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Molecular dynamics study of a tripeptide Z-Ala-Ala-Leu-pNA

RAVINDRANATH SINGH RATHORE
Department of Physics, Indian Institute of Science, Bangalore 560 012, India.
email: newdrugdesign@yahoo.com; Phone: 91-80-2932315; Fax: 91-80-3602602.

Abstract

Conformations of a substrate of subtilisin enzyme, N-benzyloxycarbonyl-L-alanyl-L-alanyl-L-leucyl-p-nitroanilide have been explored using molecular dynamics simulations. The dynamic structure is characterized by overall extended and folded conformations of the peptide.

Keywords: Molecular dynamics, tripeptide, protease–substrate, AAL.

1. Introduction

Molecular dynamics with other conformational search procedures have been proved useful in exploring solution state and receptor-bound biologically active conformations. The author had previously examined crystal structure [1] of a serine protease substrate [2], Z-L-Ala-L-Ala-L-Leu-pNA (ZAALN) (Fig. 1), which forms an antiparallel β-sheet packed by means of extensive network of $\sigma \cdots\sigma$, $\sigma \cdots\pi$ and $\pi \cdots\pi$ interactions. The high flexibility of peptide is demonstrated in the crystal by the fact that four independent molecules are co-crystallized in the unit-cell. Database examinations of AAL sequences in proteins, however, revealed two densely populated regions (Fig. 2), occupied by left-handed helical and extended conformations. This differential behavior of AAL sequence, in contrast to previous investigations, prompted us to study and explore further conformations of AAL sequence using molecular dynamics simulation.

2. Methods

2.1. *Minimization and dynamics conditions*

The starting system for our study was constructed by taking the peptide coordinates from refined crystal structure of ZAALN molecules in P_1 space group. All the four different conformers in the unitcell were independently simulated for 540 picoseconds. No nonbonded cutoff distance was used. Energy minimizations were performed by using *steepest descents* at first and later by *conjugate gradient* methods. The energy of the system was minimized by using the default Biosym CVFF force field [3], until the maximum first derivative was 0.001 kcal mol^{-1} $Å^{-1}$. Newton's equations were integrated by means of Verlet algorithm with a time step of 1 femtosecond. First, each molecule was heated up to 300K for 40 ps, and when equilibrium was achieved, data were collected for 500 ps. A distance-dependent dielectric constant, 4r, was used. Database analyses in Cambridge Structural Database and Protein Data Bank were carried out using QUEST [4] and a local conformational analysis program (C. Ramakrishnan, private communication).

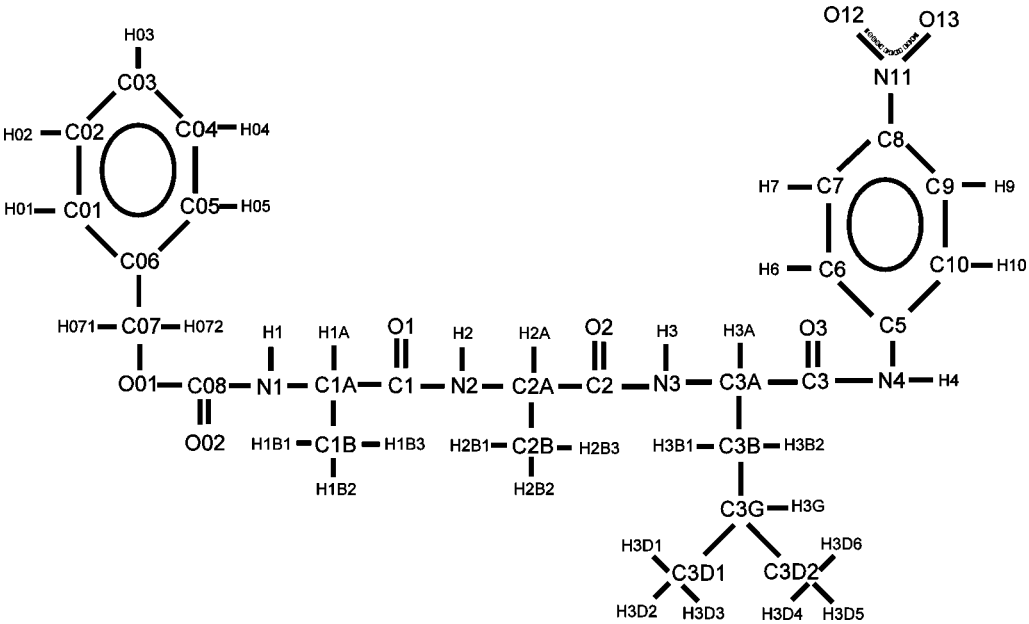

FIG. 1. Chemical structure of Z-Ala-Ala-Leu-pNA with IUPAC atomic numbering scheme.

3. Results

3.1. *Dynamics of ZAALN peptide at room temperature*

Conformational trajectories (Figs 3–5) give a clear picture of the conformations accessible to ZAALN molecules. Torsion angles are provided in Table I. In simulation experiment, carried out for 500 ps, peptide chain appears to be very flexible and adopts different conformational states. The major difference between the experimental and the simulated structures are in the Ψ torsion angles of all the residues. While the overall conformation of ZAALN-B and ZAALN-D, on an average, remains extended during simulation period, rest of the conformers differ from the crystal state observations. Individual residues assume conformations either in right-handed helical or β-regions. Ala (I) of ZAALN-C is the sole exception, and adopts relatively high energy C^{7}_{ax} conformation (69°, -86°). During simulation period, several flips of aromatic rings, on either side of the peptide, have been observed. Z-Urethane groups (θ^1, ω_0) have *trans-cis* conformations similar to that observed in the crystal structure and oscillate about 176–179° and 9–10° ranges, respectively. θ^2 values fluctuate about one of the three favored states, namely, 90±30°, -90±30° and 180±30°[5].

4. Discussion

The behavior of individual residues, in each conformer, in the dynamic structure is mentioned in the following discussion.

ZAALN-A: Fluctuations are relatively large in molecule A compared to molecules B, C and D. Fluctuations in Ala (I), Ala(II) and Leu residues are about average values - (-75°, -45°),

Table I
Torsion angles (°) of time-averaged dynamics structures of ZAALN molecules

Residues		ZAALN(A)	ZAALN(B)	ZAALN(C)	ZAALN(D)
Ala(I)	Φ_1[§]	−75(13)	−118(12), −137(14)	69(10)	−127(12)
	Ψ_1	−45(18), 34(51)	90(13), 130(18)	−86(17)	82(11)
	ω_1	176(9)	10(8)	−179(9)	11(8)
Ala(II)	Φ_2	−89(25)	−115(13), −78(11)	−89(21)	−108(12)
	Ψ_2	−52(35)	86(18), −57(15)	−68(16), −161(19)	85(21)
	ω_2	178(10)	179(10)	170(10)	−178(10)
Leu	Φ_3	−97(20)	−88(13), −115(19)	−100(18)	−120(25)
	Ψ_3	85(34)	83(13)	76(12)	−66(13)
	ω_3[¶]	−177(9)	−175(9)	−174(8)	−179(8)
	X^1	177(10), −67(9)	−172(9)	−172(9)	−66(9), −173(10)
	X^2	68(11)	68(11), 164(11)	−169(12)	68(12), 171(9)
		−170(11), −65(10)	−170(11), −73(12)	68(12)	−170(12), −67(10)
NCbz	ω_0	9(7)	10(8)	9(6)	10(7)
	θ^1	178(14)	179(13)	176(12)	179(13)
	θ^2	173(35)	−152(32)	177(16)	−124(32)

[§]C08-N1-C1A-C1 and [¶]C3A-C3-N4-C5; RMS deviations are given within parentheses in their least significant digits.

(−89°, −52°) and (−97°, 85°), respectively. The overall conformation of the molecule is folded without any intramolecular hydrogen bond. *ZAALN-B and ZAALN-D*: Among all conformers, behavior of ZAALN-B closely resembles the one observed in crystal structure, with all the three residues assuming backbone torsion angles in the β-region. A short transition into helical state, approximately of 200–250 ps range for Ala(II) residue, has been observed. Residues in molecule D also have similar extended conformations with the exception of leucine. Peptide unit 1, i.e. ω_1 (Ala(I)-Ala(II)), adopts *cis* conformation [6], in molecules B and D, as against *trans* conformation in solid state. Such transition may be induced by possible $\pi \cdots \pi$ interactions in between aromatic rings at either end of the peptide. *ZAALN-C*: Residue Ala(I) of Mol C is the unique, among all conformers and oscillates about C^7_{ax} conformation (69°, −86°) after equilibration period. Fluctuations in Ala(2) and Leu(3) are around (−89°, −68°) and (−100°, 76°), respectively. There

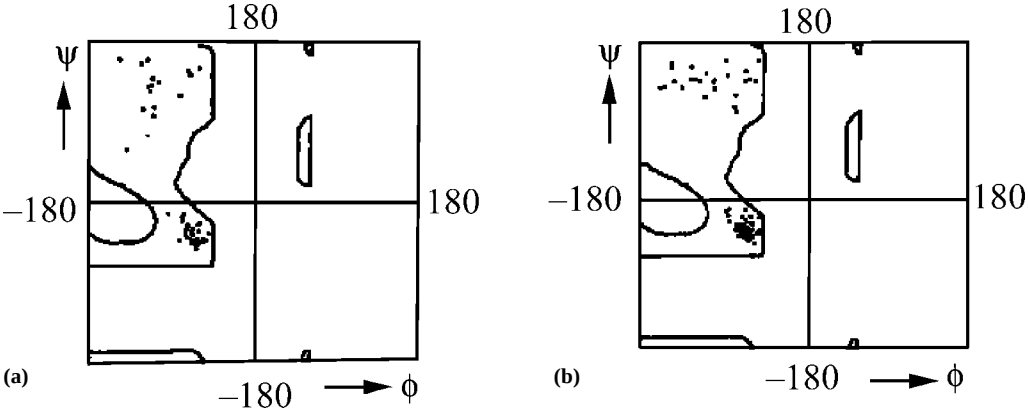

FIG. 2. Preferred conformations of: (a) leucyl and (b) alanyl residues, in AAL sequences, found in proteins. Only limited data are available for AAL sequences, in small molecule crystal structures, with a great majority of residues having conformations in β-region.

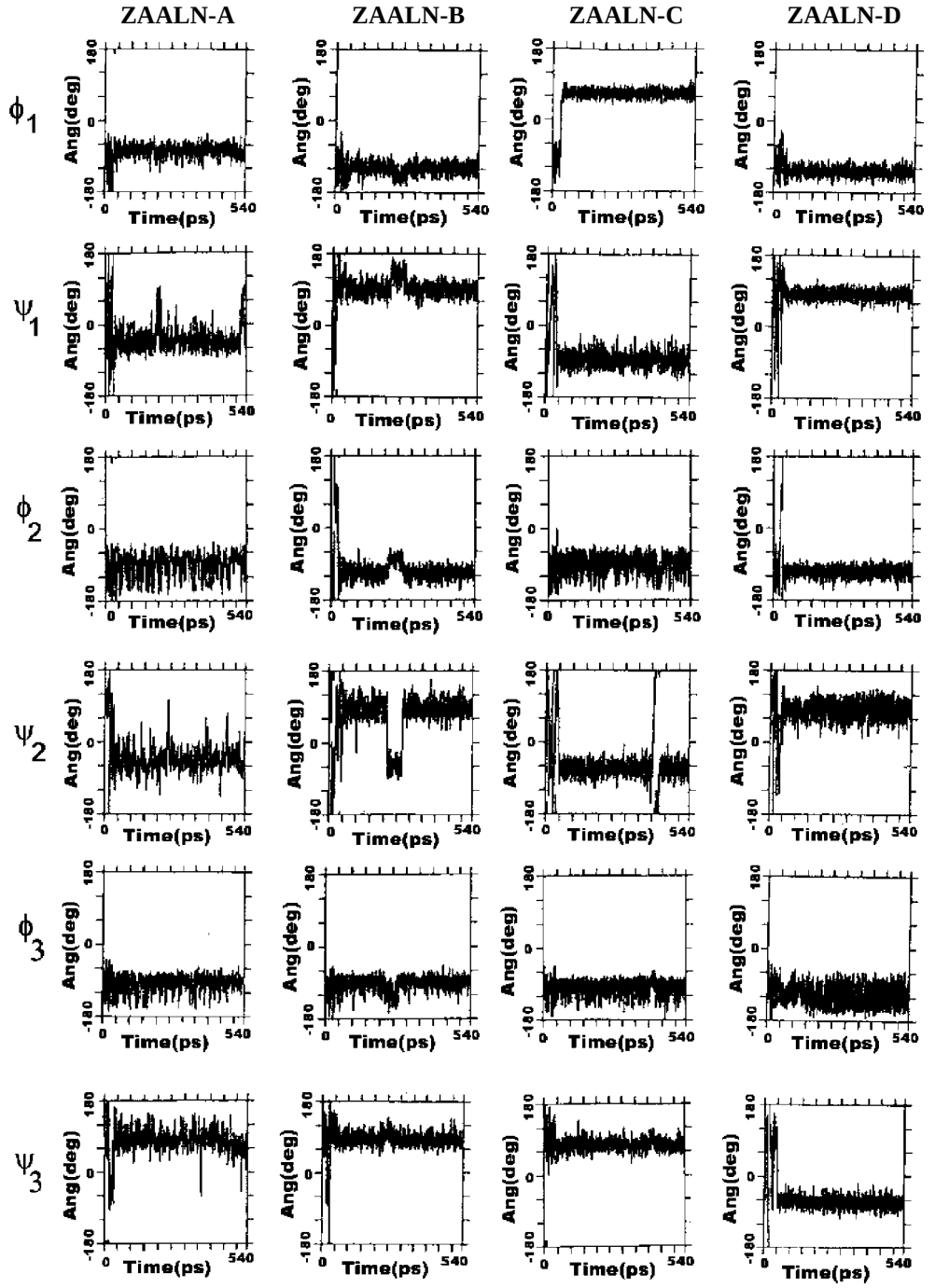

FIG. 3. Trajectories depicting fluctuations and conformational transitions of residues in the simulated molecules.

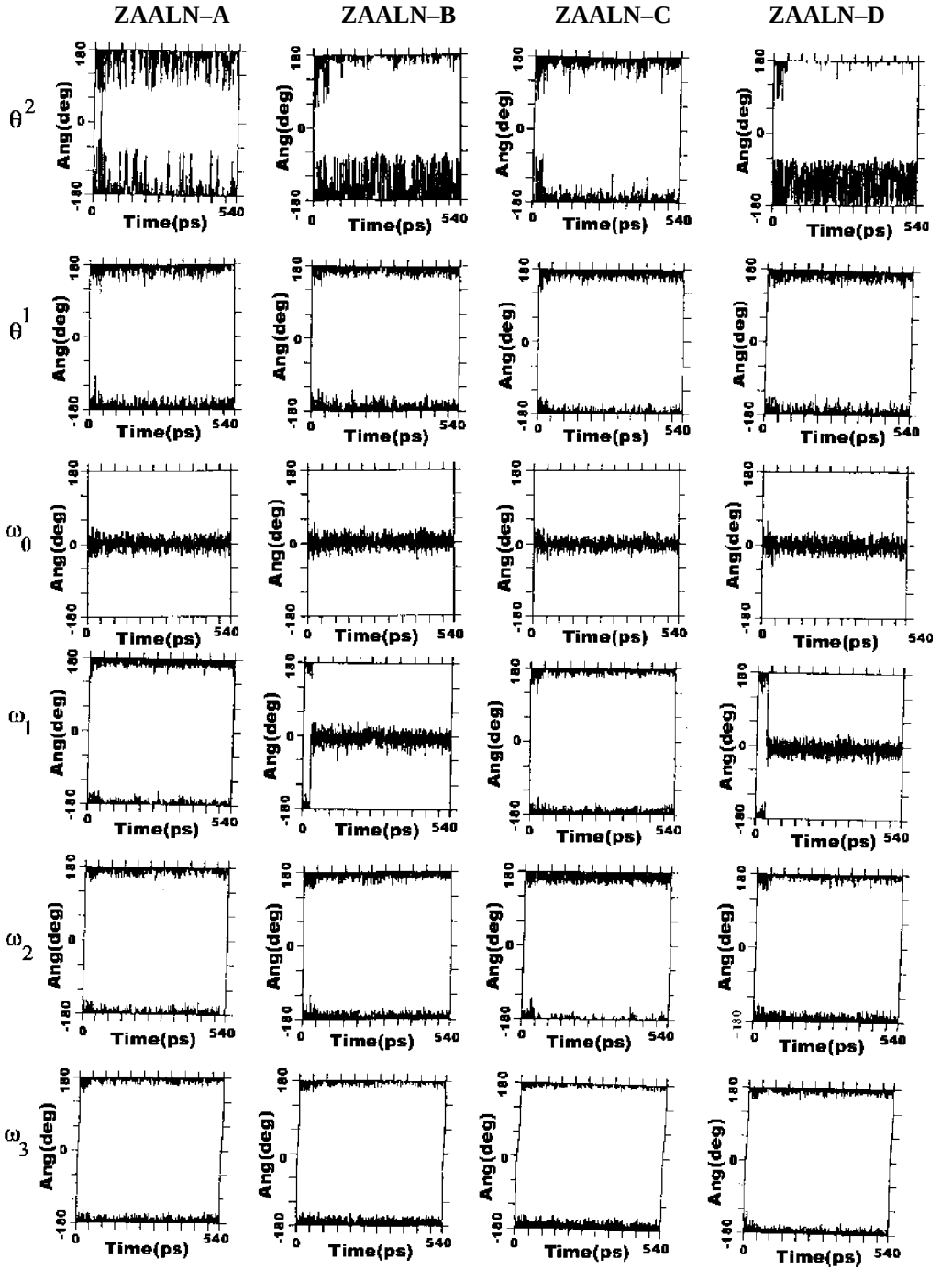

FIG. 4. Trajectories of conformational angles θ and ω.

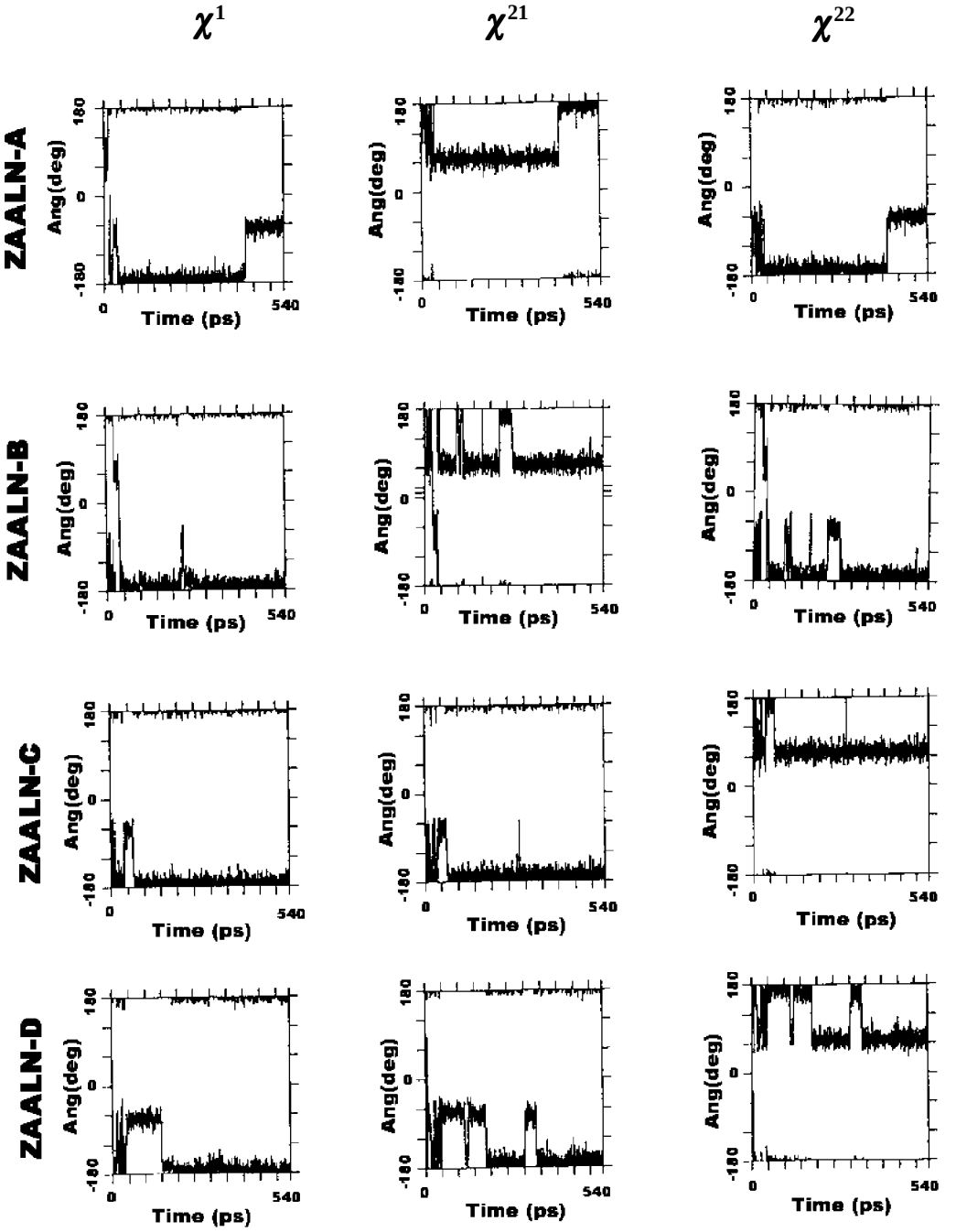

FIG. 5. Side-chain fluctuations and transitions in simulated molecules.

is no y-turn associated with Ala(I) residue, possibly due to large deviation in Ψ-angle from the standard values (Φ = 70 to 85°; Ψ = –60 to –70°) [7]. The overall conformation here also is folded without any intramolecular H-bond.

Side chain dynamics: Conformational transitions occur in all the four conformers. Torsion angles about C3A-C3B (X^1) are, by and large, *trans* in molecule B; in others, they oscillate about g^- and *trans*. Conformational transitions in torsion angles, X^2, occur either into (g^- t) or (g^+ t) states. The overall side-chain conformation of leucine is t (g^+ t) and g^- (g^- t). Both are low-energy conformations [8] and match with the crystal state conformations.

5. Conclusion

Energy minimizations and molecular dynamics simulations performed on tripeptide, in partial agreement with previous crystallographic observation, demonstrate a very flexible peptide backbone. The behavior of peptide during *in vacuo* simulation, which is characterized by ensemble of conformations with a preference for overall folded and extended backbone conformation of peptide has implications for the receptor-bound conformations of protease–substrates [9].

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