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Nanopeptides: Non - Covalent Interactions in Chemistry and Biological Functions

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Abstract

The interactions involving the side chains of weakly polar aromatic amino acid residues, e.g., Phenylalanine (Phe), Tyrosine (Tyr) and Tryptophan (Trp) generally reside at the interior of proteins and help in the stabilization of globular protein structures. The aromatic electron cloud of the aromatic rings of these amino acids are delocalized on both sides of the planer rings, so that there is a small partial negative charge on the face and a small partial positive charge on the hydrogen atoms of the edge, which leads to the possibility of electrostatic interactions. These interaction play a vital role nanofiber based vaccine adjuvants, and cocaine vaccine development and increasing interest and structure based drug development. Apart from electrostatic forces, aromatic interactions also consist of van der Waals and hydrophobic forces. These weakly polar interactions are enthalpically comparable to a hydrogen bond. Protein engineering methods have revealed that introducing aromatic pairs and aromatic clusters increases the thermal stability of proteins and it has been demonstrated that the introduction of an additional aromatic interaction improved the thermophilicity and thermostability of the family of 11 xylanase. These weakly polar interactions also have a significant role in the stability of DNA. Different types of weakly polar interactions involving the aromatic side chains are discussed below.

Keywords: Aromatic interactions; Peptide; Helix; β – sheet; Dynamics; Folding

π…π Interaction

The weakly polar nature of aromatic residues leads to π…π interactions wherein the positively polarized hydrogen atoms of one ring can interact with the δ^- π-electron cloud of a second aromatic ring. In 1985, Burley and Petsko observed, "that on an average about 60% of aromatic side chains in proteins are involved in aromatic pairs, 80% of which form networks of three or more interacting aromatic side chains [1-6]. Phenyl ring centroids are separated by a preferential distance of between 4.5 Å and 7 Å, and dihedral angles approaching 90° are most common"

This π…π interaction has a quadrupole - quadrupole character (distance dependence ~ $1/r^5$). Subsequently, a large number of analyses of aromatic π…π interactions in the context of peptides as well as in proteins have re-emphasized the importance of aromatic residues in the stability of structures and they have pointed out the occurrence of several potential orientations of closely packed aromatic rings (Figure 1). The π…π interactions contribute free energy between -0.6 kcal.mol^{-1} and 1.3 kcal.mol^{-1} towards the stability of protein structures. It has been suggested from the experiments as well as from the theoretical analysis that van der Waals and the electrostatic forces play important roles in the stability of π…π interactions.

Aromatic Interactions in Synthetic Peptides

One of the earliest monomeric β - hairpins to be examined was derived from the B1 subunit of protein G1. The characteristic stabilizing agent of this peptide was the 'hydrophobic core' constituted by three aromatic residues, namely, Tyr, Phe and Trp, along with the apolar Val residue. A noticeable feature was also the strong Phe-Tyr interaction at the first non-hydrogen bonding position following the turn, stabilizing the strand segments (Figure 2). Several peptide hairpins have subsequently been designed using aromatic amino acids as stabilizing agents. One of the most investigated of the designed peptides is the 'Trpzip' sequences. These tryptophan zipper peptides are 16 residues in length and contain two pairs of interacting Trp residues that interact in a T-shaped manner (Figure 3).

These interactions not only stabilize the peptide but also confer protein-like properties to the sequence, including a high Tm of 79°C and requiring high concentrations of urea and guanidine hydrochloride for denaturation. Other studies on peptide hairpins containing interacting Phe residues clearly indicate that the energy of interaction

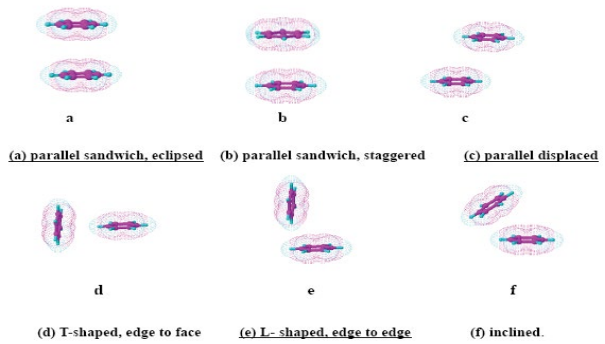

Figure 1: Possible models of aromatic interaction in benzene dimer. Aromatic pairs calculated with a fixed centroid-centroid distance of 5.5 Å.

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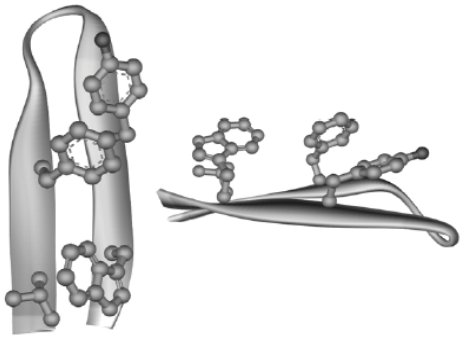

Figure 2: Structure of residues 42 - 56 of the B1 subunit of staphylococcal protein G in the solid state, determined using X-ray crystallography, showing a strong cluster of hydrophobic residues required for stability of the hairpin. This aromatic interaction was also shown to be preserved in the isolated hairpin, using solution NMR studies. Two views of the hairpin are shown (PDB code: 1PGA).

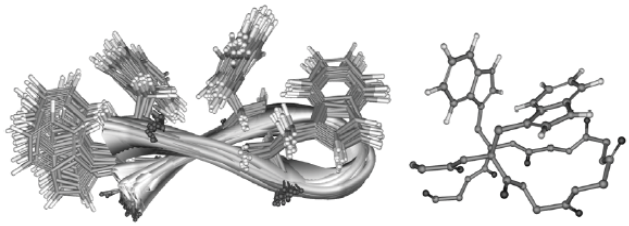

Figure 3: (Left) Superposition of 20 best structures calculated for the tryptophan zipper peptide using NMR (PDB code: 1LE0) [13,14]. Two pairs of strong aromatic interactions at the non-hydrogen bonding position of the hairpin were found to stabilize the structure and impart protein-like properties to the 16-residue peptide. (Right) Illustration of the T-shaped aromatic interaction in 1LE0, using the Trp residues immediately after the turn as an example.

Note that the T-shaped interaction is also present in the second aromatic pair towards the termini.

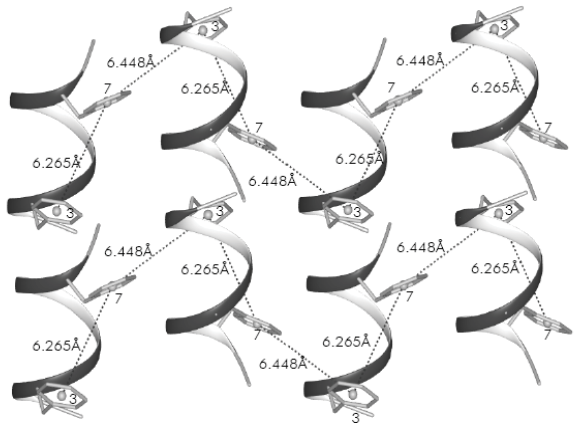

Figure 4: Example of aromatic interactions in peptides. Figure adapted from Aravinda [16]. Interaction between Phe3 and Phe7 in the peptide was observed in the crystal. Centroid - centroid distances are marked.

of Phe-Phe pairs is about -0.5 kcal/mol compared to its Cha analog [7-9]. A study comparing aromatic interactions with salt bridges, demonstrated that the removal of an aromatic ring caused significant hairpin destabilization whereas the replacement of a salt bridge did not cause any major change in peptide stability [10-12]. In all these peptides, a consistent edge-to-face geometry was observed indicating that this interaction was preferred despite the conformational flexibility allowed for the side chains of aromatic residues.

Aromatic interactions have also been demonstrated in helical peptides. In these cases, however, the interactions are largely intermolecular, as against the intramolecular stabilization provided by interacting aromatic pairs in hairpins. For example, several reports of crystal structures of peptide helices exist wherein aromatic interactions aid in three-dimensional propagation of crystals (Figure 4) [13-15]. Interestingly, such interactions are also found to largely populate the T-shaped geometries and very few examples exist wherein strong stacking interactions between side chains of Phe / Trp / Tyr are seen (Figure 5). In another study, an interaction between residues at the 'i' and 'I + 4 / 5' residues was implicated in stabilizing helical structures. Such interactions are uncommon in peptides, but are of greater incidence in proteins. In a recent study, it was shown that aromatic interactions do not contribute significantly towards peptide stability. This observation was also supported by a previous investigation, which revealed that aliphatic - aromatic interactions are more stabilizing than aromatic pairs in peptides. The stability observed was of the order (Ile / Val) – Tyr > Phe–Tyr > Tyr–Tyr > Trp-Tyr. Reports, however, do exist in which aromatic interactions serve as structure stabilizing agents and the observation of aromatic interactions might be dependent on the system under investigation, with the twist of the backbone in peptide hairpins also playing a role in the formation of favorable aromatic interactions.

Helix Packing

Preferred modes of aromatic interactions have also been observed in the case of peptides, as has been noticed for proteins and benzene dimers. The possible modes of Trp - Trp interactions are illustrated in Figures 6 and 7. The ability of Trp residues to not only stabilize secondary structure but also involve in interaction with other aromatic systems has been exploited in the design of a peptide with the ability to bind ATP and FAD for specific recognition of ssDNA in solution.

C-H…π Interaction

C-H…π interactions involving the aromatic groups either as donor or as an acceptor serves as a potential contributor to the overall stability of proteins. The interactions between aromatic C-H donor groups and

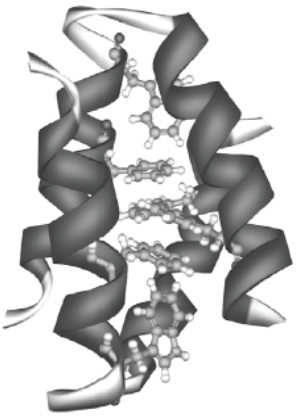

Figure 5: Example of aromatic stacking and edge to face interactions stabilizing a dimeric helix bundle in a *de novo* designed peptide. Aromatic residues have been indicated as ball and stick. Solution structure of this peptide has been solved using NMR (PDB: 1QP6) [17,18].

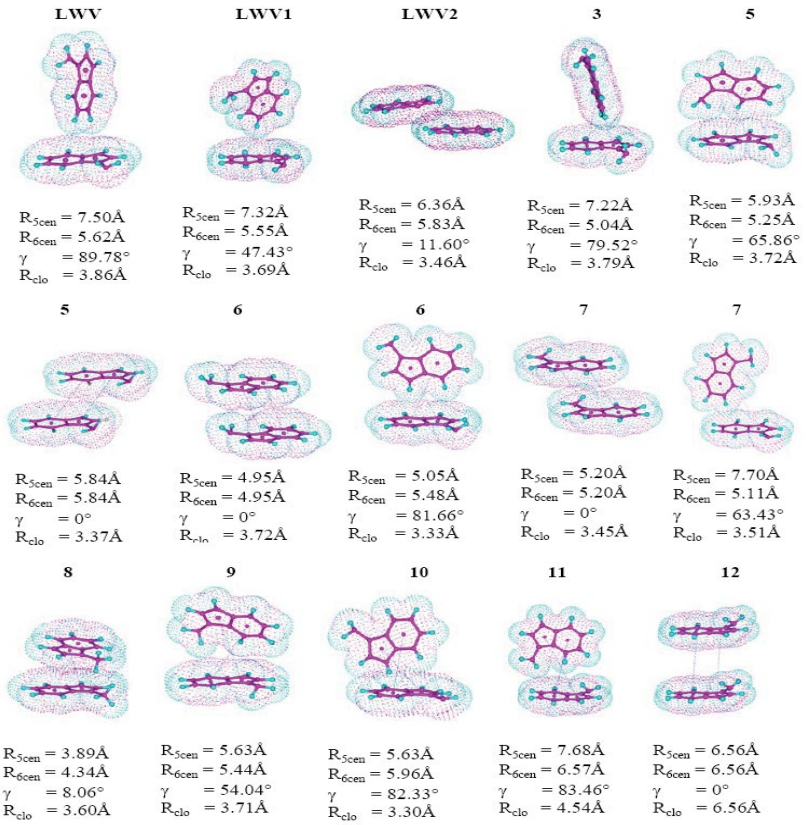

Figure 6: Examples of Trp - Trp interactions in crystal structures of peptides. Distances between the centroids of the 5-membered and 6-membered rings along with their interplanar angles (γ) are indicated below each figure. Distances between the aromatic rings are largely distributed between 5 Å – 7 Å.

aromatic π- acceptors and the interactions between aliphatic C-H donor groups and aromatic π- acceptors are most widely observed in proteins and in peptides. C-H…π interactions involving aromatic π-systems are generally found in the interior of proteins and the overall stabilization energy for potential C-H…π interaction of about 0.5 kcal mol^{-1}- 1.0 kcal mol^{-1} [16-22]. This interaction can play an important role in the molecular recognition and in the binding of DNA or RNA. C-H…π interactions might also be responsible for the stabilization of secondary structures such as α or 3_{10}-helices (Figure 9).

N-H…π Interaction

Burley and Petsko first described N-H…π interaction based on a data set of 33 high resolution (2 Å or better) protein crystal structures (Figure 10). They examined the interactions between side chain amino groups (lysine, arginine, asparagines, glutamine, histidine) and π-acceptors (phenylalanine, tyrosine, tryptophan) and concluded that there was a significant enthalpic contribution towards the protein stability. The N-H…π interactions play a role in the stabilization of peptide and protein structure and protein - ligand binding, which were described experimentally. The interactions between aromatic ring and NH of a nearby amide can be characterized as either aromatic - NH (side chain) or aromatic - NH (backbone). Both these interactions have a quadruple - dipole nature (distance dependence ~ $1/r^4$). The preferred geometry of N-H…π interaction is the one in which the NH group is positioned directly above the ring center. The distance between the N- atom and the ring center is ≤ 3.8 Å. The strength of the energy for N-H…π interaction is of the order of 1 kcal mol^{-1} - 4 kcal mol^{-1} [23].

Amide…π Interaction

Amide…π interactions have been suggested to be an important stabilizing factor of folded structures in proteins. There have been detailed analyses of interactions between the aromatic ring and backbone amide group in proteins and peptide structures. For example, the *ab initio* quantum mechanical calculations using a model system consisting of benzene ring and amide plane have been used to analyze the aromatic - amide interaction. The theoretical studies suggest that aromatic - amide interaction contributes about 1 kcal mol^{-1} - 4 kcal mol^{-1} for the net stabilization (Figure 11).

Cation…π interactions play an important role in the stability of protein structures. These interactions are basically an ion-quadrupole interaction (distance dependence ~ $1/r^3$), which occurs between a positively charged group (ammonium or guanidium group) and the electron rich π-cloud of an aromatic ring. These interactions are energetically favorable in protein structures and the electrostatic interaction energy is calculated to be ~ 3 kcal mol^{-1} [24]. Several studies have been reported on the occurrence of cation…π interactions in the context of protein structures and protein-ligand interactions (Figures 12 and 13) [24].

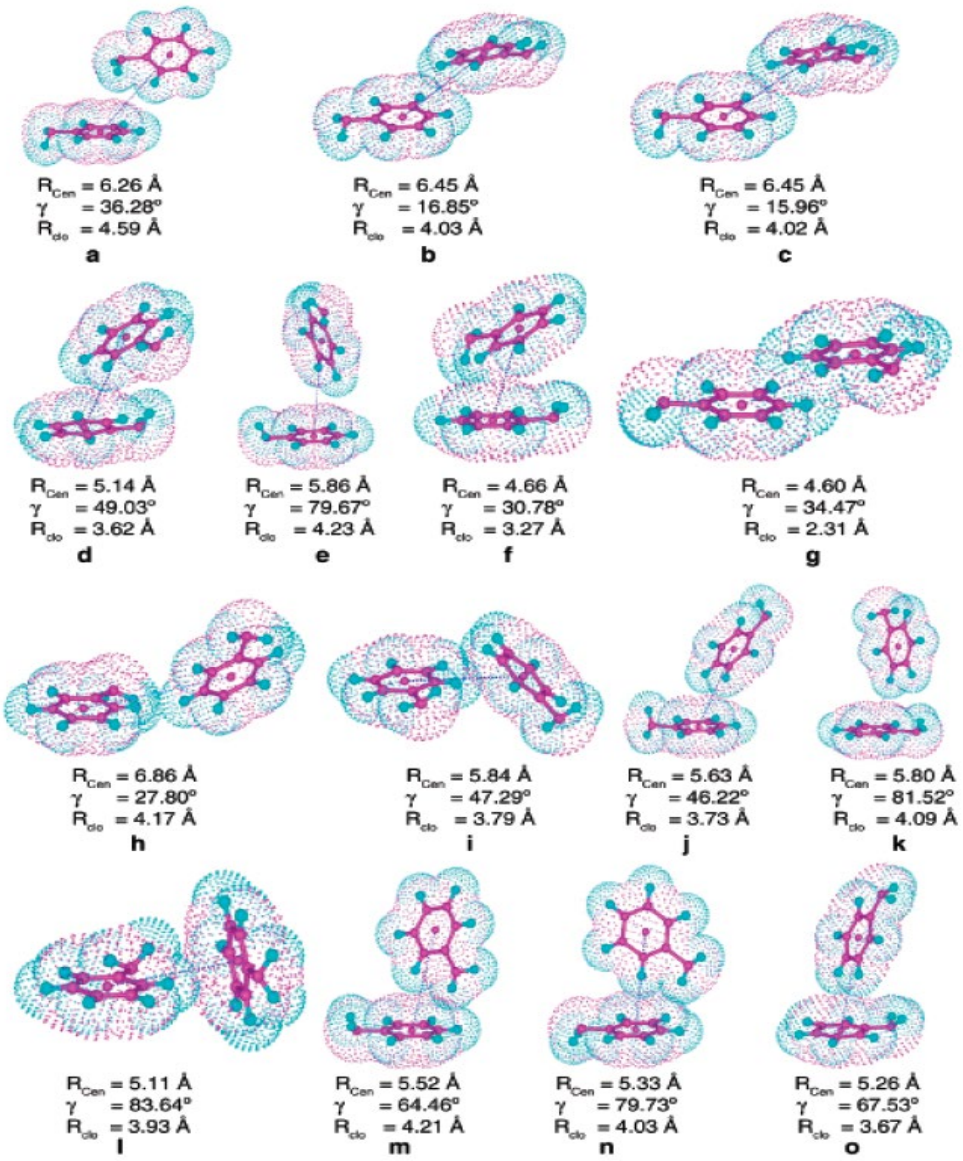

Figure 7: Summary of the unique aromatic-aromatic interactions observed in the crystals of peptides **I-III**. The parameters *R*cen, *ç*, and *R*clo are indicated: (a-c) peptide **I**; (d-k) peptide **II**; (l-o) peptide III. Figure 8 clearly shows van der waals interactions and molecular conformation of the peptides in crystal the benzyl group-the side chain molecular structure packing is attached with hydrogen. All the crystal was grown in water / methanol equimolar combination [19-21].

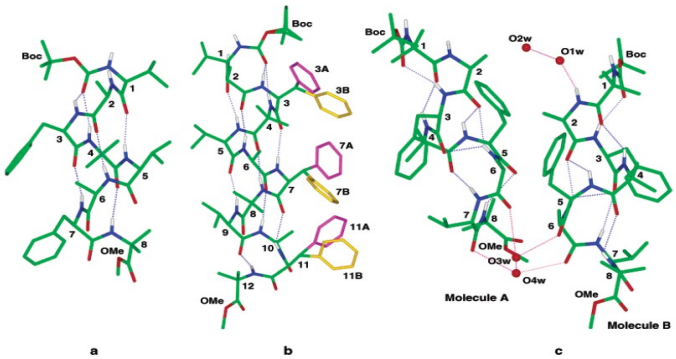

Figure 8: Molecular crystals of peptides I-III. All the hydrogen bonds are shown as dotted lines: (a) I, Boc-Val-Ala-Phe-Aib-Val-Ala- Phe-Aib-OMe; (b) II, Boc-Val-Ala-Phe-Aib-Val-Ala-Phe-Aib-Val-Ala-Phe-Aib-OMe; (c) III, Boc-Aib-Ala-Phe-Aib-Phe-Ala-Val-Aib-OMe.

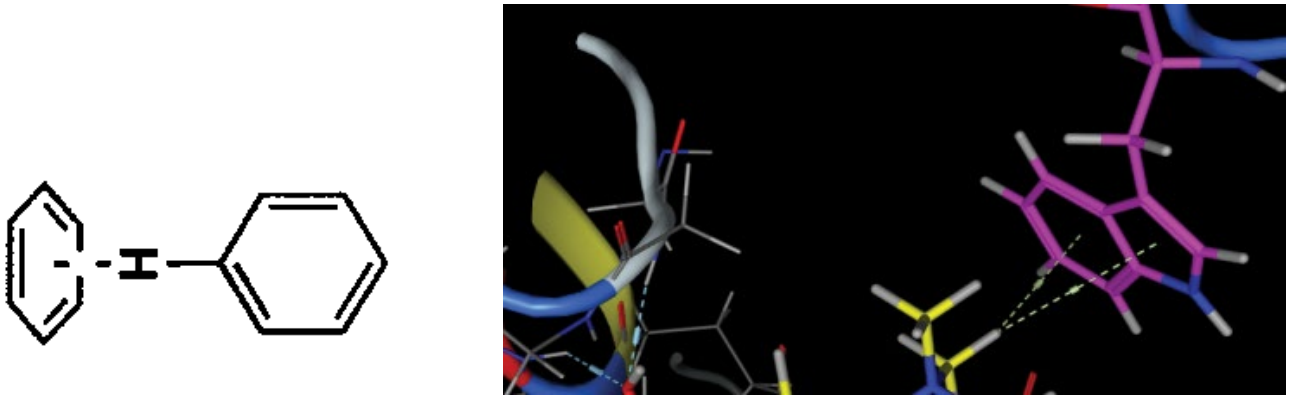

Figure 9: Folded proteins were stabilized by C-H…π interactions.

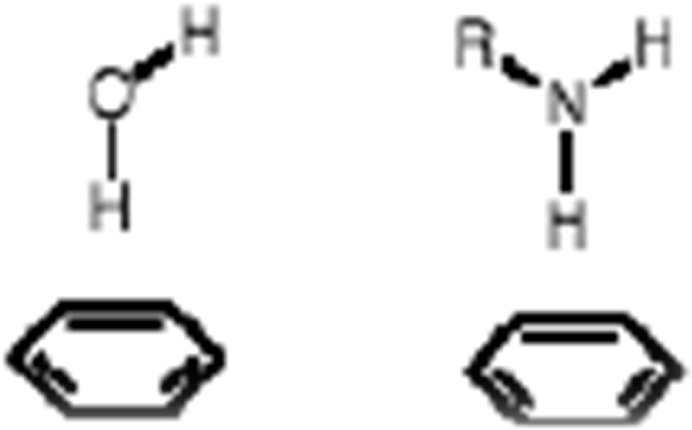

Figure 10: Simple example for the close proximity in benzene water, and benzene and amine.

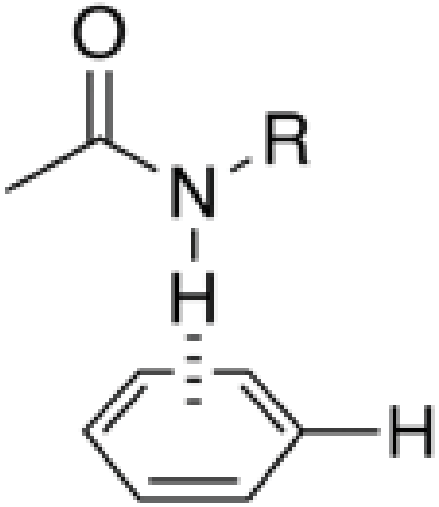

Figure 11: Benzene and Amide interaction.

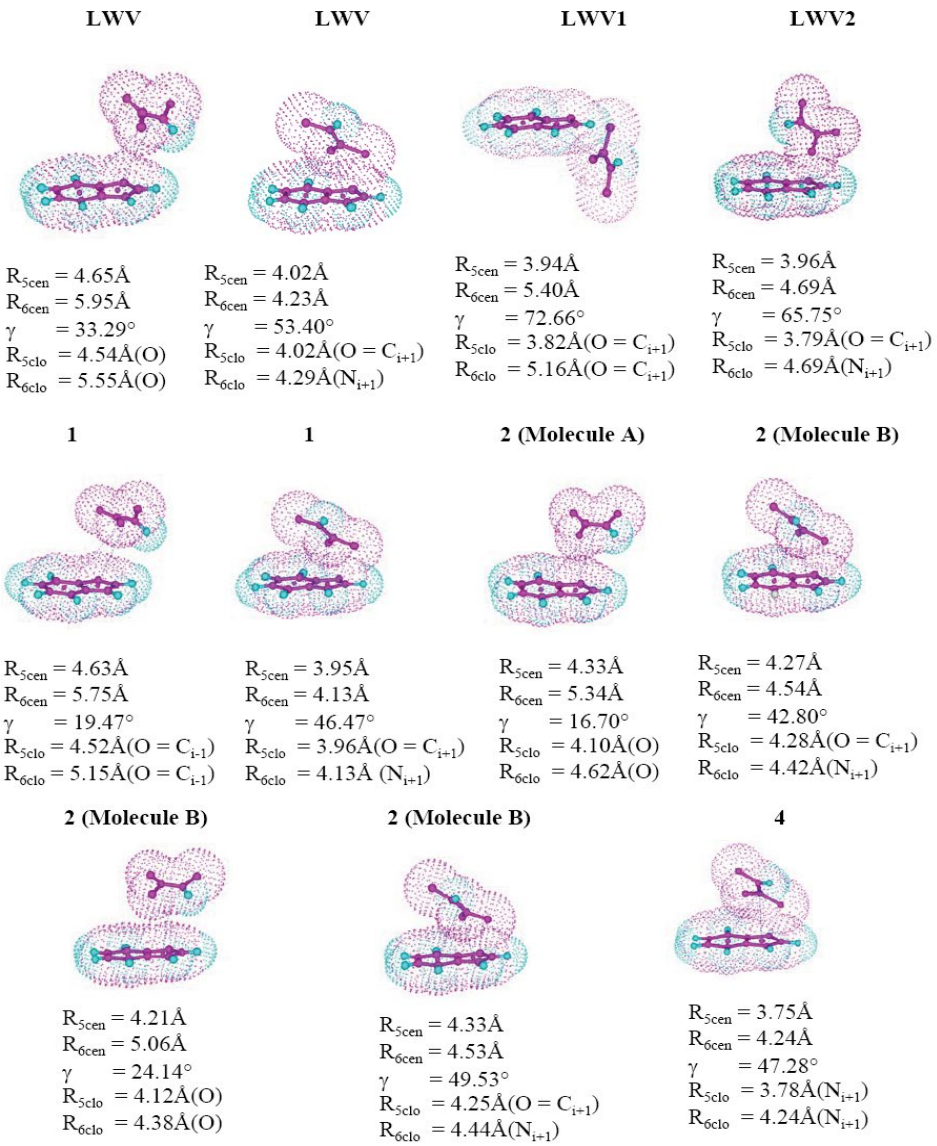

Figure 12: Aromatic - amide interactions observed in the crystals of peptides. The van der Waals surfaces are shown. The parameters R_{5cen}, R_{6cen}, γ and R_{5clo}, R_{6clo} are indicated. Table 1 lists the relevant peptide sequences obtained from Cambridge Structural Database.

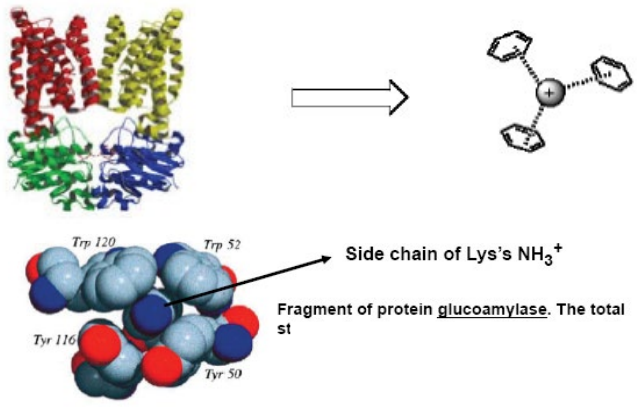

Figure 13: 623 amino acids protein significantly stabilized by Cation…π interactions.

Sl No.	Sequences	Database Identification No.	Role of Indole
1	Boc-Gly-Trp-Ala-OBu	TUPGOA	Aromatic - Amide Interaction
2	Z-Aib-Trp-Aib-OMe (Two Molecules)	ROHVEP	Aromatic - Amide Interaction
3	Z-Aib-Aib-Trp-Aib-OMe	ROHVIT	Aromatic - Aromatic Interaction
4	Boc-Aib-Trp-(Leu-Aib-Ala)2-Phe-Aib-OMe	HICKEJ	Aromatic - Amide Interaction
5	Gly-Trp-dihydrate	GLTRDH01	Aromatic - Aromatic Interaction
6	Ala-Trp monohydrate	FUJZUF	Aromatic - Aromatic Interaction
7	Trp-Gly monohydrate	FULGEY	Aromatic - Aromatic Interaction
8	Leu-Trp-Leu.HCl dihydrate	FUDFUF	Aromatic - Aromatic Interaction
9	Trp-Gly-Gly dihydrate	FIZWOA01	Aromatic - Aromatic Interaction
10	Trp-Gly-Leu	GUBDIQ	Aromatic - Aromatic Interaction
11	Trp-Met-Asp-Phenylalanylamide	GASTRN10	Aromatic - Aromatic Interaction
12	7-methylguanosine-5"-phosphate-Trp-Glu Complex	SEKXIP10	Aromatic - Aromatic Interaction

Table 1: List of Trp peptides from Cambridge Structural Database[a].

[a] The database contained 31 entries with Trp residues. Among them, only acyclic structures with short aromatic - aromatic (centroid - centroid distance between six membered rings of indole ≤ 6.5 Å) and / or aromatic - amide (centroid - centroid distance of five membered ring of indole and amide centroid ≤ 4.5 Å) distances are listed.

References

1. Georis J, Brasseur LJ, Bougnet V, Devreese B, Giannotta F, et al. (2000) An additional aromatic interaction improves the thermostability and thermophilicity of a mesophilic family 11 xylanase: Structural basis and molecular study. Protein Sci 9: 466-475.
2. Rajagopal A, Charles BC, Alexey YK, Joshua DS, Frederick JK, et al. (2015) Enhancing the magnitude of antibody responses through biomaterial stereochemistry. ACS Biomater. Sci Eng 1: 601-609.
3. Koyfman AY, Appavu R, Sheller S, Rudra JS (2015) Self-assembly of heterochiral peptides with varied sequence patterns.
4. Rudra JS, Ye Ding, Neelakantan H, Ding C, Appavu R, et al. (2016) Suppression of cocaine-evoked hyperactivity by self adjuvanting and multivalent peptide nanofiber vaccines.
5. Appavu R, Mohan D (2016) Bortezomib in Anti-Cancer Activity: A Potential Drug. Glob J Cancer Ther 2: 5-8.
6. Rajagopal A, Aravinda S, Raghothama S, Shamala N, Balaram P (2012) Aromatic interactions in model peptide ß-hairpins: Ring current effects on proton chemical shifts. Biopolymers (Peptide Sciences) 98:185-194.
7. Tatko CD, Waters ML (2002) Selective aromatic interactions in beta-hairpin peptides. J Am Chem Soc 124: 9372-9373.
8. Tatko CD, Waters ML (2004) Comparison of C-H...pi and hydrophobic interactions in a beta-hairpin peptide: impact on stability and specificity. J Am Chem Soc 126: 2028-2034.
9. Rajagopal A, Aravinda S, Raghothama S, Shamala N, Balaram P (2011) Chain length effects on helix-hairpin distribution in short peptides with Aid-DAla and Aib-Aib Segments. Biopolymers (Peptide Sciences) 96: 744-756.
10. Kiehna SE, Waters ML (2003) Sequence dependence of beta-hairpin structure: comparison of a salt bridge and an aromatic interaction. Protein Sci 12: 2657-2667.
11. Kiehna SE, Laughrey ZR, Waters ML (2007) Evaluation of a carbohydrate-pi interaction in a peptide model system. Chem Commun (Camb) 39: 4026-4028.
12. Raghavender US, Chatterjee B, Saha I, Rajagopal A, Shamala N, et al. (2011) Entrapment of water wire in a hydrophobic peptide channel with an aromatic lining. J Phys Chem B 115: 9236-9243.
13. Cochran AG, Skelton NJ, Starovasnik MA (2001) Tryptophan zippers: stable, monomeric beta -hairpins. Proc Natl Acad Sci U S A 98: 5578-5583.
14. Appavu R (2016) Expanding the loop Segments in ß-hairpin nano peptides in protein folding and biological functions. Transcriptomics 4: e116.
15. Aravinda S, Shamala N, Das C, Sriranjini A, Karle IL, et al. (2003) Aromatic - aromatic interactions in crystal structures of helical peptide scaffolds containing projecting phenylalanine residues. J Am Chem Soc 125: 5308-5315.
16. Aravinda S (2003) Indian Institute of Science, Bangalore, India.
17. Stewart AL, Waters ML (2009) Structural effects on ss- and dsDNA recognition by a beta-hairpin peptide. Chembiochem 10: 539-544.
18. Appavu R, Mohan D, Kakumanu R, Munisamy G (2016) Fundamental of Secondary Structures in Peptide Based Synthetic Nanovaccine Development. Transcriptomics 4: 131.

19. Mareque Rivas JC, Schwalbe H, Lippard SJ (2001) Interchain hydrogen-bonding interactions may facilitate translocation of K+ ions across the potassium channel selectivity filter, as suggested by synthetic modeling chemistry. Proc Natl Acad Sci USA 98: 9478-9483.

20. Waters ML (2004) Aromatic interactions in peptides: impact on structure and function. Biopolymers 76: 435-445.

21. Krishnayan B, Rajagopal A, Raghothama S, Shamala N, Balaram P (2012) ß-Turn analogues in model aß-hybrid peptides: structural characterization of peptides containing ß^{2,2}Ac6c and ß^{3,3}Ac6c residues. Chem Asian J 7: 1671.

22. Brandl M, Weiss MS, Jabs A, Sühnel J, Hilgenfeld R (2001) C-H...pi-interactions in proteins. J Mol Biol 307: 357-377.

23. Ringer AL, Senenko A, Sherrill CD (2007) Models of S/pi interactions in protein structures: comparison of the H2S benzene complex with PDB data. Protein Sci 16: 2216-2223.

24. Crowley PB, Golovin A (2005) Cation-pi interactions in protein-protein interfaces. Proteins 59: 231-239.