Preface

The study of synthetic peptides aid in improving our current understanding of the fundamental principles for the *de novo* design of functional proteins. The investigation of designed peptides has been instrumental in providing answers to many questions ranging from the conformational preferences of amino acids to the compact folded structures and also in developing tools for understanding the growth and formation of the protein secondary structures (helices, sheets and turns). In addition, the self-assembly of peptides through non-covalent interactions is also an emerging area of growing interest. The design of peptides which can mimic the protein secondary structures relies on the use of stereochemically constrained amino acid residues at select positions in the linear peptide sequences, leading to the construction of protein secondary structural modules like helices, hairpins and turns. The use of non-coded amino acid residues with strict preferences for adopting particular conformations in the conformational space becomes the most crucial step in peptide design strategies. In addition the crystallographic characterization and analysis of the sequences provides the necessary optimization of the design strategies. The crystallographic characterization of designed peptides provides a definitive and conclusive proof of the success of a design strategy. Furthermore, the X-ray structures provide an atomic view of the interactions, both strong and weak, which govern the growth of the crystal. The information on the geometric parameters and stereochemical properties of a series of peptides, through a systematic study, provides the necessary basis for further scientific investigation, like the molecular dynamics and can also aid in improving the force field parameters meant for carrying out molecular simulations. This can be further complemented by constructing biologically active peptide sequences.

The focus of this thesis is to characterize crystallographically the conformational and structural aspects of peptide nanotubes and encapsulated water wires and the β-hairpin peptide models of β-sheets. The systematic study of a series of pentapeptide and octapeptide sequences, containing Aib and D-amino acid residues incorporated at strategic positions, establish the conformation and structural properties of designed peptides as mimics of protein secondary structures and hydrophobic tubular peptide channels and close-packed forms.

The structures reported in this thesis are given below:

1. Boc-\textsuperscript{D}Pro-Aib-Leu-Aib-Val-OMe (DPUL5) \(C_{20}H_{33}N_5O_8\)
2. Boc-\textsuperscript{D}Pro-Aib-Val-Aib-Val-OMe (DPUV5a) \(C_{29}H_{51}N_5O_8\cdot(0.5)H_2O\)
The crystal structure determination of the peptides presented in this thesis provides a wealth of information on the folding patterns of the sequences, in addition to the characterization of many structural and geometric properties. In particular, the study sheds light on the growth and formation of peptide nanotubes and the structure of encapsulated water wires, and also the structural details of Type I' and Type II' β-turn nucleated hairpins. The study provides the backbone and side chain conformational parameters of the sequences, highlighting the varied conformational excursions possible in the peptide molecules.

The thesis is divided into 6 chapters and one appendix.

**Chapter 1** gives a general introduction to the stereochemistry of the polypeptide chain, description of backbone torsion angles of α-amino acid residues and the major secondary structures of α-peptides, namely α-helix, β-sheet and β-turns. The basic structural features of
helices and sheets are given. A brief introduction to polymorphism and weak interactions is also presented, followed by a discussion on X-ray diffraction and solution to the phase problem.

Chapter 2 is divided into two parts. PART 1 describes the crystal structures of a series of eight related enantiomeric peptide sequences (Raghavender et al., 2009; Raghavender et al., 2010). The crystal structures of four sequences with the general formula Boc-Pro-Aib-Xxx-Aib-Val-OMe (Xxx = Ala/Val/Leu/Phe) and the enantiomeric sequences provided a set of crystal structures with different packing arrangements. The structure of the peptide with Xxx = Leu revealed a nanotube formation with the Leu lining the inner walls of channel. The channels were found to be empty. The sequence with Xxx = Val revealed a solvent-filled water channel. Investigation of the water wire structures on the diffraction data collected on the same crystal over a period of time revealed the existence of two different kinds of water wires in the channels. Comparison with the peptide tubular structures available in the literature and the water structure inside the aquaporin channels are contrasted. Close-packed structures are observed in the case of Xxx=Ala and Phe. The backbone conformations are essentially identical. Enantiomeric sequences also revealed similar structures. Polymorphic forms were observed in the case of DVal(3) containing sequence. One form is observed to have water-filled channels forming a nanotube, as opposed to the close-packed structure in the polymorphic form.

Crystal parameters

DPUL5: \( C_{30}H_{53}N_{5}O_{8} \); \( P6_3 \); \( a = b = 24.3673 \) \( \text{Å} \), \( c = 10.6844 \) \( (13) \) \( \text{Å} \); \( \alpha = \beta = 90^\circ \), \( \gamma = 120^\circ \); \( Z = 6 \); \( R = 0.0671 \), \( wR_2 = 0.1446 \).

DPUV5a: \( C_{29}H_{51}N_{5}O_{8} \cdot (0.5) \) \( H_2O \); \( P6_5 \); \( a = b = 24.2920 \) \( (13) \) \( \text{Å} \), \( c = 10.4838 \) \( (11) \) \( \text{Å} \); \( \alpha = \beta = 90^\circ \), \( \gamma = 120^\circ \); \( Z = 6 \); \( R = 0.0554 \), \( wR_2 = 0.1546 \).

DPUV5b: \( C_{29}H_{51}N_{5}O_{8} \cdot (0.17) \) \( H_2O \); \( P6_5 \); \( a = b = 24.3161 \) \( (3) \) \( \text{Å} \), \( c = 10.1805 \) \( (1) \) \( \text{Å} \); \( \alpha = \beta = 90^\circ \), \( \gamma = 120^\circ \); \( Z = 6 \); \( R = 0.0617 \), \( wR_2 = 0.1844 \).

DPUA5: \( C_{27}H_{47}N_{5}O_{8} \); \( P2_12_12_1 \); \( a = 12.2403 \) \( (8) \), \( b = 15.7531 \) \( (11) \) \( \text{Å} \), \( c = 16.6894 \) \( (11) \) \( \text{Å} \); \( Z = 4 \); \( R = 0.0439 \), \( wR_2 = 0.1249 \).

DPUF5: \( C_{33}H_{48}N_{5}O_{8} \); \( P2_12_12_1 \); \( a = 10.3268 \) \( (8) \), \( b = 18.7549 \) \( (15) \) \( \text{Å} \), \( c = 18.9682 \) \( (16) \) \( \text{Å} \); \( Z = 4 \); \( R = 0.0472 \), \( wR_2 = 0.1325 \).
PART 2 describes the crystallographic characterization of the tubular structure in a tripeptide sequence. The arrangement of the single-file water molecules in the peptide nanotubes of FPW could be established by X-ray diffraction. In addition, the energetically favoured arrangement of the water wire inside the peptide channels could be modeled by understanding the construction of the peptide nanotube. In particular, the helical macrodipole of the peptide nanotube and the water wire dipoles prefer an antiparallel arrangement inside the peptide channels as opposed to parallel arrangements, is established by the classical dipole-dipole interaction energy calculation. In addition, the growth of the nanotubes and the arrangement of the water wires inside the channels could be correlated to the macroscopic dimensions of the crystal by the indexing of the crystal faces and contrasted with the structure of DPUV5.

Crystal parameters

FPW: $C_{28}H_{32}N_4O_6\cdot(0.33)H_2O$; $P6_1$; $a = b = 21.5674$ (3) Å, $c = 10.1035$ (2) Å; $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$; $Z = 6$; $R = 0.0786$, $wR_2 = 0.1771$.

Chapter 3 provides the crystal structures of five octapeptide $\beta$-hairpin forming sequences and a tripeptide containing a modified amino acid, with modification in the side chain (pseudo-proline, $\psi^{H,CH_3}$Pro). The parent peptide, Boc-Leu-Phe-Val-$^{D}$Pro-Pro-Leu-Phe-Val-OMe (DPLP8), was observed to form a strong Type II' $\beta$-turn at the $^{D}$Pro-Pro segment, and the strand segments adopting a $\beta$-sheet conformation. Two molecules were observed in the asymmetric unit, inclined to each other at approximately 70°. Modification in the strand sequence Phe(2) to Tyr(2) also resulted in a hairpin with identical conformation and similar
packing arrangement. The difference was in the solvent content. In both the cases the molecules were packed orthogonal with respect to each other, resulting in the formation of ribbon-like structures in three dimensions. The replacement of Phe(2) and Phe(7) with Valine residues, with the retention of DPro-Pro β-turn segment, results in an entirely different packing arrangement (parallel). Modification of Pro(5) residue of the turn segment to Aib(5) and ψPro, also results in the molecules packing orthogonally to each other. The tripeptide with a modified form of ψPro, namely ψH,CH3Pro, resulted in a folded structure with a Type VIa β-turn, with the amide bond between the Pro-ψH,CH3Pro segment adopting a cis configuration (Kantharaju et al., 2009).

Crystal parameters

DPLP8: $C_{56}H_{84}N_{8}O_{11} \cdot (0.5)H_2O$; $P\overline{2}_1$; $a = 14.4028$ (8), $b = 18.9623$ (11) Å, $c = 25.4903$ (17) Å, $\beta = 105.674^\circ$ (4); $Z = 4$; $R = 0.0959$, $wR_2 = 0.2251$.

YDPP8: $C_{56}H_{84}N_{8}O_{12} \cdot (1.5)H_2O$; $P2_12_12_1$; $a = 14.4028$ (8), $b = 18.9623$ (11) Å, $c = 25.4903$ (17) Å, $Z = 8$; $R = 0.0989$, $wR_2 = 0.2064$.

PSIP8: $C_{57}H_{86}N_{8}O_{11}S_1 \cdot (1.5)H_2O$; $C2$; $a = 34.6080$ (2), $b = 15.3179$ (10) Å, $c = 25.6025$ (15) Å, $\beta = 103.593^\circ$ (3); $Z = 4$; $R = 0.0931$, $wR_2 = 0.2259$.

DPPV8: $C_{48}H_{84}N_{8}O_{11}$; $P1$; $a = 9.922$ (3), $b = 11.229$ (4) Å, $c = 26.423$ (9) Å, $\alpha = 87.146$ (6), $\beta = 89.440^\circ$ (6), $\gamma = 73.282$ (7); $Z = 2$; $R = 0.1058$, $wR_2 = 0.2354$.

DPUF8: $C_{57}H_{88}N_{8}O_{11}S_1 \cdot (1.5)H_2O$; $P2_1$; $a = 18.410$ (2), $b = 23.220$ (3) Å, $c = 19.240$ (3) Å, $\beta = 118.036^\circ$ (4); $Z = 4$; $R = 0.1012$, $wR_2 = 0.2061$.

PSPL3: $C_{22}H_{37}N_{3}O_{5}S_1$; $P3_1$; $a = b = 14.6323$ (22), $c = 10.4359$ (22) Å, $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$; $Z = 3$; $R = 0.0597$, $wR_2 = 0.1590$.

Chapter 4 describes the crystal structure and molecular conformation of Type I’ β-turn nucleated hairpin. The incorporation of Aib-DPro segment in the middle of Leu-Val-Val strands in the peptide sequence Boc-Leu-Val-Val-Aib-DPro-Leu-Val-Val-OMe results in an obligatory Type I’ turn containing hairpin. The molecular conformation and the packing arrangement of the molecules in the crystal are contrasted with the only Type I’ β-hairpin reported in the literature and with a sequence where the turn residues are flipped and strand residues replaced with Phe(2) and Phe(7).

Crystal parameters

UDPV8: $C_{47}H_{84}N_{8}O_{11} \cdot 2(C_3H_7NO)$; $P2_1$; $a = 11.0623$ (53), $b = 18.7635$ (89) Å, $c = 16.6426$ (80) Å, $\beta = 102.369$ (8); $Z = 2$; $R = 0.0947$, $wR_2 = 0.1730$. 
Chapter 5 provides the crystal structures of three polymorphic forms of β-hairpins. The structure of BH1P8 provides new insights into the packing of hairpins inclined orthogonally to each other. The two polymorphic forms differ not only in their modes of packing in crystals but also in the strong and weak interactions stabilizing the packing arrangements. The polymorphic forms of DPUFP8 differ only in the content of the solvent in the asymmetric unit and the role it plays in bridging the symmetry related pairs of molecules. The polymorphic form YDPPP8 crystallized in a completely different space group, revealing a completely different mode of packing and also the cocrystallized solvent participating in a different set of interactions.

Crystal parameters
BH1P8: \( C_{54}H_{78}N_8O_{11}.H_2O; P2_1; a = 18.7511 \text{ (9), } b = 23.3396 \text{ (11) Å}, c = 28.1926 \text{ (13) Å}; Z = 8; R = 0.1208, wR_2 = 0.2898. \)

DPUFP8: \( C_{55}H_{84}N_8O_{11}. (0.5) H_2O; P2_1; a = 18.0950 \text{ (4), } b = 23.0316 \text{ (5) Å}, c = 18.6368 \text{ (5) Å}, \beta = 117.471 \text{ (2)}; Z = 4; R = 0.0915, wR_2 = 0.2096. \)

YDPPP8: \( C_{56}H_{83}N_8O_{12}. (1.5) H_2O; P2_1; a = 14.3184 \text{ (8), } b = 18.9924 \text{ (9) Å}, c = 25.1569 \text{ (14) Å}, \beta = 105.590 \text{ (4)}; Z = 4; R = 0.1249, wR_2 = 0.2929. \)

Chapter 6 provides a comprehensive overview of the β-hairpin peptide crystal structures published in the literature as well as those included in the thesis. The hairpins are classified based on the residues composing the β-strands and the mode of their packing in the crystals. In the crystal structures the hairpins are observed to adopt either a Type II’ or Type I’ β-turns. The indexing of the crystal faces of a few representative hairpin peptides crystallographically characterized in this thesis, provides a rational explanation for the preferential growth of the crystals in certain directions, when correlated with the strong directional forces (hydrogen bonding) and weak interactions (van der Waals, aromatic-aromatic) observed in the crystal packing. The insights gained by these studies would be highly valuable in understanding the nucleation and growth of β-hairpin peptides and the formation of β-sheet structures.

Appendix I describes the Cambridge Structural Database (CSD) analysis of the conformational preferences of the proline residues found in the peptide crystal structures. The frequency distributions of the backbone \( \phi, \psi \) and \( \omega \) and side chain \( \chi^1, \chi^2, \chi^3, \chi^4 \) and \( \theta \) torsion angles of the proline residues are calculated, tabulated and represented as graphical plots. The correlation between the backbone and endocyclic torsion angles provides for a clear evidence
of the role of a particular torsion variable $\chi^2$ in deciding the state of puckering. In addition, the endocyclic bond angles also appear to be correlated, relatively strongly, with the $\chi^2$ torsion. This provides a geometrical explanation of the factors governing the puckering of the proline ring.

List of Publications

a) In Journals


   Kantharaju, S. Raghothama, **U.S. Raghavender**, S. Aravinda, N. Shamala and P. Balaram

3. Hydrophobic peptide channels and encapsulated water wires.
   **U. S. Raghavender**, Kantharaju, S. Aravinda, N. Shamala and P. Balaram

4. Peptide hairpin nucleation with the obligatory Type I’ $\beta$-turn Aib-$^D$Pro segment
   **U. S. Raghavender**, S. Aravinda, R. Rai, N. Shamala and P. Balaram

   **U. S. Raghavender**, B. Chatterjee, I. Saha, N. Shamala and P. Balaram (Communicated)

b) Manuscripts Under Preparation

1. The Use of Diproline Templates as Models for $\beta$-Hairpin Nucleation. Implications for the Growth of $\beta$-Sheet like Structures (in preparation)

c) Conference Presentations

1. X-ray crystallographic investigation of β-hairpin peptides.  
   **U. S. Raghavender**, S. Aravinda, R. Rai, P. Balaram and N. Shamala  
   36th National Seminar on Crystallography, Chennai, India (2007)

d) Participation in workshops

1. **CCP4 workshop on Computational Crystallography.**  

2. **Workshop on Cryo-Electron Microscopy of Biological Substances.**  
   Indian Institute of Science, May 2-6, 2005.