EXPRESS COMMUNICATION

Ultrafast folding and molecular dynamics of a linear hydrophobic β-hairpin

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The first computational study of the folding and dynamics of a hydrophobic β-hairpin containing a central heterochiral diproline segment is reported. Linear hydrophobic sequences containing centrally positioned diproline motifs, heterochiral (DL/LD) and homochiral (LL/DD)), are investigated for their ability to form β-hairpins. Heterochiral diproline motifs (LD/DL) reveal the formation of stable β-hairpins with the backbone adopting β-turn conformation and the formation of backbone hydrogen bonds with antiparallel cross-strand registry, whereas the homochiral diproline (LL/DD) containing sequences tend to adopt PPII helix conformation. The competition between the β-turn formation and the backbone H-bond ladder of the antiparallel β-strands in heterochiral diproline containing sequences is employed to validate the hypothesis that β-turn formation precedes inter-strand registry in the folding of a β-hairpin (“zipper” mechanism). The observation of noncanonical hydrogen bonds leads to a folded β-hairpin-like conformation and points to the existence of relatively stable transition state intermediates, between the unfolded (extended) and folded (β-hairpin) states. The MD simulations are in excellent agreement with the experimental studies on the model system and constitute the very first computational investigation of the folding and dynamics of a completely hydrophobic synthetic β-hairpin containing heterogeneous residues of mixed chirality.

Keywords: β-hairpin; protein design; β-turns; molecular dynamics; hydrophobic; chirality

Protein folding problem has been addressed by the structural mimicry of α-helices and β-turns by designed polypeptide sequences. The desired fold (α-helix or β-sheet) is readily realized with the designed sequence adopting characteristic backbone conformations. Designed polypeptide sequences, with a handle on the conformational flexibility of short stretches, have provided a considerable wealth of information on the stability and flexibility of helical and turn conformations (Degrado, 1988; Schneider & Kelly, 1995; Venkatraman, Shankaramma, & Balaram, 2001). The nonnatural amino acid, α-amino isobutyric acid (Aib), has been experimentally shown to nucleate tight helical turns, in particular 3_10-helix, by establishing its conformational preferences in a large body of crystal structures (Karle & Balaram, 1990; Toniolo & Benedetti, 1991). The construction of conformationally stable β-turns has also received the undivided attention of protein design experts for a very long time. The implication of turns in protein–protein/protein–ligand interactions places it in the gold category of rational protein design. In the context of model systems employed to study regular secondary structures, Type I and Type II β-turns (and their prime counterparts) are the most thoroughly studied and extensively characterized reverse turns (Rose, Gierasch, & Smith, 1985; Venkatachalam, 1968). On the other end of the protein folding spectrum, the studies on the β-sheets have been somewhat limited due to the tendency of the polypeptide sequences to aggregate and form fibrillar materials (Lacroix, Kortemme, De La Paz, & Serrano, 1999). The formation of isolated β-sheets has received considerable attention during the last two decades, with the focus directed at understanding the stability and kinetics of β-hairpins (Eaton et al., 2000; Munoz, Henry, Hofrichter, & Eaton, 1998; Munoz, Thompson, Henry, Hofrichter, & Eaton, 1998; Munoz, Thompson, Hofrichter, & Eaton, 1997; Richardson & Richardson, 2002). β-hairpin is the simplest model system of antiparallel β-sheet, with the topology of two linear antiparallel β-strands connected by a tight reverse β-turn (Balaram, 1999; Gellman, 1998; Hughes & Waters, 2006; Kaul & Balaram, 1999; Smith & Regan, 1997). Studies on the kinetics of β-hairpin

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model systems have established that the rate of formation is considerably slower than an α-helix (approx. 30 times slower). Peptidomimetics that fold into a β-hairpin have been characterized experimentally in recent years, using both solution and solid-state structural studies (Blanco, Rivas, & Serrano, 1994; Chung, Dou, Baldi, & Nowick, 2005; Karle, Awasthi, & Balaram, 1996; Malalakshmi, Raghothama, & Balaram, 2006; Searle, Williams, & Packman, 1995). The preponderance of prime turn (Type I/Type II) β-hairpins in protein crystal structures provided impetus to tackle the design of polypeptide hairpins with similar topology (Gunasekaran, Ramakrishnan, & Balaram, 1997; Sibanda, Blundell, & Thornton, 1989; Sibanda & Thornton, 1985). The design and structural characterization of a large number of β-hairpin peptide sequences were made possible with the availability of stable structural templates for β-strands and β-turns (Espinosa & Gellman, 2000; Haque, Little, & Gellman, 1996). Insight into the formation and stability of the synthetic model systems of β-hairpins are, in general, in the context of hydrophobic sequences. Heterochiral diproline motifs (Figure 1) have been shown to adopt stable β-turn conformations (Nair, Vijayan, Venkatachalapathi, & Balaram, 1979; Robinson, 2000; Shankaramma et al., 2002). Conformational search methods favor Type II β-turn for the heterochiral diproline template (Chalmers & Marshall, 1995). In a brilliant use of synthetic turn templates, peptidomimetics were constructed by transplanting loops from antibody complementary determining regions (CDRs) in IgG antibodies onto a DPro-LPro template (Spath, Stuart, Jiang, & Robinson, 1998). In a recent study, involving the mimicking of interaction sites of the platelet receptor glycoprotein (GPIIb with von Willebrand factor (vWF)), a cyclic peptide containing a heterochiral diproline motif was identified as a lead compound for the generation of a novel class of antiplatelet agents (Bernard et al., 2012).

The realization of β-sheets in protein design relies on the proper assembly of stable supersecondary structural motifs, and it is imperative that the folding and dynamics of the designed peptides forming β-hairpins be put in the context of the β-hairpins in proteins. With the emergence of heterochiral diproline segment as a conformationally constrained motif (Favre, Moehle, Jiang, Pfeiffer, & Robinson, 1999; Robinson, 2008, 2009; Robinson, Demarco, Gombert, Moehle, & Obrecht, 2008), the present investigation is the first step in quantifying the chirality of the turn segment in protein design. The role of chirality, homochiral (LL/DD) and heterochiral (DL/LD), in nucleating stable β-hairpin conformations in polypeptide sequences by performing molecular dynamics simulations on extended sequences is investigated. The choice of the sequence is based on the availability of experimental structural data (Rai, Raghothama, & Balaram, 2006). The formation of a β-hairpin in Ace-Leu-Phe-Val-DPro-LPro-Leu-Phe-Val-NMA (1), using MD simulations in both vacuum and bulk solvents (water/organic solvents), is addressed. Surprisingly, the MD simulations reveal ultrafast folding of the peptide 1 into a β-hairpin (in a few ns) as opposed to few μs in proteins (Eaton, Muñoz, Thompson, Henry, & Hofrichter, 1998). The role of structural and conformational factors responsible for the stabilization of β-hairpin formation in 1 is quantified. The route to achieving the folded state is mediated by

Figure 1. Chemical structures of diproline peptide motifs. (a) DPro-LPro (DL), (b) LPro-LPro (LL), (c) LPro-DPro (LD), and (d) DPro-DPro (DD).
the formation of intermediate folded and twisted hairpin structures. The simulations reveal that stable turn conformations are readily accessible to the sequence during the simulations, and the analysis of the turn formation and the inter-strand registry of the β-strands in the β-hairpin lends support to the “zipper” mechanism (Eaton et al., 1998). Preliminary 1 ns simulations revealed the folding of 1 into a β-hairpin-like structure. Intrigued by this observation, longer simulations were performed in vacuum and/or bulk water (SPC)/methanol/DMSO, to explore the conformational dynamics. Simulations were also carried out on the control sequences, Ace-Leu-Phe-Val-DPro-Pro-Leu-Phe-Val-NMA (2), Ace-Leu-Phe-Val-Pro-Leu-Phe-Val-NMA (3), and Ace-Leu-Phe-Val-Pro-Leu-Phe-Val-NMA (4). Replica exchange molecular dynamics (REMD) was performed on 1 in bulk water, to investigate the temperature dependent kinetics of the folding of the peptide. Two metadynamics simulations of 1 for 10 ns each were also carried out in vacuum using a pair of distinct collective variables. All the simulations were carried out with the sequences built in an extended conformation and with no constraints.

Both 1 and 3 were observed to fold into a β-hairpin-like structure, with the formation of native as well as noncanonical backbone–backbone (bb–bb) hydrogen bonds in vacuum and metadynamics simulations. For a linear octapeptide sequence, eight bb–bb hydrogen bonds are theoretically possible, with the number rising to 10 if capping fragments (Ace and NMA) are included as potential donor/acceptor groups. The theoretically possible H-bonds reduce to eight, with the diproline segment in the sequence, excluding bifurcated H-bonds. Of the eight possible bb–bb H-bonds, four correspond to native β-hairpin, whereas the rest are treated as noncanonical H-bonds. A considerable number of H-bonds are found to be formed in vacuum simulations (33.4% in 1 and 34.5% in 3), in contrast to negligible H-bonds in bulk solvent simulations (Table 1). Of the total number of H-bonds observed, H-bonds ≥4 are 7.2% in 1 and 8.0% in 3. In 1, the first (Val(3) CO···HN Leu(6)) and the last (Val(8) CO···HN Leu(1)) canonical native H-bonds are observed in all instances of β-hairpin formation (Figure 2).

Surprisingly, the inspection of H-bonds along the trajectory reveals that the noncanonical H-bond between Leu(6) NH and DPro(4) CO is persistent throughout, making a few minor excursions into the native H-bond topology. The preponderance of noncanonical bb–bb, yet cross-strand stabilizing, H-bonds leads to the formation of registry slipped and strand twisted β-hairpins, a structural feature also observed in studies of β-hairpins in solution (Rai et al., 2006). This suggests the stabilization of structure by nonnative H-bonds, hence the existence of transient state structures.

The crystal structure of β-hairpins with similar backbone reveals an ideal H-bond pattern, owing to the peptide dipole effects and also the constraints of crystal packing (Karle, Gopi, & Balaram, 2002; Roy, Gopi, Raghothama, Karle, & Balaram, 2006). The replica at 344.4 K, in REMD simulations, is also involved in the formation of a greater number of canonical hairpin H-bonds as compared to other replicas, which in turn leads to the formation of a perfect β-hairpin (Table 1). This bears close resemblance with the NMR study at variable temperatures of eight-residue-long variants of G-peptide (Lewandowska, Oldziej, Liko, & Scheraga, 2010).

The backbone torsions of the heterochiral diproline segment (DL/LD) were also monitored along the trajectory for the evidence of β-turn like conformation (Figure 2). The simulations revealed proportionately larger incidents of turn conformations, as opposed to helical or sheet conformations (Table 1). The criteria applied to extract turn conformations is very stringent (±40°), the percentage of turn conformations would increase substantially if the limits on torsions are relaxed. The replicas, from REMD runs, of 1 showed near-native turn conformations in 57.5, 66, and 76.4% cases at 300.0, 311.1, and 400 K, respectively. The replica of 1 at 344.4 K reveals perfect β-turn formation in 34.4% cases, concomitant with β-hairpin formation (Figure 2). In a related study, evaluating the influence of β-turn and nonβ-turn native H-bonds on the folding kinetics of tryptophan zipper, it was established that the turn formation is the rate limiting step, and the formation of hydrophobic cluster succeeds the turn formation (Culik, Jo, Degrado, & Gai, 2012). The diproline segment was observed to adopt PP11 conformation for DPro(4) and

Table 1. Diagnostic geometrical and conformational parameters (in %) from MD simulations of 1–4. HB (H-bonds) and NHB (native H-bonds).

|                | Vacuum       | Water        | REMD        | Metadynamics |
|----------------|--------------|--------------|-------------|--------------|
|                | 1            | 2            | 3           | 4            | 1            | 2            | C           | 1(a)         | 1(b)         |
| # HB           | 33.4         | 31.2         | 34.5        | 43.9         | 1.1          | 3.7          | 1.5         | 30.8         | 32.5         |
| # NHB          | 0.4          | 0            | 10.3        | 0            | 0            | 0            | 6.2         | 7.5          | 8.1          |
| Helical        | 0            | 1.5          | 0           | 22.5         | 0            | 0            | 0.3         | 0.0          | 0.3          |
| Sheet          | 2            | 0.0          | 2.2         | 0            | 0            | 0            | 0.3         | 10.0         | 4.0          |
| Turn           | 58.7         | 45.3         | 62.6        | 63.3         | 12.3         | 22.7         | 21.1        | 52.7         | 56.2         |
Pro(5) in 69 and 3.8% cases in 1 and .02 and 94% cases in 3.

With the promising results in plain MD simulations, it was decided to further enhance conformational sampling of the 1 by performing metadynamics simulations (Laio & Gervasio, 2008). The peptide was prepared and subjected to vacuum simulations as described previously, with two pairs of distinct collective variables in two independent 10 ns runs (see Supplementary material). In the first run (1(a)), the collective variables chosen were the virtual torsion about the β-turn (Ca (i)–Ca (i+1)–Ca (i+2)–Ca (i+3)) and the distance d (O3···N6), corresponding to the β-turn H-bond. The strain in the twisting of β-strands in a hairpin is quantified by evaluating the virtual torsion angle (Gunasekaran et al., 1997). In the second run (1(b)), the backbone torsions about the diproline segment, ψi+1 and ψi+2, were chosen as collective variables. The native hairpin H-bonds are observed to be significantly larger, and constitute a greater percentage of theoretically possible set (7.5 and 8.1%) in metadynamics, implying a relatively larger sampling of conformational space (see Supplementary material).

The X-Pro segment in the middle of the sequence presents the interesting possibility of a cis-amide bond-mediated, preceding proline, Type VIb turn nucleated β-hairpin. The cis-trans conformational transitions were not observed in MD of 1. Nonetheless, simulations of an extended polypeptide 1, with the amide bond of

![Figure 2. ϕ-ψ plots of the conformations of diproline segment in MD of 1–4, (A) DL, (B) LL, (C) LD, and (D) DD. Residue (i+1) in red dots and (i+2) in green dots. The arrows in (A) and (C) point to the Type II□ and Type II β-turn clusters. Representative structures are shown in inset. Diproline segment colored in orange.](image-url)
the DPro-LPro segment in cis configuration, reveal a preponderance of turn conformations for most of the residues. The two states of 1 with the amide bond between DPro^1-LPro being trans (ω = 180°) and cis (ω = 0°) is contrasted in Figure 3. This underlines the importance of trans geometry of the amide bond in the turn segment and that the minor energy difference, between trans and cis configurations, could tilt the conformational balance to a more open and solvent accessible helical form.

The conformational analysis of the trajectories of 2 and 4 reveals the abundance of turn and helical conformations. Native H-bonds corresponding to β-hairpin structure were not formed during MD, hence no signature of hairpin in 2 and 4. On the contrary, the all L-amino acid sequence 2 and two consecutive D-amino acids containing 4 are observed to readily adopt polyproline-like helical conformations. Peptide 2 is also seen to adopt relatively greater amount of turn conformations in bulk water as opposed to 1. Individually, in 2 the Pro(4) and Pro(5) were seen to adopt PPII 92.9 and 3.0% times, and in 4 DPro(4) and DPro(5) were seen to adopt PPII 94.7 and 48.8% times. Consecutive D-proline residues appear to adopt stable and tightly clustered conformations in the polyproline region, as opposed to consecutive L-prolines in which LPro(i + 2) is seen to be relatively more flexible about conformations around ψ (i + 2) (Figure 2). Clearly, consecutive L-proline and D-proline residues could be comfortably accommodated in the middle of a linear peptide sequence adopting helical conformations. The propensity of homochiral diproline segments to adopt PPII conformations is higher than heterochiral segments. The puckering of the proline residues is presented as scatter plots (Supplementary material). The χ′-χ′ reveal differences between the homochiral and heterochiral diproline segments. The C^α-endo and C^α-exo puckering states are in equilibrium as observed previously in the investigation of models of collagenic structures (Vasilescu, Cabrol, & Tamburro, 1988).

In conclusion, the first systematic investigation of the folding dynamics of a completely hydrophobic synthetic designed peptide β-hairpin using MD simulations is presented here. The peptides 1 and 3 fold on a timescale of a few nanoseconds as opposed to β-hairpins from proteins which fold in a few μs. It has been proposed, after a critical analysis of β-hairpin sequences, that turn formation precedes inter-strand registry with through backbone hydrogen bonds (Venkatraman et al., 2001). The crystal packing results in an optimum space-filled arrangement of the flat hairpin molecules, with greatly optimized electrostatic and weak interactions (dipole–dipole, quadrupole–quadrupole). The net result is an ideal picture of perfectly oriented and geometry optimized stable structures. The formation of such structures is, presumably, the end result of a continuous optimization of the topology of the interacting species during and after nucleation. This poses the question, if the initial structure formed during the nucleation is identical or bears any structural resemblance to the structure seen in a crystal lattice? Presumably, the thermodynamically and kinetically stable species of polypeptide with ordered structure(s), like the noncanonical β-hairpins traced in MD simulations, are formed transiently after nucleation. Such a model was put forward in the context of orthogonal ββ motifs in protein crystal structures (Sowdhamini, Srinivasan, Ramakrishnan, & Balaram, 1992). The fine balance between the conformational transitions of the polypeptide backbone can play a significant role in tilting the structural transitions between two stable states, e. g. hairpin or helix. The growth of the nucleus, the turn conformations, would result in the elimination of structures with disoriented and noninteracting groups and the retention of favorably interacting structured species, leading to the complete optimization of tertiary interactions in a crystal lattice. The present model system provides an alternative to understand the factors responsible for the structure formation, hence could provide clues to the optimization of protein design strategy. The investigation by MD simulation of a designed peptide, that folds into a β-hairpin, is the first step in addressing the question of polypeptide chain folding via the appropriate stereochemical control of constituting amino acid residues.

**Supplementary material**

The supplementary material for this paper is available online at http://dx.doi.10.1080/07391102.2012.738612.
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References
Balaram, P. (1999). De novo design: Backbone conformational constraints in nucleating helices and beta-hairpins. The Journal of Peptide Research: Official Journal of the American Peptide Society, 54(3), 195–199.
Bernard, E., Buckley, V., Momar, E., Coleman, L., Meade, G., Kenny, D., & Devocelle, M. (2012). Bioorganic & medicinal chemistry letters inhibition of platelet adhesion by peptidomimetics mimicking the interactive β-hairpin of glycoprotein ib A. Bioorganic & Medicinal Chemistry Letters, 22(9), 3323–3326.
Blanco, F. J., Rivas, G., & Serrano, L. (1994). A short linear peptide that folds into a native stable beta-hairpin in aqueous-solution. Nature Structural Biology, 1(9), 584–590.
Chalmers, D. K., & Marshall, G. R. (1995). Pro-D-{Nme-Amino} acid and D-Pro-{Nme-Amino} acid: Simple, efficient reverse-turn constraints. Journal of the American Chemical Society, 117(22), 5927–5937.
Chung, D. M., Dou, Y., Baldi, P., & Nowick, J. S. (2005). The absence of favorable aromatic interactions between beta-sheet peptides. Journal of the American Chemical Society, 127(29), 9998–9999.
Culik, R. M., Jo, H., Degrado, W. F., & Gai, F. (2012). Using thioamides to site-specifically interrogate the dynamics of hydrogen bond formation in beta-sheet folding. Journal of the American Chemical Society, 134(19), 8026–8029.
Degrado, W. F. (1988). Design of peptides and proteins. Advances in Protein Chemistry, 39, 51–124.
Eaton, W. A., Munoz, V., Hagen, S. J., Jas, G. S., Lapidus, L. J., Henry, E. R., & Hofrichter, J. (2000). Fast kinetics and mechanisms in protein folding. Annual Review of Biophysics and Biomolecular Structure, 29, 327–359.
Eaton, William A., Muñoz, V., Thompson, P. A., Henry, E. R., & Hofrichter, J. (1998). Kinetics and dynamics of loops, α-helices, β-hairpins, and fast-folding proteins. Accounts of Chemical Research, 31(11), 745–755.
Espinosa, J. F., & Gellman, S. H. (2000). A designed beta-hairpin containing a natural hydrophobic cluster. Angewandte Chemie-International Edition, 39(13), 2330–2333.
Favre, M., Moehle, K., Jiang, L., Pfeiffer, B., & Robinson, J. A. (1999). Structural mimicry of canonical conformations in antibody hypervariable loops using cyclic peptides containing a heterochiral dipeptide template. Journal of the American Chemical Society, 121, 2679–2685.
Gellman, S. H. (1998). Minimal model systems for beta sheet secondary structure in proteins. Current Opinion in Chemical Biology, 2(6), 717–725.
Gunasekaran, K., Ramakrishnan, C., & Balaram, P. (1997). Beta-hairpins in proteins revisited: Lessons for De novo design. Protein Engineering, 10(10), 1131–1141.
Haque, T. S., Little, J. C., & Gellman, S. H. (1996). Stereochemical requirements for beta-hairpin formation: Model studies with four-residue peptides and depsipeptides. Journal of the American Chemical Society, 118(29), 6975–6985.
Hughes, R. M., & Waters, M. L. (2006). Model systems for B-hairpins and B-sheets. Current Opinion in Structural Biology, 16, 514–524.
Karle, I. L., & Balaram, P. (1990). Structural characteristics of alpha-helical peptide molecules containing ab residues. Biochemistry, 29(29), 6747–6756.
Karle, I. L., Awasthi, S. K., & Balaram, P. (1996). A designed beta-hairpin peptide in crystals. Proceedings of the National Academy of Sciences of the United States of America, 93 (16), 8189–8193.
Karle, I., Gopi, H. N., & Balaram, P. (2002). Infinite pleated beta-sheet formed by the Beta-hairpin Boc-Beta-Phe-Beta-Phe-D-Pro-Gly-Beta-Phe-Beta-Phe-Ome. Proceedings of the National Academy of Sciences of the United States of America, 99(8), 5160–5164.
Kaul, R., & Balaram, P. (1999). Stereochemical control of peptide folding. Bioorganic & Medicinal Chemistry, 7(1), 105–117.
Lacroix, E., Kortemme, T., De La Paz, M. L., & Serrano, L. (1999). The design of linear peptides that fold as monomeric beta-sheet structures. Current Opinion in Structural Biology, 9(4), 487–493.
Laio, A., & Gervasio, F. L. (2008). Metadynamics: A method to simulate rare events and reconstruct the free energy in biophysics, chemistry and material science. Reports on Progress in Physics, 71(12), 126601.
Lewandowska, A., Oldziej, S., Liwo, A., & Scheraga, H. A. (2010). Mechanism of formation of the C-terminal beta-hairpin of the B3 domain of the immunoglobulin binding protein G from streptococcus. III. Dynamics of long-range hydrophobic interactions. Proteins-Structure Function and Bioinformatics, 78(3), 723–737, doi: 10.1002/prot.22605.
Mahalakshmi, R., Raghothama, S., & Balaram, P. (2006). NMR analysis of aromatic interactions in designed peptide. Journal of the American Chemical Society, 128, 1125–1138.
Munoz, V., Thompson, P. A., Henry, E. R., Hofrichter, J., & Eaton, W. A. (1998). Folding dynamics of a beta-hairpin studied by laser temperature jump and kinetic modelling. Biophysical Journal, 74(2, Part 2), A175.
Munoz, V., Henry, E. R., Hofrichter, J., & Eaton, W. A. (1998a). A statistical mechanical model for beta-hairpin kinetics. Proceedings of the National Academy of Sciences of the United States of America, 95(11), 5872–5879.
Munoz, V., Thompson, P. A., Hofrichter, J., & Eaton, W. A. (1997). Folding dynamics and mechanism of beta-hairpin formation. Nature, 390(6656), 196–199.
Nair, C. M., Vijayan, M., Venkatachalapathi, Y. V., & Balaram, P. (1979). X-Ray crystal structure of pivaloyl-D-Pro-L-Pro-L-Ala-N-Methylamide; observation of a consecutive β-turn conformation. Journal of the Chemical Society, Chemical Communications, 24, 1183–1184.
Rai, R., Raghothama, S., & Balaram, P. (2006). Design of a peptide hairpin containing a central three-residue loop. Journal of the American Chemical Society, 128(8), 2675–2681.
Richardson, J. S., & Richardson, D. C. (2002). Natural beta-sheet proteins use negative design to avoid edge-to-edge aggregation. Proceedings of the National Academy of Sciences of the United States of America, 99(5), 2754–2759.
Robinson, J. A. (2000). The design, synthesis and conformation of some new beta-hairpin mimetics: Novel reagents for drug and vaccine discovery. Synlett, 4, 429–441.
Robinson, John A. (2008). Beta-hairpin peptidomimetics: Design, structures and biological activities. Accounts of Chemical Research, 41(10), 1278–1288.
Robinson, John A. (2009). Design of protein-protein interaction inhibitors based on protein epitope mimetics. ChemBioChem, 10(6), 971–973.

Robinson, J. A., Demarco, S., Gombert, F., Moehle, K., & Oberrecht, D. (2008). The design, structures and therapeutic potential of protein epitope mimetics. Drug Discovery Today, 13(21–22), 944–951.

Rose, G. D., Gierasch, L. M., & Smith, J. A. (1985). Turns in peptides and proteins. Advances in Protein Chemistry, 37, 1–109.

Roy, R. S., Gopi, H. N., Raghothama, S., Karle, I. L., & Balaram, P. (2006). Hybrid peptide hairpins containing alpha- and omega-amino acids: Conformational analysis of decapeptides with unsubstituted beta-, gamma-, and delta-residues at positions 3 and 8. Chemistry—A European Journal, 12(12), 3295–3302.

Sibanda, B. L., Blundell, T. L., & Thornton, J. M. (1989). Conformation of beta-hairpins in protein structures: A systematic classification with applications to modelling by homology, electron density fitting and protein engineering. Journal of Molecular Biology, 206(4), 759–777.

Sibanda, B. L., & Thornton, J. M. (1985). Beta-hairpin families in globular-proteins. Nature, 316(6024), 170–174.

Smith, C. K., & Regan, L. (1997). Construction and design of beta-sheets. Accounts of Chemical Research, 30(4), 153–161.

Sowdhamini, R., Srinivasan, N., Ramakrishnan, C., & Balaram, P. (1992). Orthogonal beta beta motifs in proteins. Journal of Molecular Biology, 223(4), 845–851.

Spath, J., Stuart, F., Jiang, L. Y., & Robinson, J. A. (1998). Stabilization of a beta-hairpin conformation in a cyclic peptide using the templating effect of a heterochiral diproline unit. Helvetica Chimica Acta, 81(9), 1726–1738.

Toniolo, C., & Benedetti, E. (1991). The polypeptide 310-Helix. Trends in Biochemical Sciences, 16(9), 350–353.

Venkataraman, J., Shankaramma, S. C., & Balaram, P. (2001). Design of folded peptides. Chemical Reviews, 101(10), 3131–3152.