Research Article

Quantum Density Functional Theory Studies on Additive Hydration of Tuftsin Tetrapeptide

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Density functional B3LYP method has been used to study the molecular properties of the tuftsin tetrapeptide (threonine-lysine-proline-arginine) and its retro form (arginine-proline-lysine-threonine). The influence of single water molecule on the conformations and relative stabilities of solvated tuftsin complexes has been studied by placing the water molecule at the individual amino acid residues of both the tuftsin complexes. The contribution of four water molecules to the system energetics of tuftsin complexes has also been analyzed. The conformational changes occurred in the solvated tuftsin complexes have been explored through their dihedral angles. The tuftsin is found to be sensitive to structural changes, and our results indicate that water-tuftsin hydrogen bonds (H-bonds), in addition to intramolecular H-bonds, stabilize the β-turn structure with H-bonds between threonine and arginine residues of tuftsin. Difference in the stability of the hydrated complexes is confined to the amino acid residues at which the water molecule is attached to tuftsin. The interaction energy calculations have been used to investigate the strength of the intermolecular H-bond interactions. The AIM theory and NBO analysis were employed to survey the H-bonding patterns in hydrated tuftsin complexes. The maximum ellipticity value (0.129) is noted for the Cα-H (Arg)⋯O (W) interaction in Tuftsin⋯4W complex which indicates the higher chance of structural deformation under external perturbations. The interactions between oxygen lone pairs in water and C-H antibond orbitals of tuftsin and retro tuftsin complexes exist with $E^{(2)}$ in the range of 4.03-5.7 and 3.59-4.14 kcal/mol, respectively.

1. Introduction

Tuftsin is a naturally occurring linear tetrapeptide with amino acid sequence of threonine- (Thr-) lysine- (Lys-) proline- (Pro-) arginine (Arg-), and it was first identified by Nishioka et al. [1]. Tuftsin corresponds to residues 289-292 of leukokinin, a cytophilic γ-globulin, and it has been considered to be responsible for the activity in the stimulation of phagocytosis (mechanism of removing pathogens) [2], and any modification in the sequence results in the loss of activity. Tuftsin has been found to exhibit several biological activities connected with immune system function [3], and its deficiency is involved in severe infections in skin, lymph nodes, and lungs [4]. In addition, tuftsin shows antitumor
activity [5], and it can be used as an activator of macrophages in cancer treatment [6], and it is supposed that tuftsin deficiency leads to the risk of bacterial infection in HIV-positive patients [7]. Apart from the above natal significance, because of the broad spectrum of the other biological activities such as mobility of granulocytes, humoral antibody formation, and tumoricidal activity of phagocytic cells as well as bacterial killing properties [8], tuftsin has been considered as the subject of several theoretical and experimental studies with a view to analyze its structure-activity relationships.

Siemion et al. [9] performed 13C-NMR measurements for the tuftsin and its analogs in D2O to understand the role of proline residue in the creation of the tetrapeptides. The same research group [10] performed circular dichroism measurements with tuftsin and its few sequential isomers and showed that the tuftsin’s lowest tendency to form turn. Tzehoval et al. [11] tested tuftsin’s capability to augment phagocytosis and its function as an immunogenic stimulator. Perkowska et al. [8] investigated the antibacterial properties of tuftsin and its analogs on twenty bacterial strains and found that tuftsin possesses the strongest antibacterial activity of all the analogs tested. Fröidlin and Gottlieb [3] analyzed the structure-function relationships of tuftsin and found a direct correlation between tuftsin levels in the human blood serum of normal as well as various pathologically origin and susceptibility to bacterial infections. Najjar [4] discussed the clinical and physiological aspects of tuftsin deficiency syndromes and stated that the therapy for these syndromes has been limited to gamma globulin injection along with appropriate chemotherapy. Fitzwater et al. [12] studied the conformational energy space of tuftsin and derived the characteristics of the molecular structure of tuftsin from the group of computed minimum energy conformations. Blumenstein and coworkers [13] studied the solution conformation of tuftsin using the NMR spectroscopy and did not find any preferred conformation of tuftsin.

Nishioka et al. [14] synthesized tuftsin using the Merrifield solid-phase method and noted that the synthetic tuftsin has the same physical, chemical, and biological properties as that of its natural compound. Ursi and his coworkers [15] have studied tuftsin in water and dimethyl sulfoxide solutions using 2D NMR spectroscopy. Sekacis et al. [16] found the tendency of β-formation of tuftsin in dimethyl sulfoxide solution. O’Connor and others [17] employed high temperature quenched molecular dynamics to search the conformational space of tuftsin in dimethyl sulfoxide and water. Siemion et al. [18] studied the 13C-NMR and circular dichroic spectra of tuftsin in connection with their hypothesis that β-turn is its biologically active conformation and concluded that Lys-Pro residues are important for the tuftsin conformation.

Nikiforovich [19] proposed a quasi-cyclic conformation of tuftsin with $\phi_{135} = -125^\circ$, $\psi_{135} = 120^\circ$, and $\psi_{pro} = -50^\circ$. Chipens and company [20] experimentally synthesized a biochemically active cyclic analog of tuftsin with the amide bond located between the Arg carboxyl group and the Lys amino group. Siddiqui et al. [21] studied the conformation of tuftsin in water, trifluoro ethanol, methanol, and dioxane by circular dichroism spectroscopy. Siemion and Kluczyk [22] have presented the occurrence of tuftsin and its retro sequences in proteins, their synthesis, and important properties like antigenic, antitumor, antiviral, etc. in their commendable review work on tuftsin. Kothekar et al. [23] studied the conformational flexibility of tuftsin using molecular dynamics; all atoms based potential with systematic search in addition to simulated annealing molecular dynamics techniques and concluded that irrespective of any starting conformation, tuftsin assumes highly folded structure with a week H-bond between Lys-2 CO and Arg-4 NH atoms. Sobczyk et al. [24] investigated the IR spectra of ten tuftsin analogues containing D-amino acid residues in solid state, chloroform, and carbon tetrachloride solutions. Valdeavella et al. [25] examined the conformational properties of tuftsin with 1 ns molecular dynamics simulation in water and in 1.0 M NaCl solution and compared their results with a cyclic tuftsin analog, cyclo (threonine-lysine-proline-arginine-glycine). Fröidlin and Najjar [26] analyzed the synthesis, chemical, biological, and clinical potentials of tuftsin. The primary effect of tuftsin after it binds to specific cell surface receptor (a protein molecule found on the surface of a cell which receives chemical signals originating externally from the cell, and through binding to a receptor, these signals control the activity of the cell) is to stimulate the functions of macrophages. It is interesting to note that a tuftsin receptor can recognize not only tuftsin but also retro tuftsin, a tetra peptide with amino acid sequence of Arg-Pro-Lys-Thr. Though the phagocytosis stimulating activity of the retro tuftsin was inconsistent [22] in general, Yasumura et al. [27] reported its activity as same as tuftsin. The effects of solvation on biomolecules are extremely essential since the biological processes and solution phase are inseparable. Proteins are biomolecules which are at the center of action in biological processes, and every property that characterizes a living organism is affected by proteins [28]. Water, known as universal solvent, has the ability to act as hydrogen bond (H-bond) donor and acceptor that leads to a strong preference of water as a solvent for biomolecules [29]. The biomolecule- (protein-) water interaction is predominantly important since the presence of life is strongly tied to the presence of water [30]. In this work, we describe a study of the influence of an individual water molecule positioned at each amino acid residue of unsolvated tuftsin, a phagocytosis-stimulating tetrapeptide which functions in the immune system and its retro form. In addition, the solvation effect of four water molecules on the tuftsin complexes has also been studied where our goal is to acquire a more comprehensive understanding of the interaction of water with proteins in general. Samir et al. [31] used molecular dynamics simulations to identify a tuftsin conformation with higher biological activity and found 46 super analogs with more effectiveness which leads to give semixtended conformation in the central part of the residues as observed in the crystal structure. In their study, Kovalenko et al. [32] used DFT methods to study the complexation between host (cucurbit[7]uril (CB7)) and guest (protonated tuftsin) through fluorescence and concluded that the structural binding motif engages H-bonding. Tuftsin which is a natural immunomodulator has been considered as a base to create
new drugs that are effectively used in the treatment of various diseases related to nervous system [33]. Tuftsin-containing liposomes (Tuft-liposomes) behave as a potential drug and vaccine carriers and found to increase the anticancer activity in mice [34]. Recently, tuftsin has been identified as a natural molecule against SARS-CoV-2 infection by Huang et al. [35].

2. Computational Details

The density functional theory (DFT) with Becke’s three parameter exact exchange functional (B3) [36] combined with gradient-corrected Lee-Yang-Parr correlation functional (LYP) [37] has been employed to optimize the tuftsin and its retro form using 6-31G* basis set. The structure, molecular properties, and stability of the tuftsin complexes hydrated with single water molecule attached to their every amino acid residue and complexes with four water molecules are predicted at the same level of theory. For energy comparison, the single point energy calculation has been performed at second order Moller Plesset Perturbation theory (MP2) [38] for the geometries optimized at B3LYP level of theory. The vibrational frequency analysis has been performed to characterize the stationary points. The interaction energy for optimized water interacting complexes has been corrected for the basis set superposition errors (BSSE) using the counterpoise method of Boys and Bernardi [39] using the equation

\[ E_{\text{int}}(\text{corr}) = E_{AB}(AB) - [E_A(AB) + E_B(AB)], \]

where \( E_{AB} \) (AB) is the energy of the complex and \( E_A \) (AB) and \( E_B \) (AB) are the energies of monomers A and B with full complex basis set by setting the appropriate nuclear charge to zero, which is located at the same intermolecular configuration as in the complex. Based on Bader’s theory [40], the AIM calculation was carried out. In NBO analysis, for each donor NBO (i) and acceptor NBO (j), the stabilization energy \( E^{(2)} \) associated with i \( \rightarrow \) j is given by

\[ E^{(2)} = q_i F^2(i,j) / \varepsilon_i \varepsilon_j, \]

where \( q_i \) is the orbital occupancy of the \( i \)th donor, \( \varepsilon_j \) and \( \varepsilon_i \) are diagonal elements (orbital energies), and \( F(i,j) \) are off diagonal elements associated with NBO Fock matrix. The Gaussian 03W program [41] is used to perform all the above calculations.

3. Results and Discussion

3.1. Structure and Energy. For convenience, the mono hydrated tuftsin and retro tuftsin complexes at four amino acid residues Thr, Lys, Pro, and Arg are represented as TTW, TLW, TPW, and TAW and RTTW, RTLW, RTPW, and RTAW, respectively. The solvated tuftsin complexes with four water molecules are represented as Tuftsin...4W and Retro tuftsin...4W and are depicted in Figures 1 and 2, respectively, along with the mono hydrated complexes where the atom numbering convention is detailed and the values mentioned are in Å. According to Siemion and Konopinska [42], the tuftsin was considered to be sensitive to structural changes, and it is substantiated by the energy of isolated tuftsin which is 8.16 and 4.39 kcal/mol lower than its retro form at MP2 and B3LYP levels of theory, respectively. The optimized structural parameters (selected values) of the bare and hydrated tuftsin are given in Table 1, and the structural parameters for retro tuftsin are listed in Table 2. The conformational changes are revealed through representative dihedral angles of tuftsin, and its hydrated complexes calculated at B3LYP/6-31G* level of theory and are tabulated in Table 3. Their variations are shown in Figure 3. Comparison of the geometrical parameters of the isolated and hydrated complexes suggests that the solvation influences the geometry of tuftsin and retro tuftsin complexes in the presence of H-bonds and also involves different degrees of conformational changes which are reflected by the variations in the dihedral angles (Figure 3). Our previous studies confirm the influence of solvation on the structure of an amino acid [43], dipeptides [44, 45], and a tripeptide [46].

Compared to tuftsin, all the C-N bonds of Arg are observed to be shortened in retro tuftsin except C29-N30 and N28-C20. Only marginal deviations are noted for Arg C-C and C=O bonds in the tuftsin complexes. All the dihedral angles show minor variations, and a notable difference in the dihedral angle associated with C34-C (\( \chi_{\text{Lys}} \)) is spotted in TAW compared to its parent molecule. The C1-N of Lys is found to be stretched while the C-N bond associated with the side chain is shortened in retro tuftsin compared to tuftsin. No much difference is detected for C43-C and C=O bonds. Larger variation is noted in the dihedral angle associated with the C5-C6 of the Lys side chain (\( \chi_{\text{Lys}} \)) of TLW where the water molecule makes one O-H...O H-bond with C=O of Lys (1.877 Å), another O-H...O H-bond with carboxylic C=O of Arg (1.982 Å) and one N-H...O H-bond (1.943 Å) with Thr residues which resembles to the results pertaining to H-bonds obtained in our previous studies [45, 46]. Being the end residue, there is no much variation in Thr conformations between tuftsin and its retro form.

While considering Pro residue, being at the third position of the peptide chain of tuftsin, it plays an important role in the stabilization of the biologically active conformation of tuftsin [42, 47]. Based on Siemion’s approach, the \( \Psi_{\text{pro}} = \theta + 60° \) where \( \theta = C^\beta - C^\alpha - C^\gamma - O \) of promoiety. Here, \( \theta_{\text{pro}} \) (C1 - C2 - C9 - O10) = -73.67° and \( \Psi_{\text{pro}} = -13.67° \) which exactly coincides with \( \Psi_{\text{pro}} (-13.77°) \) of our present tuftsin conformation. But Siemion has found the \( \Psi_{\text{pro}} \) values as closer to -50 and -60°, respectively, in his another work on tuftsin [18] which is comparable with \( \Psi_{\text{pro}} (-74.36°) \) of tuftsin in the present conformation. Since the observed \( \Phi_{\text{pro}} \) of the present tuftsin conformation accurately matches with the \( \Phi_{\text{pro}} \) in its crystal structure (–65 ± 15°) [48], this conformation is believed to be within the allowed region of conformational space available to the Pro backbone in 3rd position of a β-turn [31, 49]. The solvated tuftsin complexes with one and four water molecules retain the β-turn structure with \( \Phi_{\text{pro}} \) ranges from -73.03 to -82.89°.
Earlier studies suggest that one probable tuftsin conformation involves a β-turn with H-bond located between Thr and Arg [50] as is observed in the present study. Three types of intramolecular H-bonds between Thr and Arg (O57(Thr)⋅H12-N11(Arg), H61(Thr)⋅O17-C15(Arg), and O64(Thr)⋅H33-N30(Arg)) are observed in tuftsin and its mono hydrated complexes within the distances ranging from 2.07 to 2.49 Å, 2.17 to 2.41 Å, and 2.14 to 2.22 Å, respectively, which is shown in Figure 4. This yet again validates the β-turn conformation of tuftsin in its present form which is its biologically active conformation [18] of the peptide chain as previously reported by Konopinska et al. [50] and Sobczyk et al. [51] and supported by Kahn and Devens [52]. But only O57 (Thr)⋅H12-N11(Arg) H-bond is...
Table 1: Selected geometrical parameters (bond lengths (in Å), bond angles (in degree)) of tuftsin and its hydrated complexes calculated at B3LYP/6-31G* level of theory. For labeling of atoms, refer Figure 1.

| Parameter | Tuftsin | TTW | TLW | TPW | TAW | Tuftsin...4W |
|-----------|---------|-----|-----|-----|-----|-------------|
| C56-N55   | 1.352   | 1.347 | 1.346 | 1.352 | 1.352 | 1.341 |
| N55-C40   | 1.460   | 1.462 | 1.454 | 1.460 | 1.459 | 1.458 |
| C40-C38   | 1.543   | 1.542 | 1.542 | 1.543 | 1.542 | 1.541 |
| N3-C2     | 1.468   | 1.471 | 1.470 | 1.466 | 1.468 | 1.470 |
| C2-C9     | 1.542   | 1.542 | 1.544 | 1.542 | 1.542 | 1.544 |
| C9-N11    | 1.367   | 1.369 | 1.364 | 1.355 | 1.367 | 1.355 |
| N11-C13   | 1.453   | 1.454 | 1.455 | 1.458 | 1.459 | 1.459 |
| C13-C14   | 1.549   | 1.547 | 1.550 | 1.551 | 1.540 | 1.550 |
| C66-C63-C58 | 113.63 | 113.70 | 112.57 | 113.68 | 113.99 | 113.36 |
| C63-C58-C56 | 111.46 | 111.22 | 109.67 | 111.47 | 112.37 | 110.34 |
| C58-C56-O57 | 123.15 | 122.13 | 121.66 | 123.19 | 123.52 | 122.75 |
| C58-C56-N55 | 113.77 | 114.17 | 116.46 | 113.77 | 113.61 | 114.50 |
| C56-N55-C40 | 121.55 | 122.11 | 119.96 | 121.42 | 121.11 | 122.31 |
| N55-C40-C38 | 109.54 | 108.15 | 109.88 | 109.51 | 109.38 | 109.19 |
| C40-C38-N3 | 117.79 | 118.17 | 118.40 | 117.67 | 117.84 | 118.26 |
| C38-N3-C2 | 119.22 | 118.86 | 119.91 | 119.36 | 119.44 | 119.46 |
| N3-C2-C9  | 114.92 | 114.66 | 116.14 | 115.03 | 115.28 | 115.35 |
| C2-C9-N11 | 116.94 | 116.98 | 118.01 | 117.11 | 117.23 | 118.34 |
| C9-N11-C13 | 121.04 | 120.91 | 120.21 | 123.86 | 120.98 | 122.57 |
| N11-C13-C14 | 110.53 | 110.42 | 110.31 | 109.88 | 110.81 | 109.57 |
| C13-C14-C16 | 116.11 | 115.82 | 115.95 | 116.04 | 116.29 | 115.77 |
| C14-C16-C20 | 113.73 | 114.13 | 113.01 | 113.84 | 116.25 | 114.00 |
| C16-C20-N28 | 110.49 | 110.22 | 111.01 | 110.38 | 109.38 | 110.19 |
| C20-N28-C29 | 118.36 | 118.70 | 118.09 | 118.53 | 118.10 | 118.89 |

observed at 2.35 Å in Tuftsin...4W complex, and the β-turn structure is observed to be slightly disturbed with Ψ_pro = 4.22° instead of -13.67°. The H-bond between Pro-3 N (N3) and Arg-4 NH (N11-H12) has been observed in tuftsin, and its hydrated complexes with distances range from 2.33 to 2.44 Å which provide extra stabilization for this tuftsin conformation and belong to the first family (type IV β-turn) predicted by O’Connor et al. [17].

The Arg 1←Lys 3 (C15-O17...H53) type intramolecular H-bond exists in isolated and hydrated retro tuftsin complexes (Figure 5) with distances varying from 2.196 to 2.396 Å except RTLW and Retro tuftsin...4W complexes. In RTLW, the above intramolecular H-bond is replaced by two intermolecular H-bonds O76-H77(W)...O17(Arg) and O76-H78(W)...O39(Lys) at 1.917 and 1.947 Å, respectively, as a result of addition of water molecule which acts as a channel between Arg and Lys residues. This causes an increase in the Arg 1←Lys 3 intramolecular distance in RTLW up to 4.978 Å. In Retro tuftsin...4W complex, this distance is found to be 5.066 Å. In this complex also, the Arg 1←Lys 3 H-bond is replaced by two intermolecular H-bonds O76-H77(W)...O17(Arg) and O76-H78(W)...O39(Lys) at 1.958 and 1.927 Å, respectively.

Another intramolecular H-bond Lys 3←Thr 4 (C55-O56...H75) is also traced in the retro tuftsin complexes (Figure 5). The most effective intramolecular interaction takes place on O56...H75 in RTLW (2.244 Å). In RTTW, the Lys 3←Thr 4 H-bond is increased equal to 3.221 Å (from 2.318 Å in isolated retro tuftsin) due to the interaction of water molecule at O56 of Thr which acts as protonic acceptor from water molecule and increases the intramolecular distance. It is appealing to note that in the Retro tuftsin...4W complex, due to the insertion of water molecule in between Lys and Thr, the Lys 3←Thr 4 H-bond is replaced by a N54-H75 (Lys)→O85(W) and O85-H86(W)...O56 H-bonds at 2.076 and 1.82 Å, respectively. It is worth mentioning that an intraresidual N-H...NH -bond is traced at Thr in isolated and hydrated retro tuftsin complexes with distances ranging from 2.079 to 2.149 Å. A weak H-bond between Lys 2 CO (C38-O39) and Arg 4 NH (N11-H12) is noted (3.007 Å) as is observed in the previous study by Kothekar et al. [23]. Except Φ_pro, (present -74.36° and previous -79.0°), all the other dihedral angles differ from the values reported by Ursi and coworkers [15] which illustrates that the present tuftsin conformation conflicts with the previously reported structure. Structural proposals of tuftsin made by different authors differ considerably even for studies performed in the same environment [15], but globally, the tuftsin conformation is referred to as “hairpins with split ends” as observed in the
The present study (Figure 1) and supported by Fitzwater et al. [12] in addition to Scheraga and coworkers [12] as one of the low energy conformations of tuftsin. The Cα (Thr)-Cα (Arg) distance of tuftsin in this present conformation is 5.09 Å (should be ≤7.0 Å for the structures to be in their trans conformation [15] corroborate its trans configuration).

Table 2: Selected geometrical parameters (bond lengths (in Å), bond angles (in degree)) of retro tuftsin and its hydrated complexes calculated at B3LYP/6-31G* level of theory. For labeling of atoms, refer Figure 2.

| Parameter   | Retro tuftsin | RTTW | RTLW | RTPW | RTAW | Retro tuftsin…4W |
|-------------|---------------|------|------|------|------|------------------|
| C29-N28     | 1.281         | 1.281| 1.280| 1.281| 1.286| 1.282           |
| N28-C20     | 1.452         | 1.447| 1.452| 1.452| 1.451| 1.447           |
| C20-C16     | 1.535         | 1.541| 1.534| 1.536| 1.535| 1.540           |
| C14-C13     | 1.550         | 1.553| 1.554| 1.548| 1.551| 1.549           |
| N11-C9      | 1.368         | 1.368| 1.351| 1.362| 1.369| 1.355           |
| C9-C2       | 1.543         | 1.543| 1.537| 1.548| 1.547| 1.543           |
| C1-C2       | 1.542         | 1.542| 1.547| 1.539| 1.540| 1.542           |
| C42-C43     | 1.540         | 1.541| 1.540| 1.540| 1.545| 1.543           |
| C43-C44     | 1.541         | 1.541| 1.541| 1.542| 1.538| 1.537           |
| C29-N28-C20 | 118.73        | 121.45| 118.87| 118.70| 117.45| 121.06       |
| N28-C20-C16 | 110.11        | 109.90| 110.22| 110.11| 110.58| 109.79       |
| C20-C16-C14 | 116.13        | 116.28| 115.99| 115.47| 116.96| 115.84       |
| C14-C13-O18 | 112.31        | 113.25| 112.29| 112.21| 112.65| 113.20       |
| C14-C15-O17 | 125.05        | 123.07| 124.98| 125.89| 124.96| 124.06       |
| C14-C13-N11 | 110.91        | 110.98| 110.93| 109.98| 110.46| 109.36       |
| C13-N11-C9  | 122.14        | 122.15| 123.07| 120.69| 122.50| 120.71       |
| N11-C9-C2   | 115.78        | 115.78| 116.50| 117.74| 115.77| 118.20       |
| C9-C2-N3    | 113.48        | 113.42| 113.35| 115.87| 113.06| 115.28       |
| C2-N3-C8    | 119.92        | 119.96| 119.68| 121.38| 119.71| 121.29       |
| N3-C8-C40   | 116.10        | 116.06| 116.41| 116.83| 115.92| 116.50       |
| C38-C40-C41 | 112.75        | 112.93| 112.45| 112.89| 113.73| 112.81       |
| C41-C42-C43 | 114.46        | 114.30| 114.50| 114.54| 113.32| 114.42       |
| C43-C44-N45 | 116.05        | 116.27| 116.08| 116.03| 114.98| 115.00       |

Table 3: The representative dihedral angles of tuftsin and its hydrated complexes calculated at B3LYP/6-31G* level of theory.

| Dihedral angles                               | Tuftsin | TTW   | TLW   | TPW   | TAW   | Tuftsin…4W |
|-----------------------------------------------|---------|-------|-------|-------|-------|------------|
| $\Psi_{\text{Thr}}$ (N60C58C56N55)            | 39.27   | 37.65 | 43.58 | 39.23 | 43.44 | 30.05      |
| $\omega_{\text{Thr}}$ (C58C56N55C40)           | -177.74 | -171.97| 178.61| -178.18| -177.10| -176.61    |
| $\chi_{\text{1Th}}$ (N60C58C56O57)             | -142.12 | -142.98| -136.96| -142.26| -137.53| -150.55    |
| $\Phi_{\text{1Ly}}$ (C56N55C40C38)             | 70.29   | 78.69 | 57.21 | 71.72 | 66.23 | 62.70      |
| $\Psi_{\text{1Ly}}$ (N55C40C38N3)              | -118.63 | -131.17| -127.66| -117.79| -123.34| -135.44    |
| $\omega_{\text{3A}}$ (C40C38N3C2)              | -179.12 | -179.31| -176.56| -178.65| -177.64| -176.77    |
| $\chi_{\text{1Ly}}$ (N55C40C41C42)             | -58.99  | -60.50| -70.22| -59.48| -60.71| -63.06      |
| $\chi_{\text{2Ly}}$ (C40C41C42C43)             | -176.34 | -176.43| 106.41| -177.92| -178.41| 179.91      |
| $\Phi_{\text{Pro}}$ (C38N3C2C9)                | -74.36  | -76.82| -74.24| -77.08| -73.03| -82.89      |
| $\Psi_{\text{Pro}}$ (N3C2C9N11)                | -13.77  | -9.81 | -8.62 | -10.16| -10.65| 4.22        |
| $\omega_{\text{Pro}}$ (C2C9N11C13)             | 171.98  | 168.33| 176.45| 172.78| 173.58| -178.46     |
| $\chi_{\text{1Pro}}$ (N3C2C1C5)                | 31.71   | 29.32 | 34.13 | 32.11 | 32.21 | 32.57       |
| $\chi_{\text{2Pro}}$ (C2C1C5C4)                | -37.60  | -38.16| -38.05| -37.80| -37.76| -38.22      |
| $\Phi_{\text{Arg}}$ (C9N11C13C15)              | -99.29  | -90.96| -92.07| -103.79| -96.85| -95.68      |
| $\chi_{\text{1Arg}}$ (N11C13C14C16)            | 173.69  | 175.54| 170.17| 174.30| -173.89| 179.15      |
| $\chi_{\text{2Arg}}$ (C13C14C16C20)            | -94.02  | -93.54| -95.38| -94.14| -75.62| -92.33      |
This interatomic distance between the alpha carbons of Thr and Arg in the hydrated tuftsin complexes is observed to vary between 5.11 and 5.67 Å and shows that the hydration does not alter the trans structure of tuftsin tetrapeptide.

It is to be noted that TLW in which the water molecule bridges Thr, Lys, and Arg of tuftsin is the lowest energy complex (interaction energy is -13.99 and -13.05 kcal/mol at B3LYP and MP2 levels of theory, respectively) among the complexes formed from the binding of a water molecule at oxygen sites of four amino acid residues of tuftsin. In this structure, Lys C=O and Arg carboxylic C=O are acting as proton acceptor, and Thr N-H is acting as a proton donor with H-bond distances of 1.877, 1.982, and 1.943 Å, respectively. It is also interesting to examine the relative stability...
of monohydrated tuftsin and retro tuftsin complexes as a function of interaction energy calculated at B3LYP/6-31G* (Figure 6) and MP2/B3LYP/6-31G* levels of theory. For the mono hydrated tuftsin complexes, both the levels of theory predicted the same order of stability as TLW > TAW > TTW > TPW. As per the stability order, TAW turns out to be the second stable mono hydrated complex with a relative energy of 6.28 and 8.79 kcal/mol at B3LYP and MP2 levels of theory, respectively. Here, the water molecule avoided the Lys and Pro moieties and ties the two end residues of tuftsin to make a closed structure acting as arbitrator by donating one proton to Thr and Arg each (H-bonds at 1.926 and 1.976 Å, respectively) and accepting one from Arg (1.949 Å).

With -6.65 kcal/mol interaction energy, TTW stands as the third member in the stability order. The water molecule in TTW is involved in bidentate mode forming two H-bonds, one with Thr oxygen and the other with Cα-H hydrogen of Pro residues. The TPW is found to be the least stable mono solvated tuftsin complex with relative energy difference of 10.79 kcal/mol calculated at B3LYP level of theory while the TTW is found to be the least stable complex at MP2 level of theory with a relative energy of 12.93 kcal/mol. In TPW, the water molecule is attached between Pro C=O oxygen and Arg Cα-H hydrogen with H-bond distances of 1.885 and 2.308 Å, respectively. In Tuftsin…4W complex, the four binding water molecules retain similar interactions with the four amino acid residues as in their individual mono hydrated complexes with marginally longer H-bonds. Same trend has been observed in the formation of C=O oxygen and Cα-H hydrogen bonds previously [46]. The interaction energy difference between the Tuftsin…4W and the most stable mono hydrated (TLW) complexes is found to be 23.97 and 22.91 kcal/mol at B3LYP and MP2 levels of theory, respectively. This difference is obviously due to the added number of H-bonds between the tuftsin tetra peptide and water molecules. Ten strong H-bonds (two N-H…O, two C-H…O, and six O-H…O types) are traced in this four water complex with H-bond lengths varying from 1.858 Å to 2.316 Å.

In monohydrated retro tuftsin complexes, the most effective interaction takes place on the Arg carboxylic oxygen (O18-H19) in RTAW, the most stable monohydrate, resulting in the strengthening of O-H…O bonds (1.783 Å) and higher interaction energy (-14.24 kcal/mol). In RTAW, the binding water is acting as protonic acceptor and donor simultaneously and forming a closed interaction at Arg–COOH with H-bond lengths 1.783 and 1.881 Å, respectively. In the Retro tuftsin…4W complex, still stronger interactions persist at the same carboxylic oxygen of Arg (1.765 Å) along with additional eleven H-bonds (1.82-2.376 Å) render almost three times higher interaction energy (-41.85 kcal/mol) for the above complex than the most stable mono hydrated retro tuftsin complex. At all applied levels, the stability order for hydrated retro tuftsin complexes based on relative and interaction energies is found to be the same, and it is predicted to be RTAW > RTTW > RTLW > RTPW. An energy difference of -1.51 kcal/mol is noted between the most and the next stable hydrated retro tuftsin complexes exactly as that of their tuftsin counterparts (-1.5 kcal/mol).

It is interesting to note down that irrespective of the position in the peptide chain, monohydrated tuftsin complexes at Pro residue stand last at the stability order with minimum interaction energy among the monohydrates (Tables 4 and 5). The energy differences between the monohydrated tuftsin, retro tuftsin complexes at Pro, and the
corresponding most stable monohydrated complexes are found to be 8.22 and 7.59 kcal/mol and 7.97 and 6.71 kcal/mol at B3LYP and MP2 levels of theory, respectively. With -12.73 and -12.3 kcal/mol interaction energy at B3LYP and MP2 levels of theory, respectively, the RTTW has been the second nominee in the stability order of retro tuftsin complexes. In RTTW, the binding water acts as intraresidual via-duct within Arg through one O-H…O (2.041 Å) and one N-
H...O (1.98 Å) interactions, and it makes interresidual link between the two end amino acid residues Arg and Thr via another O-H...O interaction at 1.89 Å.

In RTAW, the water molecule acts as a biprotonic donor, one to Arg carboxylic oxygen (1.917 Å) and other to Lys C=O oxygen atom (1.947 Å). It accepts one proton from the Arg N-H group with H-bond distance of 2.08 Å. The water molecule acts as a biprotonic donor as well as acceptor and links the ends of the Arg carboxylic group. The bonds present in the hydrated tuftsin and retro tuftsin complexes are stretched as a result of hydration at the sites related with the atoms involving hydration interactions is stretched both in tuftsin and retro tuftsin complexes. Maximum elongation (0.021 Å) is observed for the carboxylic O-H (O18-H19) of Arg residue in Retro tuftsin...4W complex where the water molecule acts as a protonic donor as well as acceptor and links the ends of the Arg carboxylic group. The bonds present in the hydrated tuftsin and retro tuftsin complexes are stretched as a result of hydration at the sites related with the atoms involving H-bonds as that of our previous studies [44–46]. The analysis shows that the proton donor N-H involved in these hydration interactions is stretched both in tuftsin and retro tuftsin complexes with values ranging from 0.005 to 0.009 Å and 0.001 to 0.009 Å, respectively. The variation of X – Y distances (X = O, N, C and Y = O) in the hydrated tuftsin complexes is found to be 2.807-3.401 Å and 2.704-3.609 Å in the retro tuftsin complexes.

The dipole moment of the tuftsin is found to be 7.3 Debye at B3LYP and 8.23 Debye at MP2 levels of theory, respectively. This value is found to lie between 5.65 and 8.74 Debye at B3LYP and 6.32 and 9.79 Debye at MP2 levels of theory, respectively. It is noteworthy that the least stable
Table 7: Hydrogen bond parameters (bond length R (in Å), bond angles θ (in degree)), electron density ρ(r) (in a.u.), Laplacian of electron density \(\nabla^2p(r)\) (in kcal/mol) involved in H-bond interactions in hydrated retro tuftsin complexes calculated at B3LYP/6-31G* level of theory. For labeling of atoms, refer Figure 2.

| Complex        | Hydrogen bond | Monomer | \(R_{X-H}\) |
|----------------|---------------|---------|-------------|
|                | Complex       |         | Complex     |
|                | X-H...Y       | \(\Delta R_{X-H}\) | \(\rho(r)\) | \(\nabla^2p(r)\) | ε | \(E^{(2)}\) |
| RTAW           | O18-H19...O76 | 0.977   | 0.996   | 0.019 | 1.783 | 2.704 | 152.25 | 0.045 | 0.104 | 0.025 | 21.36 |
|                | O76-H78...O17 | 0.969   | 0.985   | 0.016 | 1.881 | 2.719 | 141.16 | 0.035 | 0.111 | 0.078 | 9.52  |
| RTPW           | O76-H77...O10 | 0.969   | 0.979   | 0.010 | 1.869 | 2.814 | 161.37 | 0.033 | 0.108 | 0.008 | 7.26  |
|                | C14-H24...O76 | 1.095   | 1.095   | 0.000 | 2.559 | 3.609 | 160.27 | 0.007 | 0.036 | 0.054 | 4.14  |
| RtlW           | O76-H77...O39 | 0.969   | 0.977   | 0.008 | 1.947 | 2.828 | 148.74 | 0.028 | 0.103 | 0.057 | 3.22  |
|                | N11-H12...O76 | 1.014   | 1.019   | 0.005 | 2.080 | 2.979 | 145.76 | 0.024 | 0.088 | 0.045 | 8.72  |
| RttW           | O76-H77...O56 | 0.969   | 0.974   | 0.005 | 1.894 | 2.822 | 158.14 | 0.029 | 0.110 | 0.021 | 9.83  |
|                | N30-H33...O76 | 1.012   | 1.021   | 0.009 | 1.988 | 3.002 | 172.16 | 0.027 | 0.096 | 0.036 | 12.26 |
|                | O76-H78...O17 | 0.969   | 0.972   | 0.003 | 2.014 | 2.984 | 176.08 | 0.022 | 0.091 | 0.019 | 4.43  |
| Retro tuftsin  | O79-H81...O10 | 0.969   | 0.976   | 0.007 | 1.962 | 2.905 | 161.84 | 0.025 | 0.080 | 0.039 | 5.40  |
| 4W             | O79-H80...O18 | 0.969   | 0.970   | 0.001 | 2.330 | 3.118 | 137.96 | 0.012 | 0.042 | 0.054 | 1.89  |
|                | C13-H23...O79 | 1.092   | 1.093   | 0.001 | 2.322 | 3.315 | 150.16 | 0.015 | 0.044 | 0.057 | 3.59  |
|                | O18-H19...O82 | 0.977   | 0.998   | 0.021 | 1.765 | 2.694 | 153.31 | 0.042 | 0.123 | 0.020 | 22.84 |
|                | O82-H83...O17 | 0.969   | 0.983   | 0.014 | 1.911 | 2.727 | 138.59 | 0.030 | 0.094 | 0.049 | 7.25  |
|                | N30-H33...O82 | 1.012   | 1.014   | 0.002 | 2.376 | 3.313 | 153.37 | 0.001 | 0.038 | 0.028 | 3.17  |
| Retro tuftsin  | N11-H12...O76 | 1.014   | 1.017   | 0.003 | 2.289 | 3.050 | 130.67 | 0.015 | 0.049 | 0.127 | 3.94  |
| 4W             | O76-H77...O17 | 0.969   | 0.976   | 0.007 | 1.958 | 2.905 | 162.77 | 0.026 | 0.080 | 0.019 | 5.50  |
|                | O76-H78...O39 | 0.969   | 0.977   | 0.008 | 1.927 | 2.842 | 154.79 | 0.027 | 0.088 | 0.034 | 3.55  |
|                | N54-H53...O76 | 1.021   | 1.020   | 0.001 | 2.369 | 3.223 | 140.68 | 0.011 | 0.039 | 0.128 | 1.33  |
|                | N54-H75...O85 | 1.022   | 1.024   | 0.002 | 2.076 | 3.100 | 178.38 | 0.023 | 0.062 | 0.026 | 10.27 |
|                | O85-H86...O56 | 0.969   | 0.984   | 0.015 | 1.820 | 2.783 | 165.18 | 0.035 | -1.713 | 0.018 | 6.05  |

complex TPW is observed to possess the higher value of dipole moment calculated at MP2 and B3LYP levels of theory (9.8 and 8.7 Debye, respectively). The dipole moments of the retro tuftsin complexes are lower than tuftsin, and it lies in between 3.77 and 8.02 Debye at B3LYP and 4.71 and 9.0 Debye at MP2 levels of theory, respectively.

Pictorial representation of HOMO and LUMO of isolated tuftsin, TLW, and the most stable tuftsin monohydrate and Tuftsin...4W complexes is presented in Figure 7. Figure 8 shows the HOMO and LUMO for isolated retro tuftsin along with its most stable monohydrate RTAW and Retro tuftsin...4W complexes. The analysis of these frontier molecular orbitals of tuftsin shows that the HOMO and LUMO concentration of strong positive potential charge (-0.006 a.u.) that confirmed the presence of electrostatic interaction. In Tuftsin...4W complex, the nitrogen atom which belongs to the guanidinium group of Arg shows the large electron density in its region. The amino group of Lys side chain in tuftsin and one end of Thr side chain in retro tuftsin is white denoting the concentration of modest positive charges. The electrostatic potential map for tuftsin, retro tuftsin, and their four water complexes is shown in Figures 9 and 10, respectively. The C=O oxygen atom of Thr residue in tuftsin, Lys residue in retro tuftsin, and Retro tuftsin...4W complexes are colored red corresponding to the strong negative potential charge (-0.006 a.u.) that confirmed the presence of electrostatic interaction. In Tuftsin...4W complex, the nitrogen atom which belongs to the guanidinium group of Arg shows the large electron density in its region. The amino group of Lys side chain in tuftsin and one end of Thr side chain in retro tuftsin is white denoting the concentration of strong positive potential charge (0.006 a.u.) that confirmed the presence of electrostatic interaction. In Tuftsin...4W complex, the nitrogen atom which belongs to the guanidinium group of Arg shows the large electron density in its region. The amino group of Lys side chain in tuftsin and one end of Thr side chain in retro tuftsin is white denoting the concentration of strong positive potential charge (0.006 a.u.) that confirmed the presence of electrostatic interaction. In Tuftsin...4W complex, the nitrogen atom which belongs to the guanidinium group of Arg shows the large electron density in its region. The amino group of Lys side chain in tuftsin and one end of Thr side chain in retro tuftsin is white denoting the concentration of strong positive potential charge (0.006 a.u.) that confirmed the presence of electrostatic interaction. In Tuftsin...4W complex, the nitrogen atom which belongs to the guanidinium group of Arg shows the large electron density in its region. The amino group of Lys side chain in tuftsin and one end of Thr side chain in retro tuftsin is white denoting the concentration of strong positive potential charge (0.006 a.u.) that confirmed the presence of electrostatic interaction. In Tuftsin...4W complex, the nitrogen atom which belongs to the guanidinium group of Arg shows the large electron density in its region. The amino group of Lys side chain in tuftsin and one end of Thr side chain in retro tuftsin is white denoting the concentration of strong positive potential charge (0.006 a.u.) that confirmed the presence of electrostatic interaction.

3.1.1. AIM Analysis. The nature of bonding between the atoms can be characterized through AIM theory by the values of electron density at the bond critical point (BCP). The low values of charge density \(\rho(r)\) are the indication of closed shell interactions typically found in H-bonds as...
observed in the present study. Koch and Popelier proposed the criteria of H-bonding, which is the value of $\rho(r)$ at BCP of H...Y lies within the range of 0.002-0.04 a.u. [54, 55]. The charge density ($\rho(r)$), Laplacian of charge density ($\nabla^2 \rho(r)$), and ellipticity ($\varepsilon$) at BCP for the hydrated tuftsin and retro tuftsin complexes are summarized in Tables 6 and 7, respectively. From Table 6, it can be seen that for O-H...O interactions in monohydrated tuftsin complexes, the distance of hydrogen bond varies between 1.849 and 1.982 Å, which are found to be relatively short with bond angles vary in the range of 150.68-172.89°. The $\rho(r)$ of these O-H...O interactions at bcp is found to be 0.02-0.035 a.u. which are higher than the other two (N-H...O and C-H...O) bonds. This shows that the O-H...O interactions between tuftsin and water are strong. For N-H...O interactions, the hydrogen bond length at bcp (1.941-1.974 Å), the hydrogen bond angle (159.21-171.22°), and electron density at bcp (0.029-0.033 a.u.) are observed. The AIM calculation yielded the values of $\rho(r)$ in the range of 0.015-0.017 a.u. with hydrogen bond distance ranges from 2.254 to 2.316 Å and hydrogen bond angles between 167.78 and 172.63° for C-H...O interactions in the hydrated tuftsin complexes.

For retro tuftsin complexes, the H-bond distance, bond angle, and $\rho(r)$ at BCP for O-H...O interactions are within the range of 1.765-2.33 Å, 137.96-176.08°, and 0.012-0.042 a.u., respectively. For N-H...O interactions, these values are found to be in the range of 1.988-2.376 Å, 130.67-178.38°, and 0.011-0.027 a.u., respectively. For C-H...O interactions in the hydrated retro tuftsin complexes, the H-bond distance between retro tuftsin and water molecules is observed to vary from 2.322 to 2.559 Å, which are relatively longer with smaller bond angles varying between 150.16 and 160.27°. The $\rho(r)$ for these weakest interactions is comparatively smaller which range from 0.007 to 0.015 a.u. Comparison of these density values of tuftsin complexes with the standard values confirms the existence of intermolecular H-bonds between tuftsin and binding water molecules. In tuftsin, the maximum charge density (0.035 a.u.) is observed for (O-H)Water...O (Thr) interaction whereas for retro tuftsin, it is observed for O-H- (Arg carboxylic group-) OWater (0.045 a.u.) which are due to the charge localization. The H-bonds in the hydrated tuftsin are observed to be stronger than those present in the retro tuftsin complexes.

The positive values of $\nabla^2 \rho(r)$ point out that the interactions in the hydrated tuftsin complexes are governed by the contraction of the charge density towards each of the interacting nuclei which shows that the electronic charge is...
**Figure 8**: HOMO and LUMO orbitals of retro tuftsin, its most stable monohydrated, and Retro tuftsin...4W complexes.

**Figure 9**: The electrostatic potential map of tuftsin and Tuftsin...4W complex.

**Figure 10**: The electrostatic potential map of retro tuftsin and Retro tuftsin...4W complex.
depleted in the inter atomic surface [56]. The Laplacian of charge density is found to be in between 0.087 and 0.113 a.u., 0.087 and 0.101 a.u., and 0.043 and 0.068 a.u. for O-H...O, N-H...O, and C-H...O interactions, respectively, found in the hydrated tuftsin complexes. For hydrated retro tuftsin complexes, the Laplacian of charge density $\nabla^2 \rho_{H\ldots O}$ of O-H...O is found to be in between 0.042 and 0.123 a.u., $\nabla^2 \rho_{H\ldots O}$ of N-H...O is 0.038-0.096 a.u., and $\nabla^2 \rho_{H\ldots O}$ of C-H...O is 0.036-0.044 a.u., which is indicative of closed-shell interactions, and it also compares satisfactorily with previous results that vary from 0.016 to 0.13 a.u. [55]. The large $\rho(r)$ value (0.035 a.u.) together with negative values of $\nabla^2 \rho (r)$ (-1.713) represents shared interactions and characteristics of covalent bonds as is observed for (O-H)$_{\text{Water}}$...O (Thr) interaction in Retro tuftsin...4W complex at 1.82 Å. Therefore, the presence of intermolecular H-bonds between tuftsin and water molecules is quite well justified by the $\rho(r)$ values, and this is, in fact, again supported by the Laplacian of charge density values at BCP.

The correlation between the H-bond distance, electron density, and Laplacian of electron density has been established for Tuftsin...4W and Retro tuftsin...4W complexes (Figures 11 and 12, respectively). This indicates that the bond length and electron density are inverse to each other with correlation coefficients 0.937, 0.955, 0.989, and 0.975 for the electron density and its Laplacian with H-bond lengths, respectively. The maximum ellipticity value (0.129) is noted for the C$^\alpha$-H (Arg)...O (W) interaction in Tuftsin...4W complex which indicates the higher chance of structural deformation under external perturbations. In hydrated retro tuftsin complexes, the N54-H53 (Lys)...O (W) interaction in Retro tuftsin...4W complex has maximum ellipticity value (0.128).

3.1.2. NBO Analysis. The stabilization energies $E^{(2)}$ corresponding to prominent interactions found in the hydrated tuftsin and retro tuftsin complexes calculated using equation (2) at B3LYP/6-31G* level of theory are collected in Tables 6...
and 7, respectively. The presence of finite, nonzero stabilization energies in the hydrated tuftsin complexes obviously manifests the presence of a finite, nonzero overlap between orbitals. From Table 6, it can be concluded that for hydrated tuftsin complexes, the interactions where the oxygen of water offers lone pairs to the N-H antibond orbital of the tuftsin are stronger, and \( E^{(2)} \) lies within the range of 13.38-15.56 kcal/mol. It is interesting to note that all the O-H…O interactions exist in hydrated tuftsin complexes are of similar type where the oxygen of tuftsin presents lone pairs to the O-H antibond orbital of water with stabilization energies ranging from 3.14 to 10.71 kcal/mol.

In hydrated retro tuftsin complexes, the interactions where the oxygen of water offers lone pairs to the O-H antibond orbital of retro tuftsin are found to be stronger with \( E^{(2)} \) in the range of 8.72-22.84 kcal/mol. But the interaction where oxygen of retro tuftsin presents lone pairs to the antibonding orbital of water is smaller than the former; the corresponding \( E^{(2)} \) lies within the range of 1.89-9.83 kcal/mol. This shows that the oxygen of water is liable to offer electrons to the retro tuftsin than the electrons of retro tuftsin, and the formed H-bonding is stronger, and the corresponding complex is stable. The interaction between oxygen lone pairs in water and N-H antibond orbital of retro tuftsin is present in the hydrated retro tuftsin complexes with \( E^{(2)} \) between 1.33 and 12.26 kcal/mol which are found to be lower than the N-H (tuftsin)…O (W) interactions, and the corresponding H-bonds are weaker. The interactions between oxygen lone pairs in water and C-H antibond orbitals of tuftsin and retro tuftsin complexes also exist with \( E^{(2)} \) in the range of 4.03-5.7 and 3.59-4.14 kcal/mol, respectively. This demonstrates that \( E^{(2)} \) for C-H…O (W) interactions in hydrated tuftsin complexes are a little bit higher, and the corresponding H-bonding interactions are stronger, which is consistent with the previously analyzed structure data and energy analysis.

### 4. Conclusion

The tuftsin is sensitive to structural changes, and the results indicate that water-tuftsin H-bonds, in addition to intramolecular H-bonds, stabilize the \( \beta \)-turn structure with H-bonds between Thr and Arg residues. Difference in the stability of the hydrated complexes is confined to the amino acid residues at which the water molecule is attached to tuftsin. When the water molecules are attached to the oxygen sites of amino acid residues, strong H-bonds are observed to be formed between tuftsin, retro tuftsin complexes, and water molecules. The relative energy values of the hydrated tuftsin complexes match with the interaction energies where the strong H-bonds are said to possess the maximum interaction energy and hence higher stability. The H-bonds present in the hydrated tuftsin complexes are noticed to be stronger than those of retro tuftsin counterparts. It is interesting to notice that irrespective of the position in the peptide chain, monohydrated tuftsin complexes at Pro residue stand last at the stability order with minimum interaction energy among the monohydrates.

The H-bonds in the hydrated tuftsin are observed to be stronger than those present in the retro tuftsin complexes. The analysis of the frontier molecular orbitals of tuftsin shows that the HOMO is concentrated on the distal end of the Arg residue which is capped by the guanidinium group where the LUMO is across the carboxylic acid group of Arg. The AIM calculation indicates that O-H…O, N-H…O, and C-H…O interactions present in the hydrated tuftsin and retro tuftsin complexes possess low \( \rho(r) \) and positive \( \nabla^2 \rho(r) \) values and are in agreement with electrostatic characters of the H-bonds. The NBO analysis predicts the larger value of stabilization energy (15.56 kcal/mol) for the N-H (Thr)…O (W) interaction in the TLW, the most stable mono hydrated tuftsin complex. In the case of hydrated retro tuftsin complexes, the maximum stabilization energy is noted for O-H (Arg)…O (W) interactions in Retro tuftsin…4W complex (22.84 kcal/mol). The interactions between oxygen lone pairs in water and C-H antibond orbitals of tuftsin and retro tuftsin complexes demonstrate that \( E^{(2)} \) for C-H…O (W) interactions in hydrated tuftsin complexes are a little bit higher, and the corresponding H-bonding interactions are stronger.

### Data Availability

All the data supporting the results of this study have been included with in this article.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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