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# Peptide Bond Planarity

**Nandel FS*, Baskar M, Nandel V and Shafique M**

Biophysics Department, Panjab University, India

***Corresponding author:** Fateh Singh Nandel, Biophysics Department, Panjab University, Chandigarh 160014, India, Tel: 9872659689; Email: fatehhar@pu.ac.in/ fateh_nandel@yahoo.com

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## Abstract

Peptide bond is treated planar with ω=180° or ω=0° representing trans and cis peptide bond respectively. Deviations in ω from 180°and 0° are referred as distortions of individual peptide units from planarity. Different interpretations have appeared to explain the deviations/non-planarity in terms of: i) pyramidalization of carbonyl carbon and amide nitrogen atoms, ii) ψ dihedral angle, iii) handedness of protein main chain, iv) tightening default restrictions on the peptide planarity angle ω. Recently, Mathews has argued that the quality of a refined model of a protein needs to be assessed not just from its R-factors but together with the "Fo – Fc" electron density difference maps. Thus, then non-planarity of peptide bond continued to be a debatable issue.

Analysis of high resolution pdb structures for the bond angels around carbonyl carbon's and amide nitrogen's is found to be 360°.This rules out the change in hybridization and hence pyramidalization of these atoms as claimed earlier. Likewise, there is no change in the hybridization of Cα and there is only readjustment of bond angels. Thus, the peptide bond is planar.

Both expansion and contraction of the angle N-Cα-C was observed up to 10° & 5° respectively. The position of Cα's with respect to the C-N bond is governed by the angle's N-Cα-C and this will be clearly reflected in the electron density difference map. Minimum deviations both in ω and in the angle N-Cα-C (Δτ) are found in helical and compact structures.

**Keywords:** Peptide Bond; High Resolution Structures; Pyramidalization

## Introduction

One of the most fundamental assumptions in protein structure is the peptide bond being planar described in 1953 by Corey and Pauling that (i) N-C bond has 40% double bond character and (ii) planarity of the peptide bond is a "sound structural principle" [1]. The way the electron orbital's interact in this conjugated resonance system, double bond has restrictions for rotation, as this weakens the resonance interaction. This implied that the peptide unit is largely locked into one of two conformations i.e. cis, where the alpha carbons are on the same side of the peptide unit and trans, *where* they are opposite.

Distortions of individual peptide units from planarity have been examined by both semi-empirical and *ab initio* techniques for small systems [2-7]. People's group used high level Hartree-Fock *ab initio* calculations to show that peptide bonds exhibit significant flexibility about the N-C bond in the gas phase with little energetic cost [8]. Even Pauling, *et al*. was aware of the deviations from 180° or 0° could occur with an energetic cost [1,9].

MacArthur and Thornton and others surveyed peptide and protein databases to show that experimentally derived structures show significant deviation from planar peptide bonds which may be related to handedness of the protein chain [9]. They also calculated peptide bond energy wells around ω=180° from ω angle distributions using Maxwell-Boltzmann relationship, which were further updated by Edison [9]. Some computational and experimental investigations have focused on the entity of peptide bond distortions and on the role that the local context plays in this phenomenon [10-15]. The deformations in ω have also been explained in terms of pyramidalization, i.e. the extent of tetrahedral $sp^3$ geometry at the carbonyl carbon or the nitrogen atom or at both carbon & nitrogen atoms of the peptide bond and the pyramidalization of carbonyl carbon has been claimed to depend on the main chain ψ torsion angle only [3,16-18].

Surprisingly, Chellapa and Rose argued that default restrictions on the peptide planarity angle ω can significantly reduce apparent deviations from peptide unit planarity without consequent increase in R-factors [19]. It may be mentioned here that a model allowing non-planarity has more degrees of freedom than one in which planarity is enforced. Bererton & Karplus using the same set of structures analyzed by Chellapa & Rose carried out the parallel refinements to evaluate the consequences of applying standard ω restraints and tight restraints on peptide planarity [19,20]. They emphasized the importance of inspecting electron density maps when investigating the agreement between a model and its experimental data.

Mathews has recently put forward the logic that the quality of a refined model of a protein should not be just assessed from its R-factors but together with "$F_o$-$F_c$" difference electron density maps [21]. Thus the choice of model is very crucial.

Little attention has been paid to the hybridization of carbonyl carbon and amide nitrogen in terms of the bond angles and N-$C^\alpha$-C angles when high resolution protein structures are available. Further, the definition of ω involves the $C^\alpha$ atoms of two consecutive amino acids. The deviations of bond angles around $C^\alpha$ can also affect the peptide bond torsion angle ω. Here, we have examined a number of high resolution X-ray crystallographic structures of peptides/proteins to address: (i) the hybridization around carbonyl carbon and amide nitrogen (ii) $C^{\alpha's}$ maintain the $sp^3$ hybridization or not, (iii) the peptide bonds planarity (iv) Δτ and Δω dependence on Φ & Ψ values and (v) there is a relation between deviation's in ω (Δω) and deviation's in the angle N-$C^\alpha$-C (Δτ) or not (Table 1).

| PDB Code | Peptide/Protein | Resl./A° | 2° Structure |
|---|---|---|---|
| 2GUD | Griffithisin | 0.94 | β-sheets |
| 2OV0 | Amicyanin | 0.75 | β-sheets |
| 1EJG | Crambin | 0.54 | Helices |
| 1HJE | A-conotoxin SI | 0.75 | -S-S- linkage |
| 2X7R | Fusion intermediate of HIV-1 | 2 | Helices |
| 1IRO | Rubredoxin | 1.1 | Helices & β sheets |
| 1RGE | Guanyloriboneclease | 1.15 | Helices & β sheets |
| 1LKS | Lysozyme(hen) | 1.1 | Helices & β sheets |
| 3QL9 | ATRX ADD bound to histone H3K9me3 | 0.93 | Helices & β sheets |
| 1BOY | Human Tissue factor | 2.2 | β-sheets |
| 1IJV | β-defensin- chain A | 1.2 | Helices & β sheets |
| 1ZMP | α-defensin5-chain-C | 1.65 | β-sheets |
| 3LOE | α-defensin-1 | 1.56 | β-sheets |
| 2PNE | Snow Flea Antifreeze Protein | 0.98 | PPII |
| 1YJO | AmyloidlikePeptides | 1.3 | - |
| 2OL9 | | 0.85 | - |
| 2OLX | | 1.42 | - |
| 2ON9 | | 1.51 | - |
| 2ONV | | 1.61 | - |
| 2ONW | | 1.51 | - |
| 2ONX | | 1.52 | - |
| 3DGJ | | 1.8 | - |
| 4K7T | Bacitracin-A | 1.1 | Cyclic peptide |
| 4BTB | Pro9 | 1.9 | Cyclic peptide |
| | N(rch)5 | - | Peptoid(21) |
| 1CWA | Cyclosporine-A | 2.1 | N-methylated |

Table 1: Atomic resolution structures used for analysis.

## Methods

PDB structures of proteins/peptides containing only helices, only beta sheets, rich in helices, rich in beta sheets, having minimum secondary structure elements, fibril forming peptides, cyclic peptides, peptoids and

peptides rich in N-methylated amino acids were downloaded (Table 1). The analysis was done manually.

## Results and Discussion

The concept of peptide bond planarity with ω=180° or 0° is thought to be based on, that each angle around carbonyl carbon and amide nitrogen being 120°and each $C^{\alpha}$ atoms being in ideal $sp^3$ hybridization.

Deviations in $\omega$ from 180°or 0° are interpreted as distortions of individual peptide units from planarity. Analysis of the high resolution crystal structures in terms of; (a) deviation in peptide bond torsion angle (Δω), (b) deviation in bond angle N-$C^{\alpha}$-C (Δτ) and, (c) bond angles around carbonyl carbons and amide bond nitrogen for the pdb structures 1EJG & 2GUD are summarized in Table 2. The results for some other pdb structures are presented in (Table ST1).

**1EJG**

| Residue | $\Delta\tau$ | $\Delta\omega$ | $C_i^{\alpha}$-$C_i$- $N_{i+1}$ | $C_i^{\alpha}$–$C_i$-$O_i$ | $O_i$-$C_i$-$N_{i+1}$ | $C_i$-$N_{i+1}$-$C^{\alpha}$ | $C_i$-$N_{i+1}$-H | H-$N_{i+1}$-$C^{\alpha}_{i+1}$ |
|---|---|---|---|---|---|---|---|---|
| **1-T** | -2.7 | -3.8 | 118.3 | 118.8 | 122.9 | - | - | - |
| **2-T** | -1.7 | 2.7 | 112.7 | 124.1 | 123.2 | 128.2 | 120.2 | 111.5 |
| **3-C** | -2.2 | 4.2 | 117.8 | 119.5 | 122.7 | 122.4 | 118.8 | 118.8 |
| **4-C** | 1.3 | 6.7 | 115.7 | 121.4 | 122.9 | 119.7 | 120 | 120.3 |
| **5-P** | 5.3 | 0.5 | 117.7 | 118.6 | 123.7 | 119.6 | 127.5 | 111.8 |
| **6-S** | -2.3 | 4 | 114.4 | 122 | 123.6 | 122.5 | 118.7 | 118.7 |
| **7-I** | 1.8 | -4.8 | 116.6 | 116 | 127.4 | 124.2 | 117.6 | 118.2 |
| **8-V** | 4.9 | 8.1 | 120.3 | 117.9 | 121.2 | 125.5 | 117.8 | 117.7 |
| **9-A** | 0.9 | -4.3 | 117.4 | 119.8 | 122.8 | 119.3 | 120.3 | 120.4 |
| **10-R** | 2.4 | -0.3 | 117.7 | 120.2 | 122.1 | 119.9 | 120.1 | 120 |
| **11-S** | 1.2 | -0.6 | 116.1 | 121.7 | 122.2 | 118.8 | 120.5 | 120.7 |
| **12-N** | 1.3 | -4.2 | 118 | 118.8 | 123.2 | 118.7 | 118.5 | 122.8 |
| **13-F** | 0.8 | 2.7 | 115.8 | 121.1 | 123.1 | 121.4 | 119.3 | 119.3 |
| **14-N** | 2.5 | -6.1 | 118.3 | 119.8 | 121.9 | 118.7 | 120.7 | 120.6 |
| **15-V** | 1.1 | -2.3 | 116.5 | 121.1 | 122.4 | 119.2 | 120.4 | 120.4 |
| **16-C** | 2.4 | 2.1 | 116.6 | 120.7 | 122.8 | 120.6 | 119.8 | 119.6 |
| **17-R** | 3.9 | 5.5 | 119.9 | 118.6 | 122.4 | 120.6 | 119.7 | 119.7 |
| **18-L** | 4.2 | -1.7 | 121.3 | 118.7 | 120 | 119.9 | 120.2 | 119.9 |
| **19-P** | 4.6 | -0.5 | 117.8 | 118.5 | 123.6 | 119.1 | 128 | 111.6 |
| **20-G** | 5.8 | 0.4 | 117.3 | 119.4 | 123.3 | 122 | 118.9 | 119.1 |
| **21-T** | -0.2 | 0.2 | 118.4 | 119.4 | 122 | 122.6 | 118.6 | 118.8 |
| **22-P** | 0.7 | -4.6 | 120.2 | 119.1 | 121.6 | 125.6 | 126.1 | 107 |
| **23-E** | 1.1 | 5.5 | 118.2 | 119.4 | 122.3 | 122.9 | 119.8 | 117.3 |
| **24-A** | 2.5 | -4 | 117.2 | 120.7 | 122.1 | 119 | 120.6 | 120.4 |
| **25-I** | 1.3 | -2.9 | 116.9 | 121.3 | 121.8 | 120.5 | 119.9 | 119.6 |
| **26-C** | 2.7 | -3 | 117.4 | 119.5 | 123.1 | 117 | 121.6 | 121.4 |
| **27-A** | 1.2 | -0.5 | 118.1 | 119.2 | 122.7 | 121.7 | 119.2 | 119.1 |
| **28-T** | 2.1 | -1.3 | 118.5 | 119.5 | 122.1 | 116.5 | 122.7 | 120.8 |
| **29-Y** | 2.7 | 10.6 | 117.3 | 119.7 | 122.9 | 120 | 119.9 | 120.1 |
| **30-T** | 5.9 | 11.4 | 117.5 | 117.5 | 124.7 | 122.3 | 118.8 | 118.9 |
| **31-G** | 4.5 | -9.1 | 118.6 | 118.8 | 122.5 | 121.3 | 119.4 | 119.2 |
| **32-C** | -0.5 | 4 | 114.4 | 121.2 | 124.4 | 120.3 | 120 | 119.7 |
| **33-I** | -0.7 | -6.2 | 113.9 | 122.2 | 123.9 | 122 | 118.9 | 119.1 |
| **34-I** | -2.2 | -3 | 116.6 | 119.6 | 123.8 | 123.1 | 118.8 | 118.2 |
| **35-I** | -1.9 | -0.6 | 116.7 | 121.6 | 121.6 | 121.1 | 119.3 | 119.6 |
| **36-P** | 2.5 | -2.7 | 117.6 | 117.7 | 124.8 | 119.7 | 125.8 | 112.4 |
| **37-G** | 1.1 | 3.7 | 113.9 | 122.8 | 123.3 | 123.6 | 118.8 | 117.6 |
| **38-A** | 2.9 | 9.1 | 117.4 | 119 | 123.6 | 122.6 | 118.3 | 119.1 |
| **39-T** | 0.6 | 1.1 | 116.9 | 120.2 | 122.9 | 121.2 | 119.4 | 119.4 |
| **40-C** | 0.4 | -0.1 | 116.5 | 120.7 | 122.8 | 120.2 | 120 | 119.8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **41-P** | 3 | 5.8 | 114.9 | 122.1 | 123 | 119.6 | 127.6 | 112.8 |
| **42-G** | 6.4 | -2.9 | 118.6 | 118.6 | 124.7 | 121.4 | 119.3 | 119.3 |
| **43-D** | 2.6 | -10 | 116.7 | 118.6 | 124.7 | 122.4 | 118.8 | 118.8 |
| **44-Y** | -1.6 | 13.3 | 113.1 | 122.3 | 124.5 | 122 | 118.7 | 119.3 |
| **45-A** | 6.7 | 5.4 | 116.8 | 119.1 | 124.2 | 122.7 | 118.8 | 118.5 |
| **46-N** | - | - | - | - | - | 123.8 | 118.3 | 117.9 |

**2GUD**

| Residue | $\Delta\tau$ | $\Delta\omega$ | $C_i^{\alpha}$-$C_i$- $N_{i+1}$ | $C_i^{\alpha}$–$C_i$-$O_i$ | $O_i$-$C_i$-$N_{i+1}$ | $\varphi$ | $\psi$ | $C_i$-$N_{i+1}$-$C^{\alpha}$ |
|---|---|---|---|---|---|---|---|---|
| **1** | -1.5 | 2 | 117.8 | 120.8 | 123.4 | - | 120.3 | 121.6 |
| **2** | 2 | 1 | 115.9 | 120.4 | 123.7 | -77.9 | 130.1 | 121.4 |
| **3** | -0.6 | -9.8 | 115.4 | 122.6 | 122 | -154.8 | -177.6 | 120.9 |
| **4** | -1.1 | -0.7 | 114.1 | 121.9 | 124 | -149.1 | 156 | 122.1 |
| **5** | -1 | -4.8 | 114.1 | 121.7 | 124 | -140.1 | 137.1 | 123.9 |
| **6** | 0 | -2.2 | 115.5 | 121.3 | 123.2 | -99.4 | 136.4 | 124.2 |
| **7** | -3.2 | -4.6 | 117.3 | 120.4 | 122.2 | -126.7 | 142.6 | 121.8 |
| **8** | 3.1 | 8.8 | 113.9 | 121.6 | 124.4 | 107.8 | -163.1 | 121.5 |
| **9** | 1.8 | -8.2 | 115.3 | 121.1 | 123.6 | 100.5 | -167.1 | 122.3 |
| **10** | 8.1 | 2 | 118.9 | 119.5 | 121.7 | -98.1 | -1.8 | 122.8 |
| **11** | 7.3 | -2.8 | 112.1 | 124 | 123.9 | -84.6 | -176.2 | 122 |
| **12** | 2.1 | -0.9 | 112.9 | 124.3 | 122.8 | 80.7 | -179.3 | 123.4 |
| **13** | -1.4 | 2.2 | 118.3 | 119.5 | 122.1 | 112.6 | 141.7 | 120.1 |
| **14** | 5.6 | -8.8 | 115.3 | 119.6 | 125.2 | -65.6 | 148 | 121 |
| **15** | -0.8 | 8.3 | 113.6 | 112.4 | 124 | -139.2 | 168.1 | 121.8 |
| **16** | 1.1 | -5.2 | 115.4 | 121.2 | 123.3 | -115.5 | 146.5 | 121.9 |
| **17** | 7 | -1.5 | 117.6 | 117.8 | 124.6 | -69.9 | -15.5 | 124.3 |
| **18** | -0.8 | 6.7 | 117.4 | 119.6 | 122.9 | -98.7 | 117.4 | 124 |
| **19** | 7.9 | 2 | 119.4 | 119.3 | 121.3 | -114.4 | -33 | 118.8 |
| **20** | 1 | -2.1 | 115 | 122.2 | 122.8 | -157.5 | 157.4 | 123.3 |
| **21** | -0.6 | -16 | 116.7 | 121.3 | 121.9 | -138.7 | 138 | 123.9 |
| **22** | -3.2 | -7 | 116.6 | 120.8 | 122.5 | -137.4 | 169.2 | 121.2 |
| **23** | -0.4 | -23 | 115.1 | 121.4 | 123.3 | -136.3 | 158.7 | 120.1 |
| **24** | -3.4 | 3.8 | 116.4 | 120.2 | 123.4 | -104.4 | 136.1 | 119.2 |
| **25** | 3.1 | -8.1 | 113.9 | 121.9 | 124.1 | -147.4 | 153.2 | 124.2 |
| **26** | -3.4 | -4.4 | 116.6 | 120.8 | 122.5 | -144.8 | -112.1 | 121.6 |
| **27** | 2.1 | -3.6 | 117 | 120.3 | 122.7 | -73.9 | -23.5 | 123.1 |
| **28** | -1.6 | -23 | 116.3 | 121.7 | 121.9 | -125.4 | 159.2 | 120.2 |
| **29** | -1.4 | -7.3 | 116.5 | 121.2 | 122.4 | -82.1 | 112.6 | 123.6 |
| **30** | 3.6 | 3.9 | 117.4 | 121 | 121.5 | -71.3 | -32.3 | 121.3 |
| **31** | -1.1 | -15 | 114.6 | 121.2 | 124 | -160.6 | 158.4 | 120.8 |
| **32** | -2.5 | -2.8 | 115.5 | 121.6 | 122.8 | -121.6 | 142 | 123.1 |
| **33** | -4.1 | 7.8 | 116.2 | 121.4 | 122.4 | -119.4 | 113.1 | 121.6 |
| **34** | -2.8 | 3.5 | 116.6 | 121.6 | 121.7 | -114.7 | 118.4 | 121.5 |
| **35** | 1.8 | -1.7 | 116.1 | 120.9 | 122.9 | 56.7 | 36 | 123.5 |
| **36** | 5.3 | 7.2 | 118.5 | 118.7 | 122.7 | 90.3 | -16 | 122.4 |
| **37** | -0.2 | 4.4 | 115.8 | 122.4 | 121.8 | -89.5 | 123.4 | 123.7 |
| **38** | 4.4 | -5.7 | 116 | 120.5 | 123.4 | -82.8 | 133.9 | 121.6 |
| **39** | 0 | 0.4 | 116.2 | 120.3 | 123.5 | 133.7 | 112 | 122.5 |
| **40** | 1.2 | 7.1 | 115.1 | 121.7 | 123.1 | 131.2 | -161.5 | 122.5 |
| **41** | 2.3 | 6.1 | 115 | 122 | 123 | -94.5 | -168.4 | 123.4 |
| **42** | 5.9 | -2.1 | 116.2 | 121 | 122.8 | -109.2 | 2.7 | 120.6 |
| **43** | 3.8 | 1.6 | 115 | 122.4 | 122.6 | -76.6 | -178.4 | 121 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **44** | 3.5 | 1.2 | 114.8 | 120.8 | 124.4 | 65.7 | -162.3 | 122.4 |
| **45** | -1.8 | 1 | 115.9 | 120.5 | 123.6 | -124.8 | 143.8 | 121 |
| **46** | 2 | -5.7 | 116.7 | 120.4 | 123 | -60.7 | 125.1 | 122.1 |
| **47** | 1.3 | -16 | 116.9 | 120 | 122.9 | -70.9 | 161.7 | 119.2 |
| **48** | 0 | -3.4 | 114.8 | 122.4 | 122.8 | -59 | 159.3 | 121.8 |
| **49** | 1.5 | -7.2 | 114.6 | 120.5 | 125 | -88.1 | 131.7 | 123 |
| **50** | -1.9 | 1 | 116 | 119.9 | 124.1 | -95.7 | 117.1 | 120.9 |
| **51** | -2 | -0.2 | 115.4 | 120.9 | 123.7 | -109.9 | 123.9 | 120.5 |
| **52** | 0.4 | -10 | 115.5 | 121.4 | 123.1 | -69.7 | 131.6 | 120.3 |
| **53** | 1.9 | -4.1 | 114.8 | 123.3 | 121.9 | -75.8 | 175.4 | 119.9 |
| **54** | 1.4 | 0.8 | 116.5 | 120.9 | 122.6 | -58.8 | 129 | 122.7 |
| **55** | 5.9 | -0.2 | 117.6 | 119.9 | 122.5 | 69.1 | 12.5 | 120.8 |
| **56** | 0.9 | 1.7 | 115.2 | 120.8 | 123.9 | -98.8 | 137.8 | 120.7 |
| **57** | -0.5 | -5.8 | 115.2 | 121.3 | 123.5 | -150.1 | 159.1 | 120.2 |
| **58** | -0.6 | -1.6 | 114.8 | 120.2 | 125 | -69 | 124.5 | 124.8 |
| **59** | 5.2 | -3 | 117.2 | 120.2 | 122.6 | -106.9 | -21 | 120.9 |
| **60** | -2.6 | 0.1 | 116.1 | 120.4 | 123.5 | -150.5 | 147.1 | 119.6 |
| **61** | 0.2 | -5.2 | 114.8 | 120.9 | 124.3 | -153.7 | 144.5 | 121.9 |
| **62** | -1.9 | -1.6 | 114.3 | 120.4 | 125.2 | -123.5 | 123.3 | 121.8 |
| **63** | -2.4 | -13 | 116 | 120.6 | 123.4 | -120.5 | 130.3 | 120 |
| **64** | -2.5 | 3 | 118.1 | 119.8 | 122 | -99.1 | 134.3 | 119.7 |
| **65** | -1.1 | -6 | 112.9 | 120.6 | 126.4 | -153.4 | 162.6 | 123.5 |
| **66** | -2.2 | -2.1 | 117.9 | 120.3 | 121.6 | -150.7 | -112.6 | 121.7 |
| **67** | -2.2 | -2.1 | 117.1 | 117.4 | 125.5 | -12.4 | -24.8 | 123 |
| **68** | -1.6 | -14 | 115.4 | 121.7 | 122.9 | -126.6 | 178.9 | 120.6 |
| **69** | -2.5 | -0.2 | 115.6 | 121.3 | 123.2 | -81.1 | 115.3 | 121.8 |
| **70** | 2.3 | 11.3 | 117.5 | 120.6 | 121.8 | -82.3 | -35.7 | 119.2 |
| **71** | -2.8 | -11 | 115.2 | 121.9 | 122.9 | -160.7 | 145.3 | 122 |
| **72** | 0.3 | -11 | 114.2 | 122.6 | 122.9 | -133 | 145.2 | 122.7 |
| **73** | -3 | -6.7 | 116.8 | 120.6 | 122.6 | -145.3 | 148.9 | 119.6 |
| **74** | 0.9 | -6.7 | 114.5 | 123 | 122.4 | -149 | 164.4 | 122.5 |
| **75** | -3.8 | -0.3 | 114 | 122 | 124 | -134 | 148.7 | 119.5 |
| **76** | 2.2 | -5.1 | 114.1 | 121.9 | 124.1 | -100.5 | 171.2 | 119.2 |
| **77** | 3.3 | -2.1 | 118.6 | 120.8 | 120.5 | -59.4 | -22.7 | 123.8 |
| **78** | 3.2 | -1.8 | 117.4 | 121 | 121.6 | -86.3 | 2.9 | 122.8 |
| **79** | 4.4 | -3.1 | 118.3 | 119.4 | 122.2 | 79.8 | 11.5 | 120.6 |
| **80** | 0.3 | 6.5 | 115.9 | 121.9 | 122.2 | -86.5 | 147.1 | 123.2 |
| **81** | 0.2 | -8.7 | 113.5 | 122.7 | 123.7 | -117.7 | 145.3 | 124.1 |
| **82** | -2.4 | -2.7 | 116.2 | 120.9 | 122.9 | -124.1 | 125.5 | 120.1 |
| **83** | 0.8 | 6.2 | 116 | 122.7 | 121.2 | 82.1 | -167.2 | 121 |
| **84** | 3.7 | -9.9 | 114.6 | 120.2 | 125 | -64 | 147.4 | 123 |
| **85** | -1.8 | -0.3 | 115.4 | 120.9 | 123.8 | -132 | 137.9 | 122.2 |
| **86** | 0.7 | 5.9 | 114.9 | 120.9 | 124.2 | 118.6 | -159.5 | 123.8 |
| **87** | 4.8 | 9.3 | 115.8 | 121.2 | 123.1 | -107.8 | 168.2 | 123.2 |
| **88** | 4.3 | -1 | 117.6 | 119.4 | 123.1 | -122.4 | 1 | 122.4 |
| **89** | 4.3 | -1.8 | 114.9 | 122.5 | 122.6 | -75 | -176 | 120.6 |
| **90** | 4.8 | -3.9 | 114.4 | 121.7 | 123.8 | 73.7 | -157 | 120 |
| **91** | -2.2 | 7 | 117.2 | 120.3 | 122.5 | -137.1 | 150.9 | 122.6 |
| **92** | 2.2 | -9.6 | 114.4 | 123.5 | 122 | -93.2 | 143.1 | 122.6 |
| **93** | -3 | 4.1 | 116.5 | 120.5 | 123 | -138.9 | 147.3 | 122.1 |
| **94** | 1.7 | -14 | 114.4 | 121.5 | 123.8 | -129.5 | 142 | 123.1 |
| **95** | -2.7 | 2.3 | 116.7 | 120.3 | 122.9 | -105.5 | 137.6 | 122.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **96** | -0.7 | -1.3 | 116.3 | 119.7 | 124 | -135.9 | 143.4 | 122 |
| **97** | 1.7 | -0.7 | 114.3 | 122.6 | 123.1 | 56.1 | 49.3 | 120.8 |
| **98** | 0.2 | -16 | 114.1 | 122.5 | 123.4 | -117.3 | 175.3 | 122.6 |
| **99** | -4.1 | -1.2 | 117.6 | 120.2 | 122.2 | -131.5 | 108.9 | 119 |
| **100** | -0.9 | 0.7 | 114.3 | 120.6 | 125.1 | -74.6 | 129.7 | 123.6 |
| **101** | 2.4 | 2.6 | 118.4 | 119 | 122.5 | -98.5 | -39.6 | 120.6 |
| **102** | -1.3 | -6.7 | 114.1 | 121.1 | 124.7 | -157.9 | 156.5 | 124.2 |
| **103** | -1.4 | -12 | 115.5 | 120.6 | 123.9 | -126.1 | 116.1 | 122.5 |
| **104** | -3 | -4.1 | 118.7 | 121 | 120.4 | -110.2 | 179.5 | 119.4 |
| **105** | 2.6 | -2.5 | 115 | 120.7 | 124.3 | 175.3 | -162 | 120.8 |
| **106** | -2.3 | 4.3 | 115.5 | 120.1 | 124.4 | -143.9 | 141.9 | 118.8 |
| **107** | 0.4 | -5.6 | 114.2 | 121.7 | 124 | -150.4 | 153 | 123.2 |
| **108** | -2.2 | -12 | 116.7 | 121.4 | 122 | -134.7 | -113.1 | 119.8 |
| **109** | 4 | -3.1 | 116 | 120 | 124 | -71.4 | -24.6 | 122 |
| **110** | -1.5 | -7.2 | 115.3 | 120.8 | 123.9 | -128.1 | 178 | 121.1 |
| **111** | -3 | 2 | 116.3 | 120.6 | 122.8 | -86.9 | 102.7 | 122.5 |
| **112** | 2.6 | 7.2 | 117.3 | 120 | 122.7 | -71.3 | -44.6 | 119.8 |
| **113** | 0.7 | -7.4 | 114.2 | 122.4 | 123.4 | -155.6 | 156.2 | 124.8 |
| **114** | -0.2 | -8 | 115.1 | 120.9 | 124 | -125.2 | 140.1 | 124.1 |
| **115** | -2.6 | 5.1 | 115.8 | 119.2 | 124.7 | -115.6 | 106.2 | 122 |
| **116** | -1.9 | -2.4 | 116 | 120.4 | 123.6 | -98.2 | 124.2 | 122.3 |
| **117** | -3.2 | 10.3 | 116.5 | 120.2 | 123.3 | -107.4 | 125.9 | 119.6 |
| **118** | 0.3 | -11 | 114.4 | 121.4 | 124.6 | -154.3 | 161.1 | 121.7 |
| **119** | -2 | -2.1 | 116.4 | 121 | 122.6 | -99.1 | 131.9 | 123.4 |
| **120** | 0.4 | -1.1 | 116.9 | 119.7 | 123.4 | -97.3 | 117.8 | 123.2 |
| **121** | -0.2 | - | - | - | - | -101.9 | - | - |

Table 2: Deviation in bond angle, N-$C^{\alpha}$-C (Δτ) & bond angles around carbonyl carbons and amide nitrogen atoms together with the deviation in peptide bond torsion angle, **ω** (in degrees), for the PDB structure, **1EJG** and **2GUD**.

The sum of the bond angles around carbonyl carbons and amide nitrogen's is found to be ~ 360°. Likewise, sum of the angles around each $C^{\alpha}$ atoms remains 656.8° which also suggest that the hybridization remains sp3. Only, there is a readjustment of the angles around carbonyl carbons, amide nitrogen and $C^{\alpha's}$. $C^{\alpha}$-C-N angle's was found to be less up to 10° and the angles C-N-$C^{\alpha}$ greater up to 5° from 120°. Thus the resonance principle is not violated. There is both decrease and increase in the value of bond angle N-$C_{\alpha}$-C and the decrease is in N-Cα-C (Δτ) values from 109.5° is in less number of cases as compared to the number for increase. The back bone angles N-$C^{\alpha}$-C, $C^{\alpha}$-C-N and C-N-$C^{\alpha}$ appears to play an important role. These rules out any change in hybridization around these atoms i.e. the hybridization remains intact (sp2) and rules out the pyramidalization. Thus, the six atoms i.e. two consecutive $C^{\alpha's}$, carbon, oxygen, nitrogen and hydrogen (C in peptides & N-methylated peptides) remain in the same plane and the peptide bond is planar.

The deviation in bond angles $C^{\alpha}$-C-N around carbonyl carbon and C-N-$C^{\alpha}$ around amide nitrogen from 120° will lead to changes in position of carbonyl oxygen and amide hydrogen in the same plane. The peptide bond is defined $C^{\alpha}$-C-N-$C^{\alpha}$ and the positions of $C^{\alpha's}$ with respect to the C-N bond is governed by the angles N-$C^{\alpha}$-C. Decrease or increase in the values of angle's N-$C^{\alpha}$-C from 109.5° is going to effect the position of $C^{\alpha's}$ with respect to the C-N bond resulting in that the dihedral angles $C^{\alpha}$-C-N-$C^{\alpha}$ & O-C-N-H/C may not be 180°or 0°. This will be clearly reflected in the electron density difference map "$F_o$-$F_c$" and the deviation in ω from 180°or 0° may not be taken as the criterion for non-planarity of peptide bond.

A careful look at the results in tables 2 & ST1 reveals that: (i) there is a substantial deviation in the value of peptide bond torsion angle ω from 180°& 0° and the magnitude of Δω is greater than the magnitude of Δτ. To see the dependence of Δτ and Δω on the φ, ψ values, the plot of Δτ*vs*φ & Δω *v s*φ and Δτ *vs* ψ & Δω *vs* ψ is shown in Tables 2 & ST1 and Figure 1 for 2GUD & Figure S1 for 2PNE. It is obvious from the plots that there is some similarity between the Δτ *vs* φ & Δω *vs* φ plots and likewise between the Δτ *vs* ψ & Δω *vs* ψ plots. This reflects the dependence of Δτ and Δω on φ, ψ values *i.e.* on secondary structures.

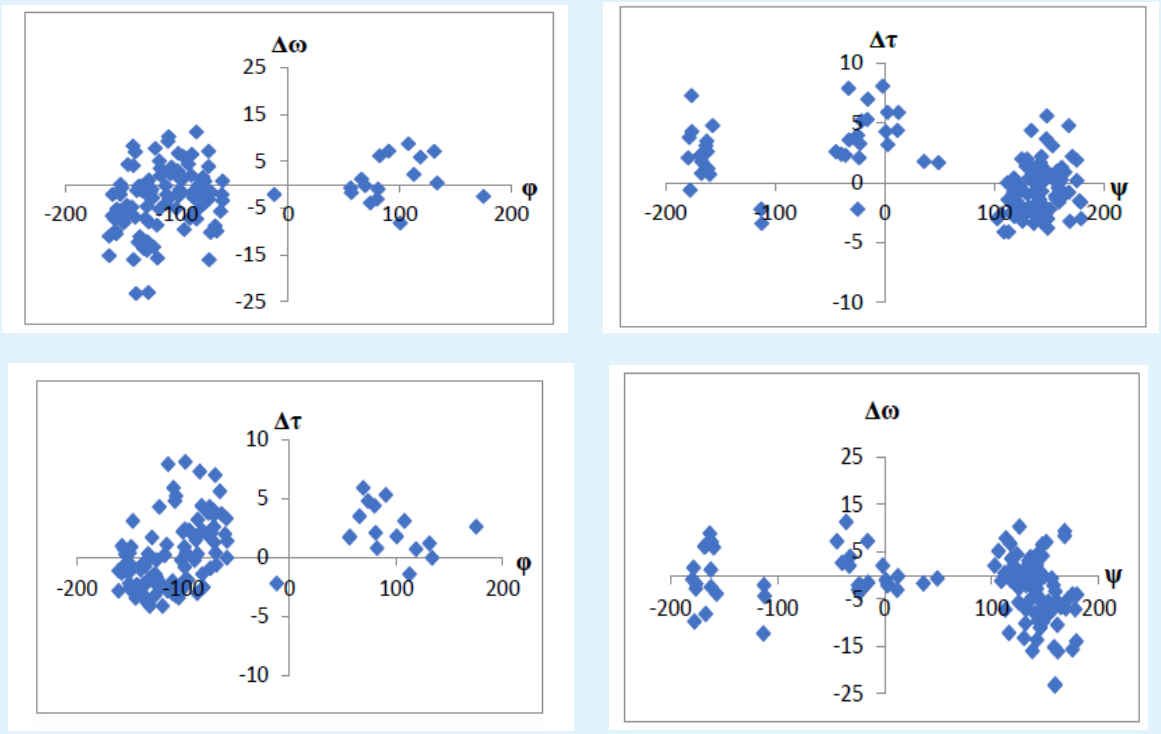


Figure1: Plot of Δτ vs φ & Δω vs φ and Δτ vs ψ & Δω vs ψ for 2GUD showing the similarities of Δτ/Δω vs φ and ψ.

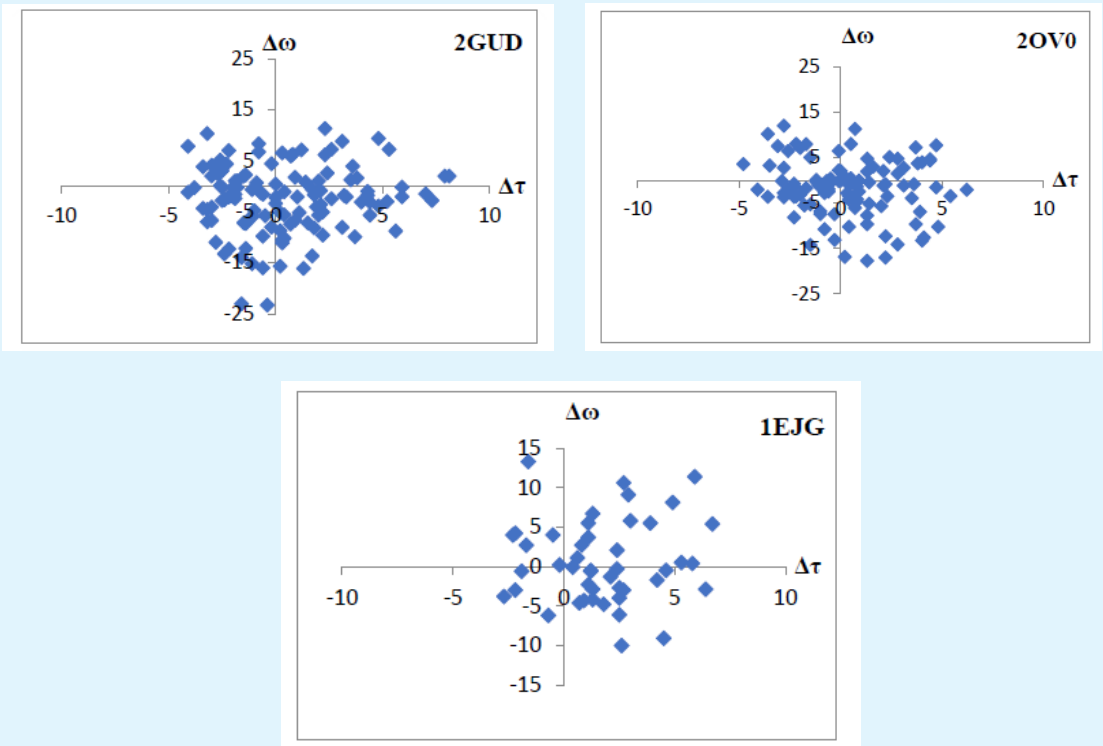


Figure2: Plot between Δτ and Δω (in degrees) for the PDB structure 2GUD, 2OV0 and 1EJG showing the relationship between the two which depends on the secondary structure element and compactness of the structure.

Plots of the deviation in N-$C^{\alpha}$-C($\Delta\tau$) vs the deviation in $\omega$ for structures (2GUD, 2PNE, 2OV0,1EJG,1LKS,2X7R,1IJV,1ZMP) having different secondary structure elements (only helices, only beta sheets, rich in helices, rich in beta sheets, the structure having minimum secondary structure elements)are shown in (Figures 2 & S2). This may points to the relation between $\Delta\tau$ and $\Delta\omega$ but the relation is not linear. This may be attributed to involvement of two $C^{\alpha}$ and local effects. The analysis of data in table's together with the graphical views of the pdb structures point out that the $\Delta\tau$ and $\Delta\omega$ values are minimum for helical segments and compact structures and maximum in loops/turns region & terminal residues of helices.

## Conclusions

Analysis of the high resolution pdb structures for angles around carbonyl carbons, amide nitrogen's and $C^{\alpha}$ atoms rules out the change in hybridization of these atoms. Hence, the six atoms i.e. two consecutive $C^{\alpha's}$, carbon, oxygen, nitrogen and hydrogen (C in peptoids & N-methylated peptides) remain in the same plane and the peptide bond is planar. The changes in the position of $C^{\alpha's}$, carbonyl carbon's & amide nitrogen's due to the changes in the back bone angles around these atoms will alter their positions and this will be clearly reflected in the electron density difference map " $F_o$-$F_c$". The deviations in $\Delta\tau$ and $\Delta\omega$ values are found to be minimum for helical segments and compact structures and maximum in loops/turns region & terminal residues of helices.

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