

THE EFFECTS OF GAS PHASE ON THE PROTEIN CONFORMATION: A MOLECULAR DYNAMICS STUDY ON EOTAXIN-3 CYTOKINE

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ABSTRACT

In the current work, the structure of the enzyme CC chemokine eotaxin-3 (1G2S) was chosen as a case study to investigate the effects of gas phase on the protein conformation using molecular dynamics simulation. Generally, simulating proteins in the gas phase tend to suffer from various drawbacks, among which excessive numbers of protein-protein hydrogen bonds. However, current results showed that the effects of gas phase simulation on 1G2S did not amplify the protein-protein hydrogen bonds. It was also found that some of the hydrogen bonds which were crucial in maintaining the secondary structural elements were disrupted.

Key Words : Molecular dynamics, gas phase simulation, CC Chemokine Eotaxin-3

1.0 INTRODUCTION

The influence of water in determining the structure and function of proteins has long been recognized [1-5]. It has been shown that water is capable of promoting conformational transitions [6] as well as catalyze the folding process of proteins [7, 8]. The protein-water relationship or the solvent effects has kept researchers puzzled for more than 40 years. This has indeed attracted intensive studies not only among experimentalists but also theoreticians [1, 9-13].

Molecular dynamics (MD) simulation has been widely applied in theoretically investigating the effects of solvation on protein structures with explicit representations of aqueous solvent [13-15]. The correct treatment of solvent is crucial in realistic simulation of proteins especially when simulating the folding process [16]. However, the presence of thousands of water molecules only renders MD impractical as it will slow down the computation due to the substantial amount of CPU cost involved in calculating the enormous amount of solvent-solvent and solvent-protein interactions. The simplest way to avoid this problem is by simulating the protein in gas-phase or in vacuum, free of solvent interactions [13, 17, 18]. While simulations in the gas phase are prone to suffer from various artifacts, there have been a lot of experimental studies investigating the behavior of proteins in the gas phase. Recent technological developments such as mass spectrometric based techniques have made it possible to study large proteins in vacuo [19-23]. These mass spectrometric based techniques however do not provide atomic details regarding the accessible conformational space. The lack of good understanding of the protein state in the gas phase provides great prospects for theoretical studies. This is because no experimental technique to date is able to provide atomistic-resolved

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information as what can be obtained through molecular dynamics simulation (MD). However, theoretical studies of proteins *in vacuo* are scarce due to the fact that simulating proteins in the gas phase suffers from severe drawbacks [13, 14, 16, 18].

The gradual rise of awareness in exploring proteins *in vacuo* was the main motivation for the current work. Thus, the main objective of the current study was to investigate the effects of gas phase on protein conformation. This method was tested both in vacuum and condensed phase. The current work was carried out using the protein, eotaxin-3 cytokine, (PDB id: 1G2S) containing 71 amino acid residues. For the record, the current work would be the first MD study ever done on the cytokine family.

2.0 MATERIALS AND METHOD

CC Chemokine Eotaxin-3

Chemokines belong to a family of about 40 small cytokines that play crucial role in immune response [24] and plays a central role in the determination of the metastatic destination of tumour cells [25]. Eotaxin-3 is a member of CC chemokines that coordinate the recruitment of inflammatory cells, eosinophils and the eosinophil-mediated tissue damage associated with respiratory disorders such as asthma. Thus, eotaxin is a potential target for anti-asthmatic drugs. The NMR solution structure of the CC Chemokine Eotaxin-3 (PDB id: 1G2S) has been solved by Ye and colleagues [26] showing a typical chemokine fold. It is a small protein of 71 amino acids consists of three antiparallel β -strands and an overlying α -helix (Figure 5.1). There are several distinct regions identified in this protein which consists of the unstructured N-terminus region (Thr1 – Thr9), the irregularly structured N-loop region (Phe12 – Pro20), the single turn of the 3-10 helix (Trp21 – Trp23), the three stranded antiparallel β -strands which formed the core part of the protein (Arg25 – Phe29; Val39 – Thr43; Lys47 – Thr51, respectively), the 30s loop (Thr30 – Ala38), the α -helix (Lys56 – Leu65) and finally the poorly defined C-terminus region (Lys66 – Leu71).

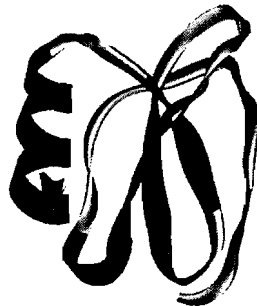


Figure 1 NMR-solution structure of CC Chemokine Eotaxin-3 (1G2S) *Simulation Details*

All the MD simulations in the current study were performed using the AMBER8 suites of programs [27] employing the amber *ff03* force field [28]. The trajectories were produced by numerical integration of the Newton's equation of motion using the Verlet-leap frog [29] algorithm with a 2-femtosecond of time step. The simulations were performed in isothermal-isobaric ensemble (NPT) at 300K and 1 atm, respectively using Berendsen thermostat [30] with coupling constants of 1 ps. Bond constraints were

imposed on all bonds involving hydrogen atoms via SHAKE [31] while the translational and rotational center-of-mass motions were removed every 1000 steps. The atomic coordinates of 1G2S were taken from the PDB [32]. For the gas phase simulation, the NMR structure was minimized employing the steepest descent for the first 500 cycles followed by 1500 cycles of conjugate gradient. A 16 Å cutoff was used together with distance-dependent dielectric constant to treat the nonbonded interactions. Meanwhile, for the solvated system, the NMR structure was immersed in a truncated octahedron water box filled with TIP3P water model [33] and counter ions to maintain the system neutrality. The system was then minimized before subjected to 50 ns of MD simulation using periodic boundary condition. The nonbonded interactions were treated using 10 Å cutoff and PME algorithm [34] for Lennard Jones and coulombic interactions, respectively.

3.0 RESULTS

This section presents results on MD simulations of the NMR native structure both *in vacuo* ($G2S_{\text{NMR-vac}}$) and explicit solvent ($G2S_{\text{NMR-wat}}$) simulations.

RMSD and RMSF Analysis

Figure 2 shows the backbone RMSD ($\text{RMSD}_{\text{back}}$) as a function of simulation time for both $G2S_{\text{NMR-vac}}$ and $G2S_{\text{NMR-wat}}$. In general, the values were large for both simulations (>3 Å) indicating strong deviations from $G2S_{\text{NMR}}$. If the highly flexible regions were excluded from the calculation, the values would decrease pronouncedly to 1.4 and 1.3 Å, for $G2S_{\text{NMR-vac}}$ and $G2S_{\text{NMR-wat}}$, respectively. For $G2S_{\text{NMR-wat}}$, a rapid increase of the $\text{RMSD}_{\text{back}}$ was observed in the first 4 ns followed by a strong fluctuation which slowly leveled off after 20 ns. This showed that the conformation was finally stabilized with no significant drift. The fluctuation corresponded to the highly mobile terminal regions (Thr1 – Thr9) and (Leu65 – Leu71) as depicted in Figure 3 (high RMSF values) which was in agreement with earlier experimental results [26, 35]. On the other hand, there were no wild fluctuations in the $\text{RMSD}_{\text{back}}$ for $G2S_{\text{NMR-vac}}$. Instead, the value remained almost plateau throughout the whole simulation indicating that the motions of the residues were less mobile as depicted from the low RMSF values for $G2S_{\text{NMR-vac}}$ (Figure 3). This behavior agreed that the removal of water molecules had increased the conformational rigidity as claimed by previous experimental study [36]. The differences between the averaged NMR structures, $G2S_{\text{NMR-vac}}$ and $G2S_{\text{NMR-wat}}$ from the native $G2S_{\text{NMR}}$ were illustrated in Figure 4 with $\text{RMSD}_{\text{back}}$ of 3.45 Å and 3.41 Å for both averaged structures, respectively.

THE EFFECTS OF GAS PHASE ON THE PROTEIN CONFORMATION

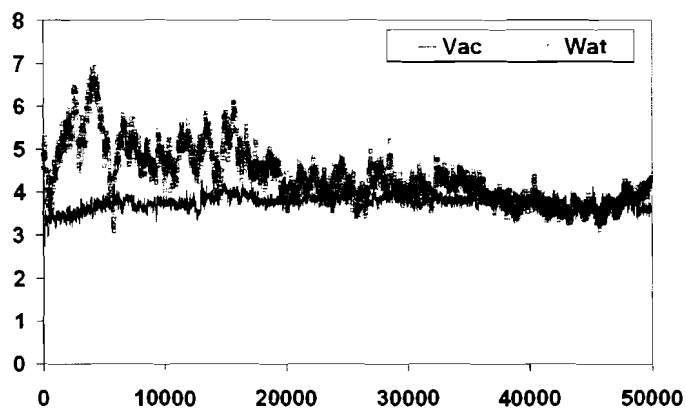


Figure 2 Time evolution (ps) of $\text{RMSD}_{\text{back}}$ (Å) for $\text{G2S}_{\text{NMR-vac}}$ and $\text{G2S}_{\text{NMR-wat}}$.

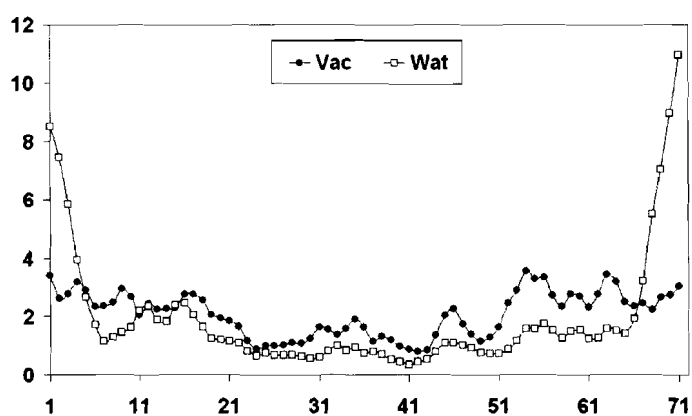


Figure 3 Root-mean square fluctuations (RMSF) (Å) shown for each residue

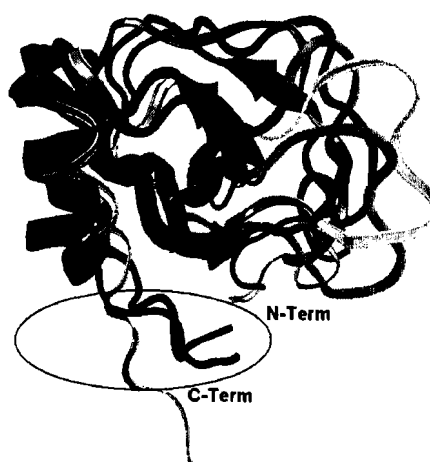


Figure 4 Superimpose between $\text{G2S}_{\text{NMR-vac}}$ (Red ribbon) and $\text{G2S}_{\text{NMR-wat}}$ (Blue ribbon) and G2S_{NMR} (yellow ribbon). N-Term (N-terminus), C-Term (C-terminus). The circled and blurred regions correspond to the highly mobile C-termini region and N-loop region, respectively.

Secondary structures analysis

Overall, the G2S_{NMR-wat} (Figure 5) shows good conservation of the α -helix and the three β -strands as compared to G2S_{NMR-vac} (Figure 6) indicating that simulation *in vacuo* tended to disrupt the stability of the secondary structures. This finding was in contrast with the previous remark that proteins in gas phase were more stable in preserving the secondary elements due to the excessive amount of protein-protein hydrogen bonds [37, 38].

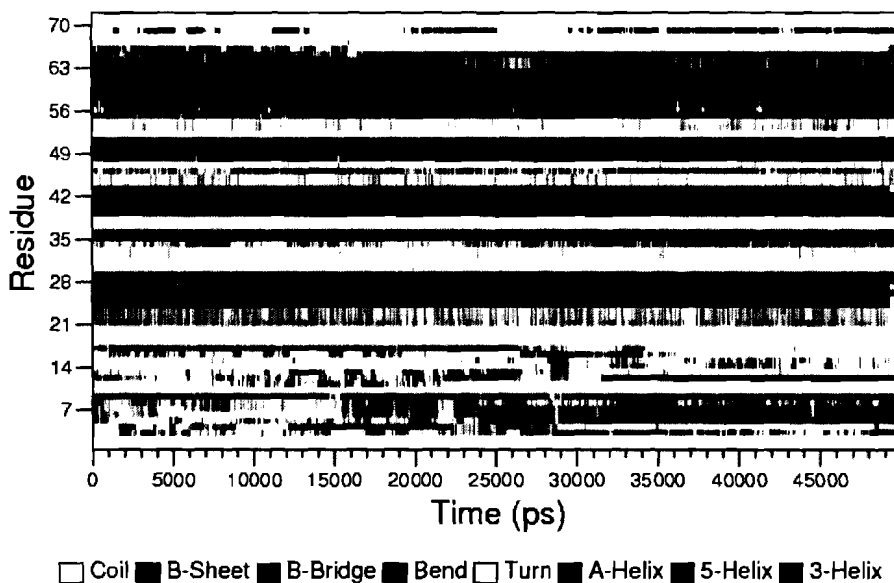


Figure 5 Time evolution (ps) of secondary structure formation for G2S_{NMR-wat}

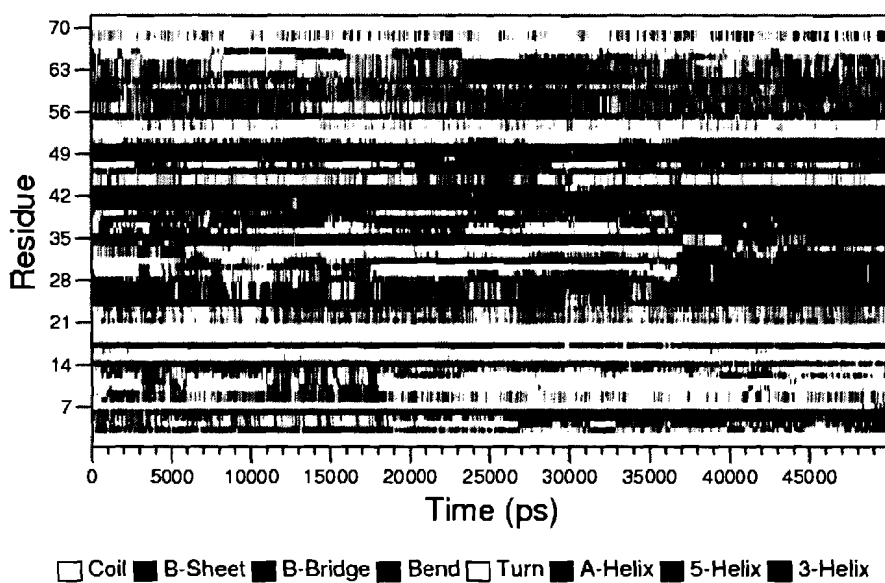


Figure 6 Time evolution (ps) of secondary structure formation for G2S_{NMR-vac}.

THE EFFECTS OF GAS PHASE ON THE PROTEIN CONFORMATION

Hydrogen bond analysis

Further investigation however revealed that the average total number of hydrogen bonds in $G2S_{NMR-vac}$ and $G2S_{NMR-wat}$ fluctuated around the same range, 38 and 42 bonds, respectively (Figure 7). Previous study also shown that 3_{10} helix was more stable in the gas phase than in solvated environment [39]. However, our finding showed that the 3_{10} helix was more preserved in the presence of solvent (Trp21 – Trp23) and almost disappeared in the gas phase simulation. *In vacuo*, the presence of the hydrogen bonds between Lys55 and Val58 and between Tyr61 and Leu65, both involved in the α -helix formation only occupied around 9% and 5% of the 50 ns simulation time, respectively. As for the condensed phase simulation, the corresponding hydrogen bonds were present for 50% and 39% of the simulation time. This might be due to the fact that in the absence of water, the hydrogen bonds that formed these helices tended to re-orientate as to satisfy those hydrogen bonding partners that were supposed to form interactions with water molecules.

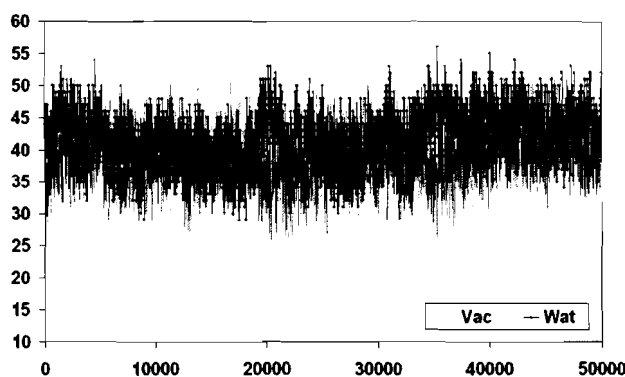


Figure 7 Time evolution (ps) of total number of hydrogen bonds for $G2S_{NMR-vac}$ and $G2S_{NMR-wat}$.

Solvent accessible surface area analysis

Figure 8 shows the development of SASA as a function of time calculated for both $G2S_{NMR-vac}$ and $G2S_{NMR-wat}$. The total SASA for the vacuum simulation was lower than the total SASA for the solvated system, which was in agreement with previous findings [37, 38]. Also shown was the total SASA contributed by the polar amino acids and as expected, the values were much higher in the solvated system than in the gas phase. This trend could be understood from the fact that these extended polar residues tended to collapse to the surface of the protein in the absence of water molecules.

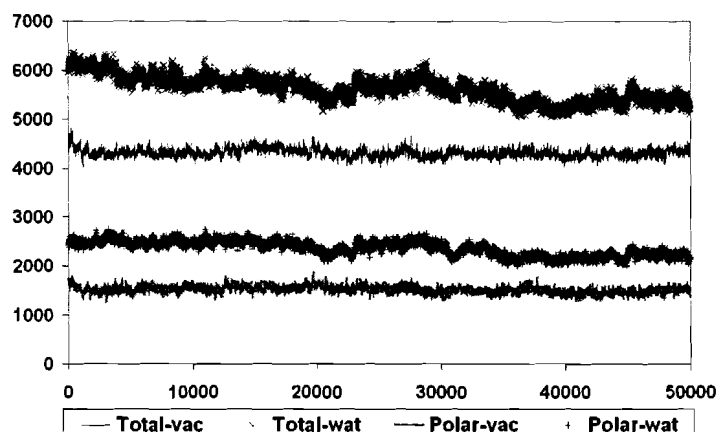


Figure 8 Time evolution (ps) of SASA (\AA^2) for $G2S_{\text{NMR-vac}}$ and $G2S_{\text{NMR-wat}}$

The consequence of the collapse led to the shrinking of the structure which was shown in Figure 9. As expected, the structure became more compact *in vacuo* than in solution that agreed well with the experimental study [22]. The R_{gyr} for $G2S_{\text{NMR-wat}}$ experienced a significant reduction compared to the more stable trend showed by $G2S_{\text{NMR-vac}}$. This showed that the protein conformations were rigid and hardly experienced significant conformational changes as to what observed in the solvated protein.

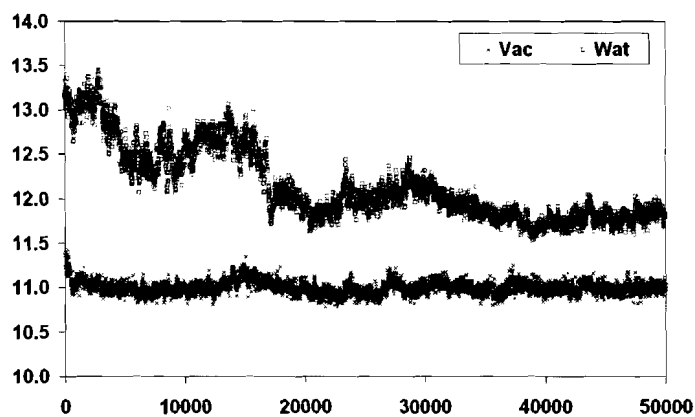


Figure 9 Time evolution (ps) of R_{gyr} (\AA) for $G2S_{\text{NMR-vac}}$ and $G2S_{\text{NMR-wat}}$

4.0 DISCUSSION

It was previously demonstrated that simulating proteins in vacuo tended to suffer from serious artifacts, among which small positional fluctuations, small radius of gyration and excessive number of protein-protein hydrogen bonds [14]. Current work showed both agreement and discrepancy on the influence of gas phase on proteins with that of earlier studies. The gas phase simulations produced highly compact structures with low SASA values than that of the solvated one. The conformers were rigid with the residues packing closely to each other. This was explained by the tendency of the extended residues especially the polar side chains to collapse together forming intra-solute interactions such

THE EFFECTS OF GAS PHASE ON THE PROTEIN CONFORMATION

as electrostatic and van der Waals interactions. Thus, the flexibilities of the N-terminal regions as well as the residues side chains were constrained by these non-bonded forces in the absence of solvent. This was shown in Table 5.4 by the low van der Waals energy for the vacuum simulation ($G2S_{NMR-vac}$) that suggested strong inter-atomic interactions as compared to that in solution ($G2S_{NMR-wat}$).

Proteins are highly charged molecules and correct treatment of the long range coulombic interactions is crucial for the protein to function properly. The presence of water solvent is important in screening the large electrostatic interactions. This phenomenon was shown in Table 5.4 in which the average protein-protein electrostatic contributions *in vacuo* were much higher than in the solvated phase. This proved that water is important in reducing the large electrostatic forces that resulted from Coulombic repulsions. The positive sign indicated that the force destabilized the protein conformations. While this causes problem in structural stability in gas phase, it will not be a problem in the solvated system since the presence of protein-solvent and solvent-solvent electrostatic contributions will reduce this high energy to a much lower value.

Table 1 Averaged values of the various energetic components for the gas phase and solvated phase simulations of the 1G2S native structures.

		Bond	Angle	Dihedral	EEl	vdW
$G2S_{NMR-vac}$	Avg	213.27	590.88	732.96	245.12	-337.58
	Max	251.35	633.42	761.91	372.73	-304.53
	Min	180.18	554.78	710.46	157.69	-361.61
$G2S_{NMR-wat}$	Avg	218.00	593.94	754.34	162.77	-240.59
	Max	247.57	650.60	779.64	335.26	-206.06
	Min	187.74	560.52	726.69	17.04	-272.52

Avg = average value, Max = maximum value, Min = minimum value, Bond = bond energy, Angle = angle energy, Dihedral = dihedral energy, EEl = protein-protein electrostatic energy, vdW = van der Waals energy, Energy terms in Kcal/mol.

In the absence of water solvent, the extended polar side chains of the protein were often found collapsed onto the protein surface to form hydrogen bonds with protein partners. As previously claimed, there should be a large increase in the intra-solute hydrogen bonds [14, 37, 38]. However, the current results showed that it was not the case. The effects of gas phase simulation on the protein 1G2S did not amplify the protein-protein hydrogen bonds; instead it resulted in almost the same number with that of the solvated protein. Furthermore, some of the hydrogen bonds that were crucial in maintaining the secondary structural elements were disrupted due to the competing of hydrogen bonding partners between the existing bonds and the collapsed polar side chains. This incurred poor stability in the secondary structures as was observed from Figure 7, which again contradicted with previous reported studies. In gas phase, the protein 1G2S formed unstable hydrogen bonds with very short residence times, suggested that these hydrogen bonds were constantly swapping partners. While the hydrogen bond donor of Glu28 formed a stable hydrogen bond with Ser7 in $G2S_{NMR-wat}$, this hydrogen bond donor however kept on swapping partner with Thr42 and Ser26 to form unstable hydrogen bond interactions in $G2S_{NMR-vac}$. In another example, the hydrogen bond between Leu19@O and Tyr61@OH in $G2S_{NMR-wat}$ appeared to be highly stable covering

90.5% of the sampling period. However, this bond was disrupted in G2S_{NMR-vac} when Leu19@O formed a hydrogen bond with the competing highly charged amino group of Lys60 that collapsed on the protein surface instead of being extended away from the protein. Furthermore, this non-native hydrogen bond was less stable with very low residence time, 6.46%.

These observations suggested that the presence of water solvent is highly crucial towards the formation of correct hydrogen bonds in protein to avoid from chaotic, less stable hydrogen bonding networks. This is in line with the perception of water acting as a lubricant that facilitates the search for correct hydrogen bonding partners in proteins [40, 41]. The current findings showed that proteins in gas phase were not necessarily forming excessive hydrogen bonds as what previously claimed. In this case, the presence of the non-native hydrogen bonds mostly resulted from the breaking of the existing native hydrogen bonds. These newly formed hydrogen bonds were unstable and kept on breaking, forming and swapping partners. This ensured consistent total numbers of hydrogen bonds for the gas and solvated protein.

5.0 CONCLUSIONS

Water played a crucial role in determining the structure and the functionality of globular proteins. This study showed that proteins in vacuum suffered from too compact structures, poor stability in preserving the secondary structural elements (especially the α -helix) and finally short life hydrogen bonding network.

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