# Making Strides in Peptide-Based Therapeutics

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**In a recent report published in *PNAS*, Gellman and coworkers describe the design, characterization, and potent activity of α/β-peptides that mimic a long α helix involved in HIV viral entry.**

Protein-protein interactions are intrinsic to many biological processes, from signal transduction to cell death, and misregulation of these interactions is implicated in many diseases. As such, these interactions are potential targets for drug discovery. However, the disruption of protein-protein interactions is a difficult challenge because the protein interfaces often occupy large surface areas and lack deep cavities amenable for small-molecule binding (Arkin and Wells, 2004).

A broad effort in the organic and medicinal chemistry community to develop suitable ligands to target protein-protein interactions has focused on fragment-based screening and the synthesis of natural products and natural product-like libraries (Stockwell, 2004). A complementary effort has centered on a rational-design approach that seeks to adapt protein recognition principles utilized by nature. The examination of the structural features of complexes of proteins with other biomolecules reveals that proteins tend to interact with partners via folded subdomains, or protein secondary structures. α helices constitute the largest class of protein secondary structures, and play a major role in mediating protein-protein interactions. Peptides adopt stable helical conformation in the context of proteins, but isolated peptides in solution rarely retain their biologically relevant structure. The chemical biology community has focused much of its attention on studying different approaches to either stabilize the α-helical conformation in peptides or mimic this domain with non-natural scaffolds. These approaches can be divided into three general categories: helix stabilization, helical foldamers, and helical surface mimetics (Henchey et al., 2008). Helix stabilizing methods based on side-chain crosslinks and hydrogen-bond surrogates pre-organize amino acid residues and initiate helix formation; miniproteins that display helical domains are also part of this category. Helical foldamers are oligomers composed of non-natural amino acid residues capable of adopting conformations similar to those found in natural proteins. Helical surface mimetics utilize conformationally restricted scaffolds with attached functional groups that resemble the *i*, *i*+4, *i*+7 pattern of side chain positioning along the face of an α helix.

The work of Gellman and others focuses on β-peptide foldamers (Figure 1), which are well-established mimics of α helices (Gellman, 1998; Seebach and Gardiner, 2008). Multiple methods for controlling the helical structure of β-peptides have been described. The Gellman group has pioneered the use of cyclic β-amino acid residues to lock the oligomer backbone into favorable positions. Constraining the desired Cα-Cβ torsional angles in cyclic compounds (e.g., trans-2-aminocyclopentanecarboxylic acid (ACPC) and trans-3-Aminopyrrolidine-4-carboxylic acid (APC); Figure 1A) improves helicity by reducing flexibility (Gellman, 1998). Other approaches for stabilizing the β-peptide conformation include the insertion of favorable salt-bridging interactions along one face of the helix and control of the helical macrodipole (Kritzer et al., 2005). More recently, α-, β- and cyclic β-amino acid residues were combined to create heterogeneous backbones with a diverse projection of side-chain functionality (Horne and Gellman, 2008). The ability of foldamers to take on a variety of helical shapes is advantageous in the design of therapeutics that better imitate protein secondary structures (Goodman et al., 2007).

Perhaps the greatest benefit of β-peptides is their inherent proteolytic stability. The β-peptide backbone is not recognized by common proteases, and the β residues in α/β-peptides offer substantial protection to neighboring amides from proteolytic cleavage (Horne et al., 2009).

In the current manuscript, Horne et al. (2009) created a panel of peptides containing α-, β- and cyclized β-amino acids that potently inhibit HIV entry. Viral entry is facilitated by conformational changes that HIV membrane protein gp41 undergoes to adopt a six-helix bundle during the fusion of the host and viral membranes. The six-helix bundle consists of three helices from the C-terminal heptad repeat domain and three helices from the N-terminal heptad repeat domain (Chan et al., 1997). Prevention of bundle formation using α-peptides (or mimics thereof) derived from the C-terminal region is central to anti-HIV entry drug design efforts. One drug in current use, enfuvirtide (Fuzeon), is a 36-amino acid α-peptide comprised of residues from the C-terminal region of gp41. As enfuvirtide is composed solely of α-amino acids, it is susceptible to protease degradation and therefore can be highly unstable as a therapeutic agent.

Discovery of potent nonnatural, peptide-based gp41 mimics or small molecules capable of disrupting six-helix bundle formation has been a difficult challenge. Gellman and coworkers began their α/β-peptide designs by mimicking a previously described α-peptide helix (Horne et al., 2009). Acyclic $\beta^3$-amino acid substitution into the α-peptide sequence provided modest inhibitors with in vitro activity in the micromolar range. Replacement of key acyclic β-residues with ACPC and APC resulted in rigidified chimeric α/β-peptides with a remarkable 380-fold binding enhancement. The optimization studies provided highly effective constructs with low nanomolar activities in cell-cell fusion and HIV anti-infectivity assays. Importantly, helical propensity in α/β-peptides correlated well with inhibitory

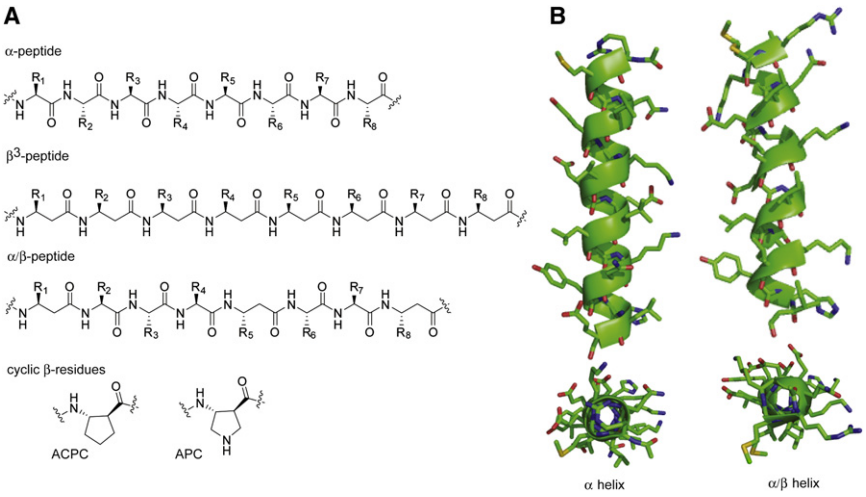


**Figure 1. α Helix and α/β-Peptide Foldamers Array Side Chain Functionality in Similar Fashion**

(A) Backbone sequences of α, β, and α/β helices, and structures of cyclic β-residues.

(B) Comparison of the α, and α/β helices (PDB codes 2ZTA and 3C3F).

potency and resistance against proteolytic degradation.

The current work highlights the molecular design strategy employed by Gellman and coworkers (Horne et al., 2009) to create faithful mimics of the α helix that are endowed with conformational and proteolytic stability. Structural studies suggest that subtle changes in the backbone composition can significantly affect the superhelical twist of the foldamer helix. Addressing these critical observations will help build a comprehensive understanding of the relationship between sequence, structure, and function in foldamers. The mechanism used by the HIV virus to enter cells is also employed by other Class I viruses to target host cells (Dimitrov, 2004). Success of the outlined strategy suggests that α/β helices may be effective scaffolds for the generation of potent inhibitors or antigens against these viruses.

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