla-DTrp-DTyr-DAla-DAsn-DPhe(3-CF_3)-DGlu-DLys-DLeu-DLeu-DArg, **38**, $K_D = 0.45$ nM (the SPR-based competitive binding assay)).[48e] The crystal structure of **38** in complex with MDM2(25−109) solved at 1.8 Å resolution (PDB ID: 3TPX) indicated that the *para*-trifluoromethylation of DPhe7 enlarges the total buried surface area of the D-peptide in the complex from 561 to 640 $Å^2$ and also suggested further ligand improvement by modification of the side chain of DTrp3 (**Fig. 17B**). The introduction of a fluorine atom at position 6 of the indole ring led to the most active compound with a $K_D = 220$ pM. One limitation of these D-α-peptides is their poor capacity to translocate across cell membranes, and thus to engage MDM2 and MDMX inside the cells (no cytotoxicity was observed in HCT116 $p53^{+/+}$ cell lines at up to 50 μM of **37**). This would require implementation of additional strategies such as conjugation to molecular transporters (see sections "**Cell Penetrating Foldamers: Intracellular Drug Delivery and Membrane Disruption**" and "**Foldamers That Target Intracellular PPIs**").

A variety of inhibitors of the p53/*h*DM2 interaction based on extended and relatively rigid scaffolds have been reported (see **Fig. 18**). Early work on the aromatic terphenyl scaffold by substituting the three ortho positions with aryl or alkyl groups for mimicking the hot-spot residues in the *i*, *i* + 4, and *i* + 7 positions of the p53 helix demonstrated the ability of these topographical mimics to engage selectively the target in vitro (fluorescence-based assays and ^1H–^{15}N HSQC perturbation shift experiments). Remarkably, several compounds in this series (exemplified by **39**) were shown to inhibit this PPI at submicromolar concentration with some selectivity over other PPIs with a similar type of binding interface such as BCL-x_L/Bak and BCL-2/Bak (14- and 82-fold selectivity, respectively for **39**).[61b] Surprisingly, the selectivity of compound **39** was reversed when substituting the 1-naphthyl moiety by a 2-naphthyl (see section **Targeting anti-apoptotic Bcl-2 family members**). Remarkably, several terphenyl p53 mimetics were found to be membrane permeable and to induce p53 accumulation and activation in tumor cells. Further elaboration led to aromatic scaffolds with heteroatoms such as oligopyridylamides and oligobenzamides whose solubility is improved over terphenyl backbone and synthesis more modular, transferable to solid-phase and amenable to the creation of larger libraries.[63,133,196] Inhibitors of p53/hDM2 in the low μM range have been reported by screening focused libraries of diversely substituted 2-*O*- and 3-*O*-alkylated,[65b,196,197] *N*-alkylated oligobenzamides (exemplified by **40**),[167,198] as well as hybrid oligoamides containing single or multiple proteinogenic α-amino acid replacements at selected positions (exemplified by **41**).[34c,196] Again, compounds with the ability to interfere with the p53/*h*DM2 interaction in cells were reported, including some potent membrane-permeable dual inhibitors (e.g., **40**) of the p53/*h*DM2 and NOXA-B/MCL-1 interactions.[167] Compounds with increased preference for *h*DM2 were obtained by substituting a proteinogenic amino acid residue for the central amino benzoic acid unit as in **41**, the resulting selectivity (i.e., *h*DM2 vs. Mcl-1) being influenced by the stereochemistry of the α-amino acid residue.[34c] In a similar fashion, aliphatic oligooxopiperazines[69a] have also been evaluated as possible p53 α-helix mimics. Computational design using the Rosetta software (https://www.rosettacommons.org/)[199] modified to enable modeling of oxopiperazines,[200] combined with experimental structure−activity relationship data, led to the discovery of p53/MDM2 inhibitors (e.g., **42**, $K_D = 0.3$ μM).[69c] The Rosetta-derived model of the complex was supported by $^1H-^{15}N$ HSQC NMR titration experiments.

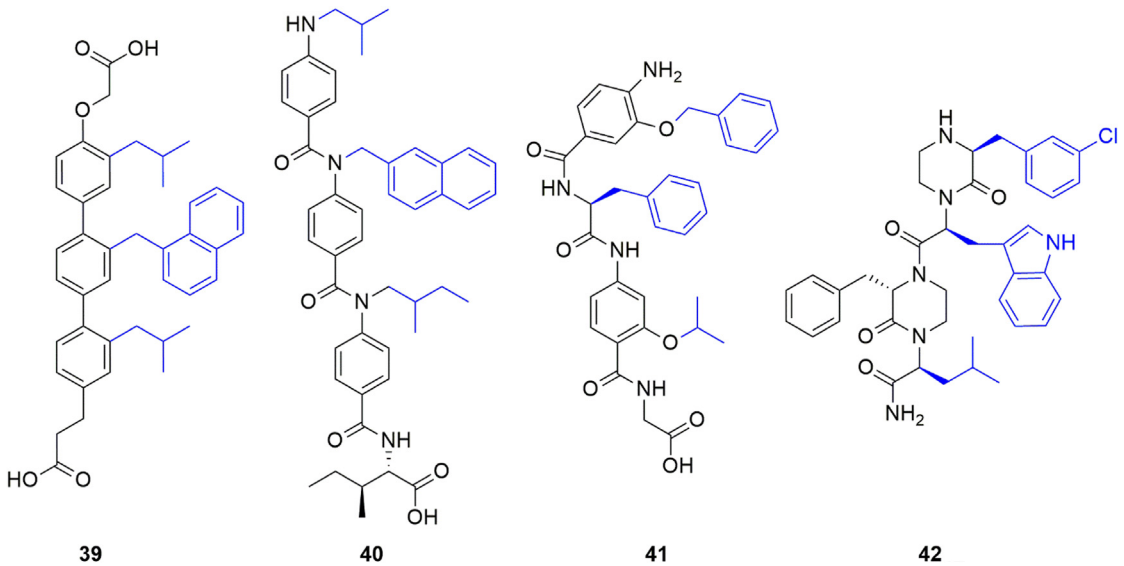


Figure 18 Inhibitors of the p53/*h*DM2 interaction based on extended and rigid α-helix mimetic scaffolds.

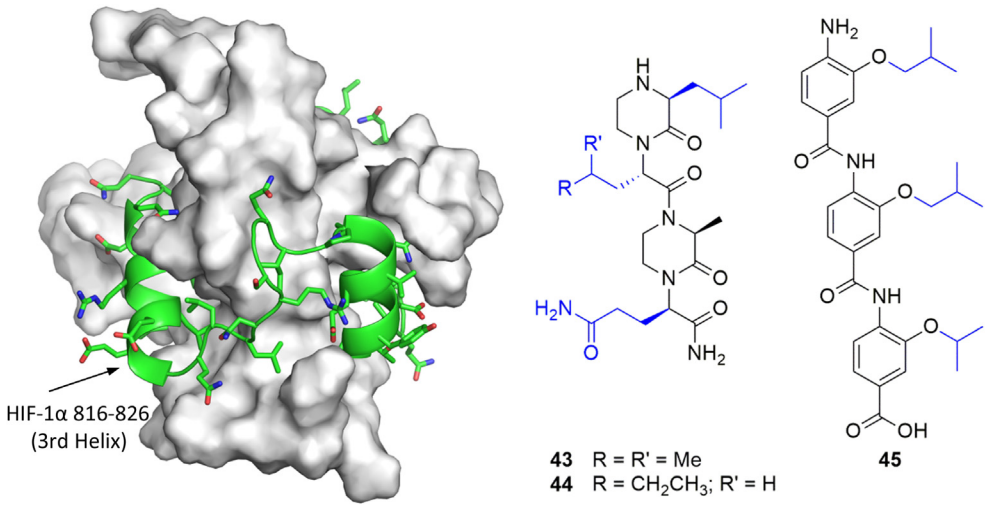


Figure 19 (Left) Solution structure of the complex between the C-terminal transactivation domain of HIF-1α and the CH1 domain of p300. (Right) Oligooxopiperazine and 3-*O*-alkylated oligobenzamide derivatives designed to mimic the HIF-1α third helix.

5.05.3.3.2.3 p300/HIF-1α interaction

Other transcription factors involving α-helical interfaces such as HIF-1α have also been targeted with helix mimetics. HIF-1α plays a crucial role in adaptation of tumor cells to hypoxia. Under hypoxia, the α-subunit of HIF-1 accumulates, dimerizes with constitutively expressed aryl hydrocarbon receptor nuclear translocator subunit, recruits coactivators (CREB-binding protein/p300 (CBP/p300)) *via* its transactivation domain, and further exerts its transcriptional role on hypoxia-responsive target genes that play key roles in cancer biology (angiogenesis, invasion, metastasis). The HIF-1α/p300 coactivator interaction represents a possible target for controlling HIF-1α-mediated hypoxia signaling. The solution structure of the complex (PDB ID: 1L8C) reveals that the C-terminal transactivation domain of HIF-1α binds to the CH1 domain of p300 *via* three short helical regions and an extensive network of hydrophobic and polar interactions (**Fig. 19**).

Arora and coworkers have reported oligooxopiperazine derivatives[69a] designed to mimic side chain display of three of the four residues in the HIF-1α third helix (816–826) that make close contact with the CH1 domain, namely, Leu818, Leu822, and Gln824.[69b] In particular, compound **43** was found to bind the CH1 domain of p300 with an affinity of 533 nM, to downregulate multiple genes implicated in angiogenesis, apoptosis, cell proliferation, and invasion in non-small-cell lung cancer cell lines (for activity in vivo see section "**In Vivo Studies of Bioactive Foldamers**"). Further optimization of compound **43** led to oligooxopiperazine **44** which exhibits a 13-fold enhancement in binding affinity and 100-fold specificity for the targeted p300-CH1 surface over MDM2.[69c]

In a similar fashion, 3-*O*-alkylated oligobenzamides designed to mimic HIF-1α third helix have been reported to inhibit the binding of a 42-residue HIF-1α-labeled peptide in a fluorescence anisotropy competition assay with activities in the μM range (e.g., **45**, $IC_{50} = 9.2$ μM).[201] These results obtained with short helix mimetics **43**–**45** are significant given that the 16-residue parent peptide encompassing the third helix is not active. A potentially useful extension of this approach to improve the activity of short topographical mimics in terms of affinity and/or target specificity is to combine the helix mimetic and the rest of the native peptide sequence in a single extended hybrid molecule. One example reported by the Wilson group is a hybrid molecule consisting of the HIF-1α second helix linked to molecule **45**.[117b] Although the resulting compound did not show improved binding compared to the native sequence, the resulting molecule exhibited much increased specificity to the target compared to **45**. This is an interesting direction that they may lead, if target peptide sequences are longer, to composite proteins with new properties, and is worth continued exploration.

5.05.3.3.2.4 Targeting vascular endothelial growth factor signaling

Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis and vasculogenesis whose biological effects are mediated by two receptor tyrosine kinases, VEGFR-1 and VEGFR-2. The important role of VEGF during tumor angiogenesis makes it an important therapeutic target, and VEGF antagonists have received considerable attention in the context of cancer therapy. Bevacizumab, a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting interactions with cell surface receptors, and aflibercept, a recombinant fusion protein consisting of extracellular domains (ECDs) of human VEGF receptors, are approved drugs to treat cancer or macular degeneration. Phage display methods have been employed to identify disulfide-constrained peptides as well as mini proteins (e.g., derived from the three-helix 58-residue Z-domain scaffold[202]) that bind to the receptor-binding domain (residues 8–109) of VEGF and antagonize binding of VEGF to its receptors.[203] Haase et al. have investigated α → β replacements in the 19-mer cyclic VEGF-binding peptide with the dual aim to increase resistance to proteolysis and to maintain the affinity for VEGF.[204] Regular patterns of α → β replacements (such as ααβααβ and αααβ) resulted in compounds with

low binding profile probably due to the absence of a well-defined and regular helical conformation in the parent peptide. Nevertheless, several α/β-hybrids containing from three to six β-replacements were found to retain significant affinity for VEGF (albeit 4–77-fold lower affinity relative to the parent peptide) and to display increased (up to 190-fold) resistance. In another study, Checco et al. started from the three-helix Z-domain scaffold VEGF ligand (Z-VEGF), which they simplified by removing one helical segment and constrained by introducing a disulfide cross-link at the other end of the loop. The resulting dimer which binds VEGF with the same affinity as Z-VEGF served as a starting point to implement α → β replacements within the helical segments. The introduction of six β-residues at sites that do not contact the target protein or interfere with tertiary packing led to α/β-peptides that can structurally and functionally mimic the binding surface of the parent peptide (affinities in the same range or slightly improved) while exhibiting significantly increased resistance to proteolysis (up to 3300-fold relative to the cognate a-peptide in a proteinase K assay). The crystal structure of the tightest VEGF-binding α/β-peptide, **46**, in complex with VEGF(8–109) (PDB ID: 4WPB) confirmed that **46** binds to the same surface of VEGF(8–109) as Z-VEGF (PDB ID: 3S1K http://dx.doi.org/10.2210/pdb3s1k/pdb[203b]) with the anticipated helix-loop-helix conformation (see **Fig. 20**).[107b] In addition, **46** was found to attenuate VEGF-induced proliferation of human umbilical vein endothelial cells. It is noteworthy that the α → β replacement approach was also successfully extended[107b] to produce effective mimics of Z-domain-derived peptides that bind to other target proteins, such as tumor necrosis factor-α, a pro-inflammatory cytokine involved in several inflammatory diseases,[205] and the Fc portion of human IgG1.[206]

Mirror image phage display was used by Kent and collaborators to engineer high-affinity mini D-protein ligands of VEGF (L-form) that can block binding of VEGF to its cognate receptor VEGF-R1. They used the 56-residue B1 domain of streptococcal protein G (GB1) as a scaffold to display a designed diversity library of mutants which was screened against synthetic D-VEGF, followed by a round of affinity maturation. The high-resolution X-ray structures of the heterochiral L-VEGF/D-protein antagonist complexes were determined by racemic protein crystallography (PDB IDs: 4GLN http://dx.doi.org/10.2210/pdb4gln/pdb and 5HHC http://dx.doi.org/10.2210/pdb5hhc/pdb).[48f,207] Detailed analysis of the interaction between the D-protein antagonist and VEGF-A revealed a binding interface of ≈ 800 Å^2 localized in the region of VEGF that interacts with VEGFR1. Plasma stability,

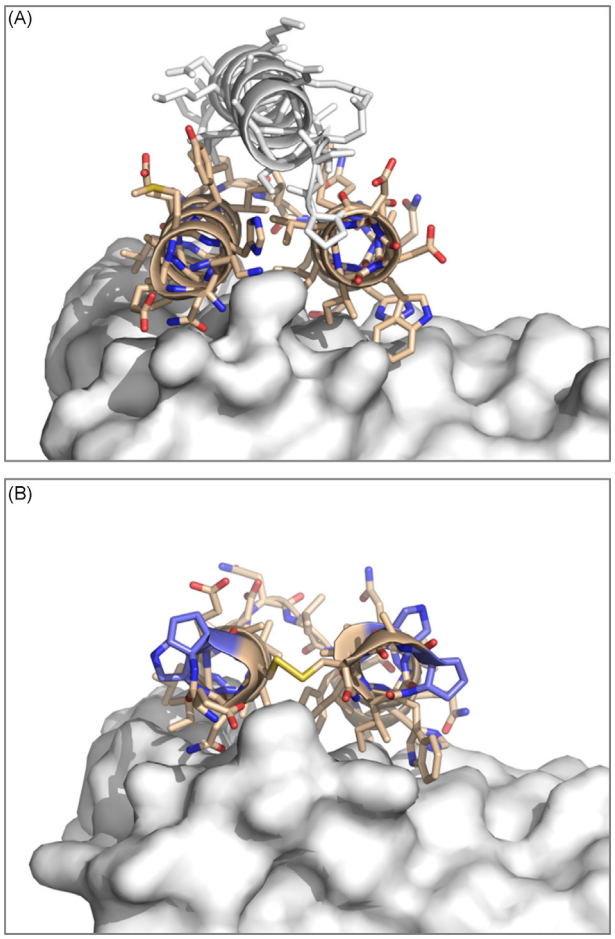


Figure 20 Crystal structures of the three-helix Z-domain scaffold VEGF ligand (Z-VEGF) (A), and α/β-peptide **46** (B) in complex with $VEGF_{8–109}$. Carbon atoms of β-residues in **46** are colored in *slate blue*.

PKs, and immunogenicity of this mini D-protein were studied in detail to further evaluate the potential of D-proteins as therapeutic agents (see section "Antigenicity and Immunogenicity of Bioactive Foldamers").[48f]

5.05.3.4 Ligands of G-Protein-Coupled Receptors: Hormone-Like Analogs

Some approaches discussed in the previous sections ("Helices mimicking viral glycoproteins to inhibit viral fusion" and "Foldamer-based inhibitors of PPI linked to cancer") have been recently extended to create analogs of peptide hormones such as ligands of class B G-protein-coupled receptors (GPCRs)[208] which include secretin, glucagon, glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), vasoactive intestinal peptide (VIP), corticotrophin-releasing factor (CRF), parathyroid hormone (PTH), and growth hormone-releasing factor, pituitary adenylate cyclase-activating peptide (PACAP), and calcitonin. Although generally unfolded in aqueous solution, the C-terminus of natural ligands of class B GPCRs adopts an α-helical structure upon binding to their cognate receptor. Endogenous peptide ligands for class B (secretin receptor family) GPCRs and their synthetic derivatives have attracted considerable interest as candidates for the treatment of several human pathologies and several of these are now marketed drugs (e.g., liraglutide, teriparatide).[209] Significant insight into the mechanism of class B GPCR activation has been provided by structural analyses of ECDs of several of these receptors (including CRFR1, CRFR2, GLP-1, PACAP, GIP receptors) in complex with bound peptide ligands (natural ligands or analogs)[208] and by the first structures of membrane-spanning domains of some of these GPCRs.[210] In the structures of ECD with bound peptide ligands, the peptide adopts an amphipathic α-helical conformation with hydrophobic side chains buried into a groove at the surface of the ECD: the C-terminal residues contacting the ECD and the N-terminal end protruding out of the ECD. This crystallographic data set suggests a two-step mechanism whereby the interaction between the ECD and the C-terminus of the peptide hormone exposes the N-terminal part to the transmembrane domain of the receptor. Extensive efforts have been made over the last decades to stabilize the α-helical conformation and the lifetime in biological fluids of these peptide hormones and generate potent agonists and antagonists.[209] Chemical approaches based on the introduction of α,α-dialkylated amino acids and side chain cross-links have been largely employed.[211]

More recently, Gellman and coworkers have shown that carefully designed α/β-peptides can also produce effective mimics of class B GPCR ligands with increased resistance to proteolytic degradation and possibly the ability to modulate receptor signaling. In a first study, they examined six analogs of PTH(1–34), a full agonist of human PTH receptor 1 (hPTHR1), and the active ingredient of the osteoporosis drug teriparatide.[212] Analogs contained between five and seven $\alpha \rightarrow \beta^3$ replacements extending from the C-terminus with the ααααβ pattern. All six α/β-peptides proved to be full agonists (by monitoring cAMP production in HEK293 cells that stably express hPTHR1). Remarkably, **47** which contains seven $\alpha \rightarrow \beta^3$ replacements is indistinguishable from $PTH_{(1-34)}$ in terms of PTHR1 affinities and agonist potency, and this compound displayed in vivo activity (see also section "α/β Peptide Analogs of Class B GPCR Agonists"). Similar periodic $\alpha \rightarrow \beta^3$ replacements in the sequence of the related [$DTrp^{12}$]PTH(7–34) were found to retain the antagonist and inverse agonist activities of this α-peptide and to increase resistance to proteolytic degradation.[213] In sharp contrast, even fewer $\alpha \rightarrow \beta^3$ replacements in the sequence of the glucagon-like peptide 1 (GLP-1) (7−37)-NH_2, which displays full activity at GLP-1 receptor, resulted in considerable loss of activity as measured by cAMP production ($EC_{50} > 100$ nM compared to $EC_{50} = 1.6$ nM for GLP-1(7–37)NH_2)). The agonistic activity at the receptor was restored by introducing ring-constrained β-amino acids such as ACPC or APC.[214] Compound **48** which contains five $\alpha \rightarrow \beta$ replacements proved to be a full agonist of GLP-1R, with native GLP-1-like potency. GLP-1(7–37)NH_2 has a very short plasma half-life (1–2 min). It is rapidly degraded by proteases such as dipeptidyl peptidase-4 (DPP4) which specifically cleaves after Ala8 and neprilysin (NEP 24.11), which cleaves after Asp15, Ser18, Tyr19, Glu27, Phe28, and Trp31. Although $\alpha \rightarrow \beta$ replacements in **48** were expected to suppress the action of NEP 24.11 to a large extent, attempts to extend $\alpha \rightarrow \beta$ modifications toward the N-terminus to provide further stabilization were found to be detrimental to the activity. Compound **49**, an analog of **48** with two Aib residues at positions 8 and 16, was found to be a full agonist of GLP-1R and to be highly resistant in vitro to degradation by DPP4. α/β-Peptide **38** was found to promote glucose-stimulated insulin secretion from freshly isolated mouse islets in a dose-dependent manner similar to GLP-1 and to be active in vivo in glucose tolerance tests (GTT) (see section "α/β Peptide Analogs of Class B GPCR Agonists"; Fig. 21).

The $\alpha \rightarrow \beta$ replacement strategy was also applied to the VIP to improve its resistance to proteolysis. VIP elicits immune-based neuroprotection but its short half-life hampers possible therapeutic applications. Furthermore, VIP activates two broadly distributed receptors, VIPR1 and VIPR2, and there is an interest for analogs which would display receptor selectivity. Two metabolically stable VIPR1 and VIPR2 agonists were developed. For example, compound **50** which contains nine $\alpha \rightarrow \beta$ substitutions (either native side chains or constrained residues) is a potent agonist of VIPR2, highly selective for VIPR2 versus VIPR1 which exhibits strong resistance to proteinase activities (pepsin, proteinase K, chymotrypsin). The two peptides were investigated for their abilities to protect mice against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurodegeneration used to model Parkinson's disease (PD) (see section "α/β Peptide Analogs of Class B GPCR Agonists").

5.05.3.5 Inhibitors of Amyloid Protein Aggregation and Fibril-Like Oligomer Assembly Formation

Misfolding and aggregation of amyloid proteins are implicated in the pathology of a range of diseases such as Alzheimer's disease, type 2 diabetes, and PD. Foldamer approaches based both on β-sheet and helix mimics have been employed to antagonize the aggregation of various amyloid proteins, and potentially reduce the toxicity of amyloid aggregates. Nowick and coworkers have focused on amyloid-β (Aβ)-sheet mimics (ABSMs) that can display Aβ-strands from different amyloid proteins including

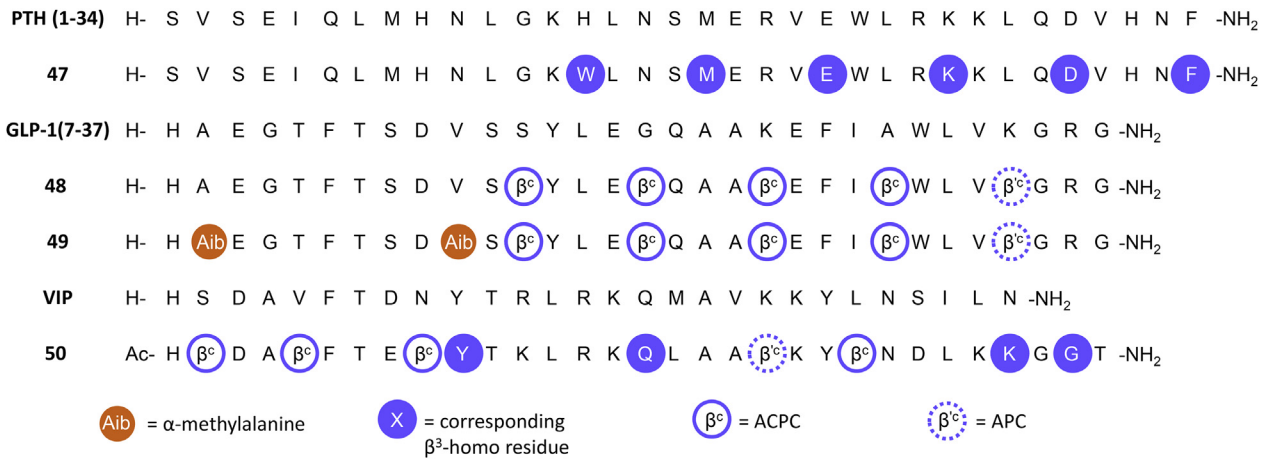


Figure 21 α/β-Peptide analogs **47–50** of peptide hormones $PTH_{(1–34)}$, $GLP\text{-}1_{(7–37)}$, and VIP. β^3- and cyclic β-amino acid residues are shown in *blue disks/circles*, respectively. Aib residues are shown as *orange disks*.

Aβ-peptide (associated with Alzheimer's disease), Tau, human and yeast prion proteins, human β2-microglobulin, human α-synuclein (associated with PD), and human islet amyloid polypeptide (IAPP, associated with type 2 diabetes).[83,215] In particular, they have designed 54-membered ring macrocyclic β-sheets (Fig. 22) comprising a recognition amyloid heptapeptide fragment (upper strand) which could bind to parent amyloid protein, one Hao unit which serves as a tripeptide β-strand mimic in the lower strand with two flanking dipeptides to promote intramolecular H-bond and two δ-linked ornithine ($^{\delta}$Orn) residues for connecting the two strands. The Hao unit plays the additional role of preventing ABSMs to aggregate in solution and form an infinite network of β-sheets by minimizing exposed H-bond groups in the lower strands.[82b] Remarkably, ABSMs with $A\beta_{17–41}$ in the upper strand were found to delay the aggregation of Aβ (monitored by thioflavin T fluorescence assays and Transmission electron microscopy (TEM)) at substoichiometric concentrations (as low as 1 μM) and to reduce the toxicity of Aβ40 and Aβ42 in PC-12 cells.[83] The current model is that ABSMs thus bind early β-structured oligomers and not the monomer to block Aβ-nucleation.

Short-length aromatic foldamers consisting of an aromatic salicylamide (Sal) or 3-amino benzoic acid (Benz) backbone and containing basic arginine (Arg), lysine (Lys), or citrulline (Cit) side chains have also been reported to prevent spontaneous and seeded Aβ-fibrillization.[216]

Miranker and coworkers have focused on inhibition of membrane-bound oligomeric intermediates of the amyloidogenic IAPP, a 37-residue hormonal peptide, which are hypothesized to contribute to β-cell pathology in type 2 diabetes (Fig. 2). They identified oligopyridine α-helix mimetics[217] and oligoquinoline helical foldamers[218] that stabilize a preamyloid α-helical conformation of IAPP and inhibit lipid-catalyzed IAPP aggregation. Oligoquinoline **51** which is water soluble and crosses biological membranes was found to rescue β-cells from IAPP-induced toxicity upon coaddition with IAPP but also more remarkably when a time delay (up to 24 h) was introduced between the addition of IAPP and its addition to cells (Fig. 23).

5.05.3.6 Cell-Penetrating Foldamers: Intracellular Drug Delivery and Membrane Disruption

Much effort has been devoted to the discovery of molecular transporters that when conjugated to a molecule of interest (e.g., a drug, a peptide, a protein, or DNA) might facilitate its entry into cells. Cationic peptides such as those derived from HIV-1 Tat, Drosophila

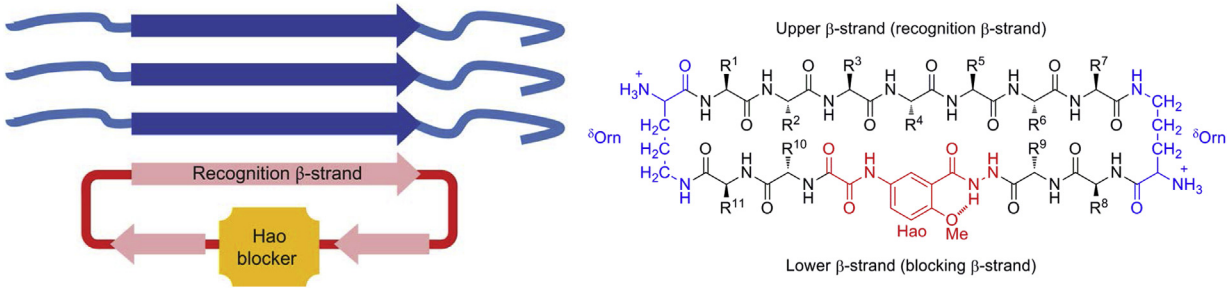


Figure 22 (Left) Representation of an amyloid β-sheet mimic recognizing and blocking amyloid aggregation through β-sheet interactions; (right) sequence of a prototypical 54-membered ring macrocyclic amyloid β-sheet mimic. Reprinted from Cheng, P.-N.; Liu, C.; Zhao, M.; Eisenberg, D.; Nowick, J. S. *Nat. Chem.* **2012**, *4*, 927–933, with permission from Macmillan Publishers Ltd. Copyright (2012).

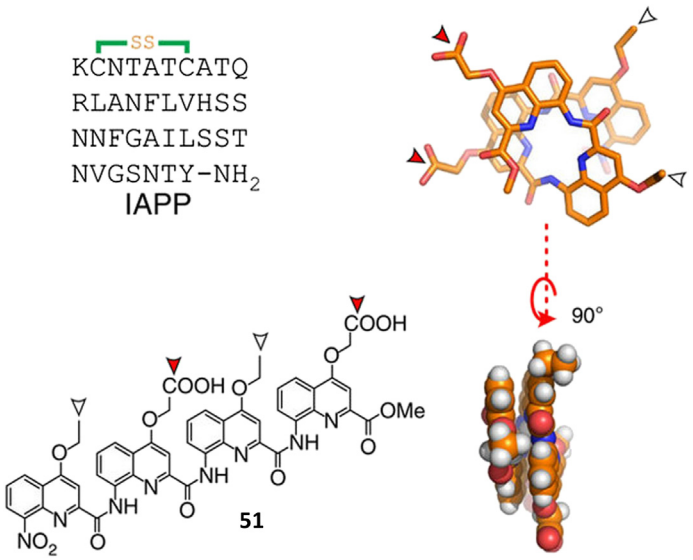


Figure 23 Approach based on the use of oligoquinoline helical foldamers (e.g., **51**) to inhibit lipid-catalyzed IAPP aggregation. Reprinted from Kumar, S.; Birol, M.; Schlamadinger, D. E.; Wojcik, S. P.; Rhoades, E.; Miranker, A. D. *Nat. Commun.* **2016**, *7*, 11412, with permission from Macmillan Publishers Ltd. Copyright (2016).

Antennapedia, and many other proteins translocate the cell membranes and mediate delivery of cargo molecules into targeted cells.[219] This knowledge of cell-penetrating sequences has stimulated the design of a wide range of cell penetrating peptide (CPPs), of which a large fraction are arginine rich, the guanidinium headgroups playing a crucial role in the cell entry process.[220] Concurrently, approaches based on nonnatural oligomeric backbones including foldamers may provide new classes of molecular transporters (i.e., cell-penetrating foldamers, CPFs) with improved stability in biological fluids. For example, oligomers of D-Arg (e.g., 8-mer and 9-mer)[221] as well as mixed L/D-octaarginine[222] display cell-penetrating properties comparable or even greater than the control $Tat_{(49-57)}$ peptide together with increased enzymatic and metabolic resistance. Similarly, guanidine-rich peptoids,[221] α-peptide/β-peptoid chimeras,[223] oligocarbamates, and oligomers of α-aminoxy acids[224] have been shown to be equally and even more potent in entering cells than the corresponding L-peptides. These molecules which do not adopt a regular structure in solution provide evidence that a defined conformation is not strictly required for α-peptides and peptoids to translocate across cell membranes. Similarly, short polycationic β-peptides including β^3-HArg/β^3-HLys oligomers[225] can rapidly enter the cytoplasm and nucleus of human cells from the extracellular medium by endocytosis. Although these β^3-peptides do not show strong conformational preference in aqueous environment, they are however largely helical in MeOH solution and in the presence of lipid micelles. Subsequent studies have shown that the conformational stability of the β-peptide and the geometry of guanidinium group display directly affect entry into live cells.[226] For example, the ACHC-containing β-peptide **52** which forms a stable 3_{14}-helix in aqueous solution was found to enter HeLa cells to a greater extent than the cognate β^3-peptide **53** with lower helix-forming propensity.[226a] A similar trend was observed with α/β-peptide analogs of $Tat_{(47-57)}$.[227] The introduction of a cyclic β-amino acid bearing a side chain guanidinium group led to a CPF with substantial helicity in methanol (**54**) that enters HeLa cells much more readily than do the corresponding Tat α-peptide and β^3-Arg-containing α/β-peptide. The introduction of a minimal cationic motif on one face of a bioactive helical β-peptide (e.g., a PPI inhibitor) is yet another approach to increase cellular entry while avoiding conjugation to an extra CPP or CPF (Fig. 24).

Bioreducible helical CPFs with high capacity to assemble with plasmid DNA and to deliver nucleic acids into the cell have been obtained by thiol-mediated dimerization of a short (8-mer) amphipathic helical oligourea bearing His and Arg side chains.[29] Compound **55** was found to compare favorably in terms of transfection efficiency with LAH4, a His-rich peptide CPP with high transfection ability.[228]

As mentioned in the previous section "**Inhibitors of Amyloid Protein Aggregation and Fibril-Like Oligomer Assembly Formation**,"[218b] aromatic oligoamide foldamers may also display unique cell-penetrating properties. Huc and coworkers have shown that helically folded aromatic oligoamides with peripheral cationic side chains are able to efficiently cross cell membranes.[55] They report that cell entry is (i) dependent on the length and the number of positive charges of these aromatic foldamers, with octamer **56** proving to be the most active CPF for all three tested cell lines, and (ii) mediated by endocytosis, like CPPs.

5.05.3.7 Nucleic Acid Recognition

Synthetic oligoamides have attracted considerable attention as nucleic acid analogs (PNAs and analogs) and B-DNA minor groove binders (hairpin Py–Im polyamides), and represent interesting candidates for applications in biology and as therapeutic

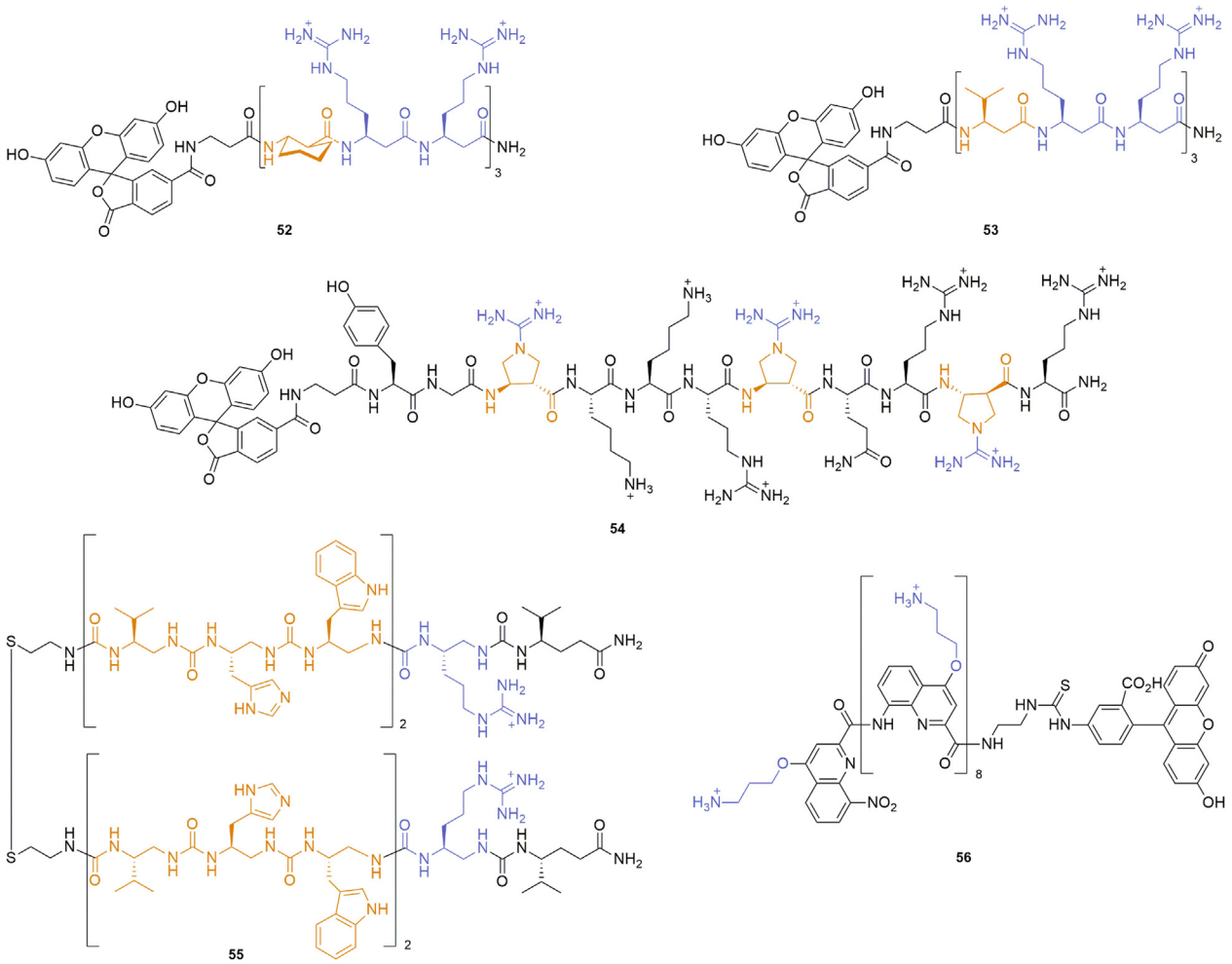


Figure 24 Examples of biotic and abiotic cationic foldamers **52–56** designed to translocate into cells.

agents (see section "Folding upon binding"). PNAs have been used extensively as tools for specific modulation of gene expression by targeting translation or transcription processes, but their development as drugs suffers from low cellular uptake and limited water solubility, although some techniques may be used to overcome these drawbacks (e.g., CPPs). Py–Im polyamides bind defined DNA sequences with affinities and specificities comparable to those of DNA-binding proteins and may induce allosteric changes in the DNA helix that can interfere with DNA binding of transcription factors (e.g., hypoxia-inducible factor 1 alpha (HIF-1α),[229], nuclear factor kappa B,[230] …). Py–Im polyamides such as **57** (Fig. 25) have been used as molecular probes in cell culture to alter gene expression profiles, and more recent studies have turned to animal models as Py–Im polyamides show toxicity against a variety of cancer cell lines (see section "In Vivo Studies of Bioactive Foldamers").

Although the literature on nucleic acid recognition by artificial oligomers is by far dominated by PNAs (and their numerous analogs) and Py–Im polyamides, new folded backbones such as helical aromatic amide foldamers have been reported to bind other DNA targets such as G-quadruplex DNA. Helical oligoamides based on 8-aminoquinoline-2-carboxylic acid and bearing cationic side chains, such as tetramer **58**, selectively interact with DNA G-quadruplexes in solution, including the human telomeric G-quadruplex (H-telo) and quadruplex sequences of several gene promoters (e.g., c-kit1, c-kit2, c-myc, k-ras, and BCL-2). This specificity of interactions was supported by the fact that a directed DNA evolution study against a related but longer helical cationic oligoamide identified G-quadruplexes as preferred targets.[231] Whereas most known G-quadruplex ligands have flat aromatic structures and stack on top of G-tetrads, the helical shape of quinoline-based oligoamides makes a similar mode of interaction unlikely, and the recent cocrystal structure of **58** with an antiparallel hairpin dimeric DNA G-quadruplex $(G_4T_4G_4)_2$ supports a binding mode driven by ammonium–phosphate electrostatic interactions between the foldamer cationic side chains and the DNA backbone loops (Fig. 25). The resolution of more selective foldamer G-quadruplex interactions will likely guide the rational design of more potent aromatic helical foldamer-based ligands for DNA G-quadruplex.

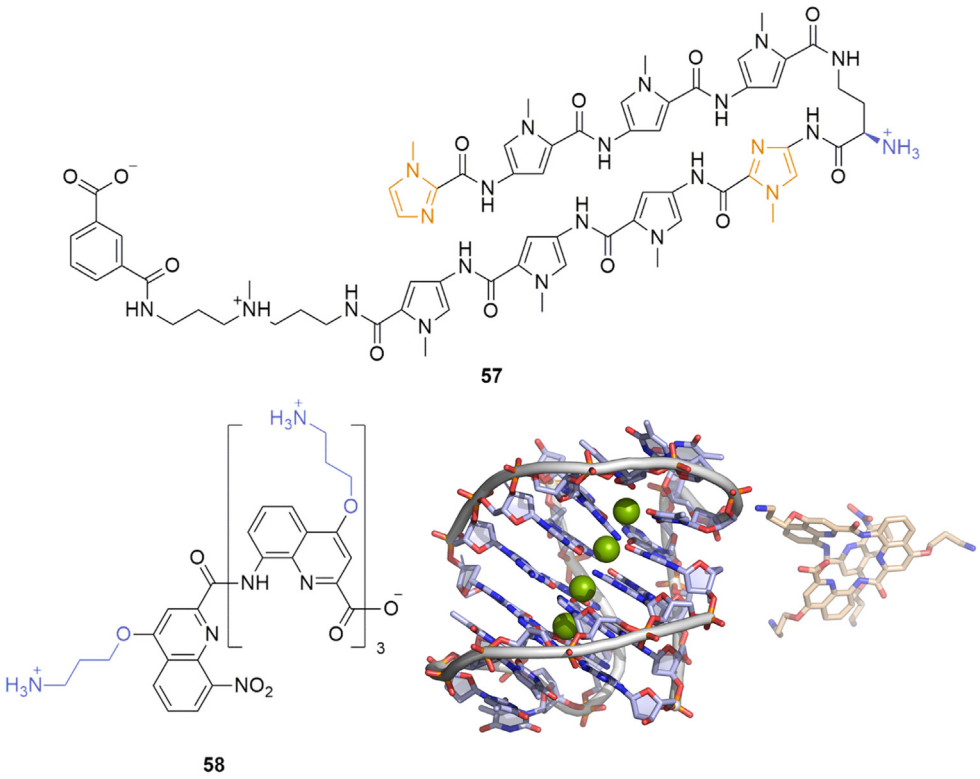


Figure 25 Aromatic oligoamides for nucleic acid recognition: Py–Im polyamide (**57**) and helical oligoamides based on 8-aminoquinoline-2-carboxylic acid (**58**).

5.05.4 In Vivo Studies of Bioactive Foldamers

Synthetic foldamers made of unnatural backbones intrinsically differ (chemically and structurally) from the natural α-peptides or biopolymer they intend to mimic. These differences are generally assessed in vitro in terms of interaction with the biological target, resistance to proteolysis, membrane permeability, and cellular activity (see previous sections), but relatively few studies have focused on activities of foldamers in vivo. In the context of developing possible therapeutic applications, there is increasing interest to know more about the properties of foldamers in vivo (efficacy in animal models, duration of action, PKs, toxicity, immunogenicity).

5.05.4.1 PKs and Tissue Distribution

Along with increased resistance to proteolytic degradation (see section "Resistance to proteolysis"), foldamer mimics of natural peptides are likely to manifest different PKs and as a result different activities in vivo. Only a limited number of studies have examined the PK properties and tissue distribution of bioactive foldamers. To a large extent, these investigations have focused on cationic amphiphilic helices (8–12 residues long) for which antibacterial activities were known, namely, antibacterial β^3-nonapeptide H-(β^3Ala-β^3Lys-β^3Phe)$_3$-OH,[232] peptoid analogs of **14**[233] and the *N*,*N*′-linked oligourea **17**[142] as well as on (β^3Arg)$_9$ a cationic CPF.[234] PK studies of the doubly ^{14}C-labeled H-(β^3Ala-β^3Lys-β^3Phe)$_3$-OH derivative in rats[232] revealed that following intravenous (i.v.) administration of 5 mg kg^{-1} (i) the concentration of β-peptide in blood and plasma decreases rapidly and the radioactivity is distributed in organs and tissues; (ii) after 7 days, residual radioactivity in organs and tissues represented ≈ 50% of the i.v. dose with high levels in the kidney, lymph nodes, and liver; and (iii) the β-peptide is highly stable against metabolic degradation in vivo. Negligible absorption took place after per os administration, and the administered dose was completely excreted *via* the feces within 96 h.[232] The same trend was observed following i.v. administration of the ^{14}C-labeled (β^3Arg)$_9$ derivative, but excretion from the body was even slower (2% of the dose in 7 days).[234] A major fraction of the radioactivity ended up in the liver (~30% of the dose was still found in the liver at 168 h postdosing).

The 12-mer peptoid analog of **14** conjugate was assayed in vivo as its ^{64}Cu-labeled DOTA by both biodistribution studies and small-animal positron emission tomography (PET).[233] After i.v. administration, biodistribution studies of ^{64}Cu-labeled peptoid demonstrated prominent uptake in the liver with a slow elimination from the liver region observed by PET images.

The PK profile and the tissue distribution of a ^{3}H-labeled analog of **17** were investigated by beta-radio imager whole-body mapping in mice.[142] Following intranasal administration, a large amount of radioactivity was sequestered but transiently within the lung and upper airways. Low excretion and recovery of the oligourea in the kidney, following i.v. injection, were found to be consistent with high stability in vivo. Overall, these observations, that is, in vivo stability and high tissue uptake, are consistent with the two studies conducted on β-peptides[232,234] and peptoids[233] and are likely to reflect specific features of these compounds: protease resistance and membrane permeability of cationic foldamers. It remains to be seen whether the PK and biodistribution properties reported for these specific foldamers can be modulated upon sequence variation and whether similar trends would be observed for other folded backbones.

Much information has also been gained about the PKs and toxicity of Py–Im polyamides in rodents.[235] Py–Im polyamides are cell-permeable molecules, and depending on their architecture, they can circulate several hours after i.v. administration and up to 48 h in some cases. A number of studies have documented activities in vivo without evidence of systemic toxicity.[236] For example polyamide **57** which was designed to target the consensus androgen and glucocorticoid response elements 5′-WGGWWW-3′ (W = A or T) is capable of trafficking to the tumor site following subcutaneous injection and modulates transcription of selected genes in vivo. An FITC-labeled version of this polyamide was detected in tumor-derived cells by confocal microscopy. This compound has demonstrated efficacy in vivo against prostate cancer xenografts in mice with limited toxicity.[236b,c]

5.05.4.2 Activities of Host Defense Peptide Mimetics

In a few cases, foldamers mimicking host defense peptides (see section "**Membrane Active Foldamers as Antimicrobials**") have been evaluated in mouse models of bacterial infection. For example, arylamide foldamers such as **22** developed by DeGrado were found to exhibit significant activity in a *S. aureus* thigh infection model (the thigh muscle of neutropenic mice is inoculated with bacteria followed by i.v. administration of the foldamer) when administered at doses of 2 mg kg^{-1} (i.e., 10^{5} reduction in viable CFU of *S. aureus* ATCC 13709).[158] Today, this family of foldamers is probably the most advanced of all in terms of clinical development. Brilacidin (**59**), a close analog of **22** originally developed by PolyMedix as a new class of antibiotic and since 2013 by Cellceutix (http://cellceutix.com), has recently completed a Phase 2b clinical trial for the treatment of acute bacterial skin and skin structure infections caused by *S. aureus*, including MRSA. In the trial, brilacidin demonstrated high clinical response rates comparable to those of daptomycin (the active control), and was shown to be safe and generally well-tolerated (**Fig. 26**).

A longer 12-mer analog of peptoid **14** was recently tested in vivo using a murine model of invasive *S. aureus* bacterial challenge.[237] At a concentration of 4 mg kg^{-1}, this compound apparently caused no medium-term toxicity, and treatment at this concentration resulted in an average two-log-order reduction in bacterial counts in the peritonium. Moreover, mortality was reduced by 75% in the peptoid-treated group compared to saline-treated controls. In another study, *N*,*N*′-linked oligourea **17** which is active in vitro against bacterial forms of *B. anthracis* encountered in vivo (i.e., germinating spores, encapsulated and nonencapsulated bacilli) was evaluated in cutaneous and inhalational models of infection with *B. anthracis*.[142] This model of infection is highly informative because the infectious process can develop through natural routes in normocompetent mice, without any immune manipulation.[238] Compound **17** was found to exert a protective activity in vivo in the cutaneous model of infection; a limited local treatment increased time of survival and led to partial protection. A similar delay in time to death was also observed following inhalational infection.

5.05.4.3 α/β-Peptide Analogs of Class B GPCR Agonists

Several studies have reported in vivo activities of α/β-peptide ligands of class B GPCR receptors (GLP-1R, hPTHR1, VIPR1, or VIPR2) mentioned in section "**Ligands of G-Protein-Coupled Receptors: Hormone Like Analogs**." The GLP-1R agonist **49** was tested in a GTT for its ability to normalize circulating glucose levels following the administration of a bolus of glucose. Mice injected α/β-peptide **49** and GLP-1$_{(7-37)}$-NH_2 at 1 mg kg^{-1} were equally effective in suppressing the rise of blood glucose concentration relative to vehicle-treated mice during the GTT. Furthermore, when the GTT was repeated 5 h after agonist administration, the glucose-lowering effect of **49** was maintained but not that of GLP-1(7−37)-NH_2.[214] Overall these results possibly reflect the higher resistance of the α/β-peptide to proteolysis and show that the α → β replacement strategy is effective to generate potent B-family

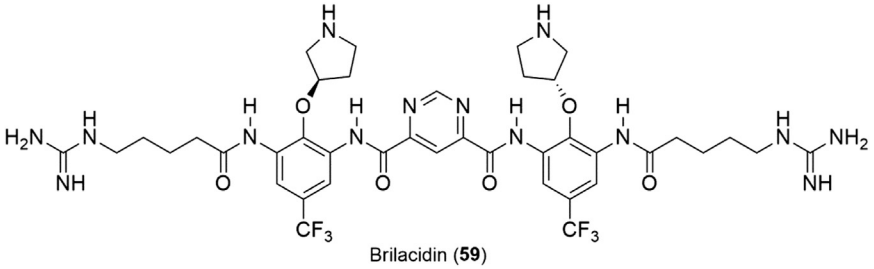


Figure 26 Formula of brilacidin (**59**).

GPCR agonists which can recapitulate hormone function in vivo and which could find useful biomedical applications. This is further supported by in vivo studies of PTHR1 agonists. Subcutaneous injection of PTHR1 α/β-peptide agonist **47** induced a calcemic response in mice longer than that caused by $PTH_{(1-34)}$, which correlates well with its affinity for a particular functional state of the receptor (i.e., R^0 state which forms independent of $G_{\alpha S}$, a heterotrimeric G-protein responsible for activating adenylate cyclase upon receptor activation). The calcemic effect duration of **47** is likely facilitated by the substantially prolonged bloodstream bioavailability of this derivative compared with $PTH_{(1-34)}$. The development of long-acting PTH analogs may find practical use for treating hypoparathyroidism, for which agents that can normalize blood calcium levels for extended periods, if not continuously, are needed.

VIPR1 or VIPR2 α/β-peptide agonists such as **50** were tested for their abilities to protect mice against MPTP-induced neurodegeneration used to model PD.[239] The metabolically stable VIPR2-specific agonist **50** displayed an improved in vivo PK profile ($t_{1/2} = 24.33$ min) in comparison to that previously reported for VIP ($t_{1/2} < 1$ min) and was found to be an effective immunomodulatory agent in a disease-relevant PD model. Treatment of MPTP-intoxicated mice with **50** significantly spared dopaminergic neuronal cell bodies, decreased the amount of reactive microgliosis, downregulated proinflammatory cytokine production, and modulated T-cell phenotypes with treatment. In contrast, treatment with the stable, VIPR1-selective agonist yielded only lesser neuroprotective responses. These results support the use of VIPR2-selective agonists as neuroprotective agents for PD treatment.

Overall these three studies demonstrated that the α/β-peptide approach is particularly well suited to mimic long peptide helices and increase biological lifetime of natural peptide hormones. It remains to be seen whether other types of foldamer backbones could be equally useful to design ligands of class B GPCRs.

5.05.4.4 Foldamers That Target Intracellular PPIs

Examples of foldamers designed to modulate/inhibit intracellular PPIs that actually display intrinsic capacity to cross the cell membrane to reach the appropriate location inside the cells and engage their target proteins are relatively sparse and are dominated by topographical mimics.[69c,167,240] Oligooxopiperazine **43** designed to mimic HIF-1α and to target the transcriptional coactivator p300 is one such example. This compound was shown to be active in a mouse tumor xenograft model. Injection of **43** (15 mg kg^{-1}) into mice bearing xenografts derived from the triple-negative breast cancer cell line MDA-MB-231 reduced the median tumor volume by roughly 50% compared with the untreated group, without showing measurable changes in animal body weight.[69b] Folded aliphatic backbones such as D-peptides, aliphatic β- and α/β-peptide helices may be endowed with cell-penetrating properties through sequence variation,[192] side chain cross-linking,[18c] and conjugation to molecular transporters.[18d] This is the case of the D-peptide ligand of MDM2 **37** which, despite biostability and effective binding to MDM2, fails to actively penetrate cells. In this particular case, conjugation to a cell-penetrating sequence ($(D\text{-Arg})_9$) *via* its C-terminus did not lead to the expected activity but resulted in necrosis of both HCT116-$p53^{+/+}$ and HCT116-$p53^{-/-}$ cell lines in a p53-independent manner.[195] However, these D-peptide ligands of MDMD2 (either in the free form or palmitylated) were shown to kill human glioblastoma cells in a p53-dependent manner and to exert p53-dependent antitumor activity in nude mouse xenograft models when encapsulated in liposomes functionalized with an integrin-targeting cyclic-RGD peptide, thus providing proof of principle for efficient D-peptide loading and delivery.[195,241]

5.05.4.5 Antigenicity and Immunogenicity of Bioactive Foldamers

Characterizing the possible interactions of biologically relevant foldamers with the immune system and making sure that they do not induce an unwanted immune response from the host are important issues that certainly need to be carefully addressed if therapeutic applications are sought. To date, there is still little information available on the possible immunogenicity of foldamers and their recognition by the specialized molecules of the immune system, that is, antibodies, major histocompatibility complex (MHC) molecules, and T-cell receptors. Early work reporting immune responses against nonnatural peptide backbones (e.g., D-peptides) was often the result of efforts to make effective immunogens after conjugation to a carrier protein and/or association with adjuvants.[242] Other studies have investigated the role of the peptide backbone in T cell recognition, by scanning the backbone of antigenic peptides with pseudopeptide bonds (or single α → β replacements) and measuring the capacity of such pseudopeptides to bind their cognate MHC molecule and subsequently to engage the T cell receptor (TCR).[145c,242b,c,243] With a completely different objective (i.e., avoiding immune response), Gellman and coworkers have investigated how bioactive α/β-peptides (e.g., Bim BH3 derived) with high β residue content and regular patterns (e.g., ααβ and ααβααβ) are recognized by the immune system and compared these with cognate α-peptides.[244] It turns out that recognition by antibodies raised against the prototype α-peptide is suppressed by periodic α → β replacements and that antibodies raised against α/β-peptides fail to recognize prototype α-peptides displaying identical side chain repertoires. Finally, periodic α → β replacements in CD8(+) T cell viral epitope suppress the formation of a productive MHC I/peptide/TCR ternary complexes that would activate cytotoxic T-lymphocytes, due in part to disruption of MHC binding. Although further studies are needed to determine whether the lack of cross-reactivity between homologous α-peptides and foldamers is a general feature, these data are supported by the recent analysis of a small D-protein scaffold selected to bind to VEGF-A that is completely resistant to proteolysis in mouse, monkey, and human plasma and had a longer in vivo half-life than the cognate L-protein when injected i.v. and a complete lack of immunogenicity, even when dosed in combination with a strong adjuvant.[48f]

5.05.4.6 Conclusion and Trends

Advances in foldamer chemistry together with the finding that nonnatural oligomeric backbones may retain folding in water and exhibit diminished susceptibility to degradation by proteases gave impetus for the use of synthetic folded oligomers in many different biologically and therapeutically relevant contexts, from structural mimics of AMPs and inhibitors of PPIs to peptide hormone mimics and DNA ligands. Remarkable progress has been made in the field since the seminal discovery that short β-peptides may adopt well-defined helical secondary structures akin to those found in proteins. Sequence-based biopolymer mimicry still has a greater role to play as a pharmacological tool and possibly toward the development of innovative medium-size therapeutic agents. The use of foldamers as protein secondary structure mimics for modulating biological interactions is currently dominated by aliphatic oligomers such as α/β-peptides for which robust and general design principles have been proposed, but many new backbones (both biotic and abiotic) are currently available for further evaluation, expanding the scope of possible approaches to address a particular biological question. Structural characterization, at high resolution, of foldamer/biopolymer interactions and molecular modeling tools are becoming increasingly important to learn more precisely how foldamers can interact with biological surfaces, mimic the information encoded by biopolymers, and guide the design of more potent bioactive foldamers. Studies reporting activities of foldamers in vivo and PK properties of foldamers in animal models are still rare, but this is a direction which will surely develop in the future, contributing to address important issues (PK, tissue distribution, toxicity, immunogenicity) for the practical development of this unique and fascinating class of molecules.

Acknowledgments

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