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Spectroscopic signatures and structural motifs in isolated and hydrated serotonin: A computational study

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The conformational landscapes of neutral serotonin and its hydrated complex were characterized by MP2, CC2 and DFT methods. The ground state geometry optimization of the twenty three lowest energy structures of serotonin have been performed employing the higher basis sets. The MP2, CC2 and DFT (M06-2X, ωB97X-D and B3LYP-D3) calculations predict that the Gph-out/anti conformation of serotonin is the most stable which is in agreement with the experimental rotational spectroscopy (Cabezas et al, *Phys. Chem. Chem. Phys.*, 2012, 14, 13618) and is in contrast to the resonance-enhanced two photon ionization (R2PI) and UV-UV hole burning (UVHB) spectroscopy results. Computed wave-numbers and intensities of the observed conformers are found in consonance with the experiment (LeGreve et al, *J. Am. Chem. Soc.,* 2007, **129**, 4028). The predicted intensity of the OH bending fundamental provides a useful diagnostic for the 5-OH anti and syn conformations. The computed hydrogen bond geometries of the experimentally observed $Sero_1$-$(H_2O)_1$ and $Sero_1$-$(H_2O)_2$ clusters are found in remarkable agreement with the experiment. The $Sero_1$-$(H2O)_2$ involving the Gph(out) conformation is used to form a strong water dimer bridge to the 5-OH with a binding energy of 104 KJ/Mol. The low-lying excited states of each experimentally observed conformer of serotonin have been determined by means of coupled cluster singles and approximate doubles (CC2) and TDDFT methods and a satisfactory interpretation of the electronic absorption spectra is obtained. One striking feature is the coexistence of the blue and red shift of the vertical excitation energies of the ^1L_b (ππ*) and the ^1L_a (ππ*) state upon forming complex with a water. The effect of hydration on lowest ^1L_b (ππ*) excited state due to bulk water environment was mimicked by a combination of polarizable continuum solvent model (PCM) and conductor like screening model (COSMO), for the different serotonin conformations which shows a red shift. The lowest excited state (^1L_b) of the most stable $Sero_1$-$(H2O)_1$ structure shows a significant shift of 1.15 Å of water molecule towards the 5-OH group due to S_0-S_1 electronic excitation.

Keywords: Conformations in Serotonin, Ab initio and DFT calculations, Hydration, Excited states;
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1. Introduction

Serotonin (5-hydroxytryptamine or 5-HT) is an important neurotransmitter for a number of brain functions and it plays a key role in the daily behavior and physiological states of human.[1,2] It is involved in the regulation of mood, stress, sleep, pain, aggression, anger, learning and regulation of body temperature and blood pressure.[3-7] Low serotonin levels or improper serotonin receptor functionalities are considered to lead to aggressive behavior, depression, migraine, bipolar disorder, anxiety and borderline personality disorder.[1,5-10] Moreover, serotonin is the target, at least partially, for most drugs that are currently used for the treatment of psychiatric disorders (e.g., depression and schizophrenia).[2] Serotonin mediates its physiological actions by binding to its receptors. The biological significance of this molecule has drawn considerable theoretical and experimental interest for exploring its structure in the gas phase.[2,6,11,12] It is generally accepted that some particular conformation of a biologically active molecule at the receptor site is decisive in order to trigger a specific biological response. At physiological pH, serotonin occurs in its protonated form, however many of the receptor sites for serotonin are non polar environment in which the NH_2 group may be neutral rather than protonated.[9,13] From the extensive study of the conformational preferences inherent to the isolated serotonin molecule, it is expected to contribute a better understanding interaction of serotonin with these receptor sites to carry out its multi biological functions.[5]

Serotonin (See Fig.1) can exist in various forms, differing from each other by the arrangements of the ethylamine side chain and hydroxyl group. The conformational flexibility of ethylamine neurotransmitters, mainly arising from facile rotations around the C-N and the two C-C bonds (C(α)- C(β) and C(β)- C(γ) bonds, shown in Fig.1) of the ethylamine side chain, is expected to be highly relevant for the drug-receptor interaction and molecular recognition.[7,14] The flexible ethyl amino side chain of serotonin gives rise to 27 conformers from rotations about the single C-N and the two C-C bonds. Of these twenty seven conformers the nine conformers were supposed to have considerably lower energies than the remaining 18 ones. A short-hand notation for the serotonin isomers, are earlier reported [5] in the following way. The amino group can reside in any of three positions, gauche on the phenyl side (Gph), gauche on the pyrrole side (Gpy) and Anti. The orientation of the amino group is labeled by the direction of the lone pair of electrons relative to the indole plane, indicated with "out" or "up' and by "ph", "py" or "up' for the

anti species. Syn (toward) or anti (away from) indexes are employed to denote the two different orientations of the OH group relative to indole NH. Neutral serotonin has been investigated theoretically by van Mourik and Emson,[11] they reported 23 low energy isomers for serotonin within 11 kJ/mol at the B3LYP/6-31+G(d) level. This includes the full set of 18 lowest energy structures, nine syn plus nine anti. They predict that, in every case, the anti configuration for the 5-OH group is lower in energy than its syn counterpart. However, Table 1 of van Mourik and Emson[11] shows a number of inconsistent results, in which syn configuration is noted as lower in energy than its anti counterpart. The calculations also predict that the Gpy(out) conformer which is the global minimum in tryptamine retains that status in serotonin as well.[11] Bayari et al.[16] and few other researchers[17-19] presented FT-IR spectra of serotonin in KBr films as well as in aqueous solution. However, these spectra display low resolution and it is unclear at present whether they correspond to the neutral or the protonated form. The earlier vibrational analysis[18] seems to be incomplete and ambiguous. Electronic absorption spectra and the singlet excited states of serotonin and its protonated form were studied by Kishi et al.[20]

Spectroscopic signatures of isolated bio-molecules and their hydrated clusters may provide insight on their preferred conformations, dynamical flexibility, and inter- and intra- molecular interactions determining their skeletal structures. Therefore, it is a great challenge to measure the spectral signatures and consequently to extract the contributions of the conformational isomers, while assigning them to specific structures The conformational preferences of the isolated serotonin molecule have been elucidated in supersonic jets by LeGreve et al [5,12] using a variety of spectroscopic techniques, including laser induced fluorescence, resonance-enhanced two photon ionization (R2PI), UV-UV hole burning and Resonant ion-dip infrared spectroscopy etc. Spectral signatures due to eight low-energy conformers were observed and assigned by comparing to its close analogue tryptamine.[5,21] The conformation-specific study of 5-HT at B3LYP/6-31+G(d) and MP2/6-31+G(d) levels showed that, there are at least eighteen possible conformational isomers.[5] The eight experimentally observed conformers split into two groups depending on the orientation of the 5-hydroxy group, either anti (theta=180) or syn (theta=0) relative to indole NH.[5] The five anti-OH origins are seen to the blue (higher wavenumber) of the three syn-OH origins. Zwier and coworkers [5] assigned the observed serotonin conformers as A,B,C,D,E,F,G and H which were linked to Gpy(out)/anti, Gpy(up)/anti, Gph(out)/anti, Anti(py)/anti, Ant(up)/anti, Gpy(out)/syn, Gpy(up)/syn, and Gph(out)/syn respectively. The ultraviolet spectrum [5] divides into two subsets due to the two distinct OH orientations, syn and anti. Within each subset, the Gpy(out), Gpy(up), and

Gph(out) conformations dominate. The most intense transition was observed in serotonin conformer A, which has a Gpy(out) ethylmine side chain conformation, the same conformation that is global minimum in tryptamine.[5,12,21] In this structure, the amino group is in the gauche position on the pyrrole side of indole, with the NH_2 lone pair oriented out away from the ring. The most dramatic change induced by the 5-OH substitution is the selective stabilization of the Gph(out)/anti conformation relative to all others. This stabilization has its most likely cause in electronic effects transmitted through the fused-ring system to the ethylamine side chain. However LeGreve et al [6] explained that MP2 calculation seems to overestimate the stabilization in Gph(out)/anti relative to the experiment as found in the case of tryptamine and followed DFT results for comparison with experiment.[5] They have concluded that the most stable conformer is Gpy(out)/anti followed by Gph(out)/anti and Gpy(up)/anti. Recently, microwave spectra of Cabezas et al[2] have provided very accurate ground state rotational constants of the most highly populated conformers of serotonin. In the rotational spectrum of serotonin[2] three lowest energy conformers, Gph(out)/anti, Gpy(out)/anti and Gpy(up)/anti, have been detected and characterized. In this study[2] the Gph(out)/anti conformer was found to be most abundant and with the help of computed MP2 energies it was concluded that in serotonin the most stable conformer is Gph(out)/anti, followed by Gpy(out)/anti Gpy(up)/anti. The stabilization of Gph(out)/anti conformer attributed to an electronic effect associated specifically with the anti OH orientation, which does not occur for the Gpy(out)/anti nor for others.

For tryptamine and serotonin, characterized by an ethylamine group attached to an aromatic system, the N-H..pi weak hydrogen bond is one of the leading structural motifs stabilizing the structure as pointed out in previous studies. These weakly polar intra-molecular interactions are forces which drive conformational preferences in serotonin. Cabezas et al[2] have expressed the need of a high level ab initio computations for the extensive study of serotonin conformation.

Because serotonin carries out its biological functions in aqueous solution, it is also important to understand how the conformational preferences of serotonin changes in the presence of water molecules. Many of the receptor sites for serotonin in which the NH_2 group may be neutral in aqueous solution.[9] Despite the nonpolar environment, water molecules are likely to be present in these binding pockets and may play a role in the way in which 5-HT interacts with the receptor. Spectroscopic signatures of $Sero_1$-$(H_2O)_{1,2}$ clusters have been also experimentally performed [22] using the same conformation-specific methods already employed on the monomer. [5] Since serotonin has both H-bond donor group (the 5-OH) and H-bond

acceptor (NH_2) group, spatially separated from one another, the water molecules can form H-bonded bridges that link these groups in serotonin, [22] as in proteins.[23]

Unfortunately, the few available theoretical studies on the lowest energy conformers of serotonin lack a real description of the nature and amplitude of the intramolecular interactions that influence the conformational stability of serotonin. The main reason comes from the small basis sets used in the calculation to describe weak non bonded interactions that need extended basis set to describe conformational preferences in 5-HT more precisely. Assignment of the experimental spectra, depends crucially on comparison with spectra computed using high level quantum chemical techniques, and the increasing size of molecules can be studied accurately with ab initio and density functional theory methods.[24-36] In quantum mechanical computations, a high degree of electron correlation must be included to reliably account for dispersion interaction. Second order Moller-Plesset perturbation theory method (MP2) [24-26] offers a better approach for describing non-covalent interactions since it can be extended to much larger systems. Second order Moller-Plesset perturbation theory (MP2),[22] second order approximate coupled cluster (CC2) [26,27] and density functional theory (DFT) [28-36] methods implemented in Gaussian [25] & TURBOMOLE [26] quantum chemical software's, provide important insights into the energetic, ground state structures and photochemistry of these systems. The goal of the present study is thus to characterize the most of the stable structural motifs of serotonin and its experimentally observed hydrated complexes. Furthermore, we will investigate the IR spectra and low-lying excited states of the eight experimentally observed serotonin. Our calculations have aided the interpretation of IR spectra more systematically and many previous incomplete and ambiguous assignments have been analyzed and amended. The application of DFT to non-covalently bound complexes has been limited due to the failure of most density functional approximation, in many case, to describe dispersion interaction. However, several approaches exist for improving existing density functionals to handle dispersion effects. In this paper, we also report a comparative study of the accuracy of the B3LYP,[28-30] B3PW91,[28-31] and X3LYP [28,29,32] density functionals and newly developed M05, M06,M05-2X M06-2X [34-36] and dispersion corrected ωb97X-D,[33] and DFT-D3 functionals [26,37] to predict the energy and/or binding energy of serotonin and its hydrated complex. The electronic absorption spectra was investigated and assigned by using the second order approximate coupled cluster (CC2)[27] and the Time Dependent Density Functional Theory (TD-DFT) calculations [38] for the vertical electronic excitation energies of serotonin. The effect of hydration on the lowest singlet ππ* excited- state of serotonin is investigated .

2. Computational Methods

Electronic structure calculations have been performed using the Gaussian 09 and Turbomole 6.4 quantum chemical software packages.[25,26] For bare serotonin, all possible conformers resulting from the rotation of ethylamine side chain and hydroxyl groups were initially optimized by the Hartrre-Fock method [25,26] employing the lower basis set. Finally all the twenty three lowest energy structures of serotonin in the ground state have been optimized using the MP2[24] and DFT-B3LYP[28-30] methods. The eight experimentally observed lowest energy ground state structures of serotonin have also been optimized using resolution of identity (RI) second order approximate coupled cluster (CC2) [26] and density functional theory[28-36] employing the B3PW91,[27-30] X3LYP [27-29,31] and newly developed M05, M06, M05-2X, M06-2X,[34-36] and ωb97XD[33] exchange-correlation functionals. The 6-31+G(d), 6-311++G(d,p) and aug-cc-pVDZ basis sets were employed in the geometry optimization and vibrational modes calculation of serotonin conformers. The two most stable ground state structures were also optimized using dispersion corrected DFT-D3 [37] implementation in TURBOMOLE V6.4 employing the basis set TZVP.[26] For hydrated serotonin, all possible locations of interactions between serotonin and water were considered, such as nitrogen atom in NH_2 group and oxygen atom in the hydroxyl group and pi cloud of the ring. The lowest energy conformers were mainly selected from the initial geometries by using the HF method, while the experimentally observed structures finally optimized by using DFT-M06-2X and MP2 methods as employing 6-311++G(d,p) basis set. The lowest excited state structure of the most stable conformer of serotonin involving Gph(out)/anti conformation and its lowest energy monohydrated complex are optimized at DFT level employing the basis set def TZVP.

Theoretical methods described above were applied to a molecular geometry without symmetry restrictions. The calculated geometry with C_1 symmetry was very close to that with C_s symmetry. All optimized structures have been verified as minima by performing frequency calculations, in order to ensure that no imaginary frequency were present. Harmonic wave numbers calculated at the B3LYP/6-311++G(d,p), B3LYP/6-311++G(2d,2p), B3LYP/aug-cc-pVDZ levels of theory have been found to give good agreement with experiment. However the fundamental frequencies calculated by DFT method with B3LYP parameterization using the MP2 optimized geometries are significantly more close to experiment than those produced by using the DFT optimized geometries. We used mainly the scaling factor 0.977 and 0.988 for the OH and the NH/CH

fundamentals respectively to scale down the vibrational frequencies of monomer, however in the alkyl CH stretching region and below there is no need of scaling.

The CC2 method is an approximation to the coupled cluster singles and doubles (ccsd) method where single equations are retained in the original form and the double equations are truncated to the first order in the fluctuating potential. [27] The X3LYP [28-31] (extended hybrid functional with Lee-Yang-Parr correlation functional[30]) extended functional for density functional theory was developed to significantly improve the accuracy for hydrogen bonded and van der waals complexes. The M05, M06, M05-2X and M06-2X[34-36] are newly developed standard hybrid DFT functionals with parameters optimized on training sets of benchmark interaction energies. According to Zhao and Truhlar,[34] the M06 series of functionals represent a significant step forward in density functionals, implicitly account for 'medium- range' electron correlation, which is sufficient to describe the dispersion interaction within many complexes. Recently, Andrew et al[37] have examined the performance of a variety of DFT procedures for the calculation of complexation energies, paying special attention to the M05-type and M06-type functionals. The mean deviation obtained with M05-type and M06 type functionals become more negative with an increasing amount of Hartree-Fock exchange, in contrast to the behavior of typical DFT methods.[37] It was reported[37] that the M05-type and M06 type functionals are generally accurate and robust and the performance generally improves in order M05→M06→M05-2X→M06-2X. The M06-2X has mean absolute deviation values that are quite small and comparable to those for other procedure PW6-B95.[37]

TD-DFT method[39] employing B3LYP [28,29] functional with 6-311++G(d,p) and aug-cc-pVDZ basis sets was used at corresponding (MP2/aug-cc-pvdz) ground state optimized geometries to predict the electronic absorption wavelengths of the eight experimentally observed conformers of neutral serotonin. RI-CC2[26,27] implementation in TURBOMOLE V6.4,[26] employing the basis set TZVP is also used to compute the vertical electronic absorption wavelengths. The effect due to the so-called bulk water molecules was taken into account within the polarizable continuum model (PCM) [40-42] and the conductor like screening model (COSMO) [43] framework. The electronic absorption spectra in aqueous solution have been calculated by employing the B3LYP hybrid exchange correlation functionals, using the PCM and COSMO continuum solvent models.[40-43]Binding energies (ΔE_{Stab}) of the serotonin -water complexes have been calculated as follows:

$\Delta E_{Stab} = E_{Complex} - (E_{serotonin} + E_{Water})$

The calculated binding energy of serotonin$_1$-(water)$_1$ complexes is corrected for the basis set superposition error (BSSE), using the counterpoise method of Boys and Bernardi.[44]

3. Results and discussion

3.1. Ground-state optimized structures of neutral serotonin

The conformational flexibility of serotonin is mainly arising from rotations of ethylamine side chain group and hydroxyl group .The most stable twenty four structures of neutral serotonin considered in the previous computational study[11] were subjected to geometry optimization using MP2 and DFT methods, employing the higher basis sets. Table 1 and Table 2 lists the ZPE corrected relative energies and the dipole moments of these twenty four lowest energy conformers at the MP2 and DFT-B3LYP levels of theory respectively employing the basis sets 6-311++G(d,p) and aug-cc-pVDZ. The twelve anti-OH structures according to the order of decreasing stability (based on the MP2/6-311++G(d,p) ZPE-corrected energy) are listed in Fig. 2a. Mourik and Emson[11] considered the three high energy conformers (conformers 9,10,11) in addition to the nine core set of conformers of serotonin corresponding to tryptamine structures. All of these twelve conformers and their corresponding syn-OH forms are stable structures verified by the absence of imaginary frequencies. As seen in Table 1 and 2, the conformers Gph(up)/syn and Conf. 10/syn have almost the same optimized energy at the each level of theory, therefore the number of independent stable structures considered in the present study is reduced to twenty three.[12] Interestingly the conformer 10 of Mourik and Emson,[11] which was reported to be a high energy conformer, is predicted to be the fourth lowest energy conformer of anti-OH serotonin at the MP2/6-311++G(d,p) level theory. As seen in Table 1 and 2 the relative stabilities of the conformers significantly change when going from B3LYP to MP2. This is likely due to the inability of B3LYP functional to describe the dispersion energy quantitatively. It was reported [11] that the Conformer 10 (labeled IV in Fig. 2a) should be labeled Gph(up), just like conformer V in Fig. 2a, therefore we have labeled these two conformers IV and V as a Gph(up)-[a] and Gph(up)-[b] respectively. The conformers XI and XII in Fig. 2a (earlier assigned as Conformers 9 and 11 respectively),[11] which have the ethylamine side chain in the indole plane are the two highest energy conformers anti-OH serotonin based on the MP2/6-311++G(d,p) corrected energies. The

eighteen core structures of bare serotonin (the nine ethylamine side chain conformers Gph (out, up, in), Gpy(out, up, in), and Anti (up, ph,py), with the 5-OH group in anti/syn configuration) are optimized at the MP2/aug-cc-pVDZ level of theory. These structures along with their corresponding relative energies are displayed in Fig. 2b. The ZPE-corrected relative energies at the MP2/aug-cc-pVDZ level of theory were obtained by including the zero-point corrections from B3LYP/aug-cc-pVDZ. The calculated energy of conformer Gph(out)/anti (earlier assigned as conformer C)[5] was found to be the lowest and the energies of the other conformers were calculated relative to them. As seen in Table 1, Fig. 2a and 2b, the MP2 calculations employing the higher basis sets predict that the Gph(out)/anti is the most stable and Gpy(out)/anti is the second most stable structures of serotonin, while the Gph(in)/syn structure is the least stable. The MP2 results are in good agreement with the result of the experimental rotational spectroscopy of serotonin,[2] in which the Gph(out)/anti was found as the most abundant species. In the two most lowest energy structures, Gph(out)/anti and Gpy(out)/anti (See Figure S1 and S2 of ESI), the lone pair is oriented away from the indole ring, and one of the NH_2 hydrogen is directed towards the pi-cloud of indole moiety, providing additional stabilization. The more stabilization of the Gph(out)/anti conformer attributed to an electronic effect associated specifically with anti-OH orientation which does not occur for the Gpy(out)/anti, Gpy(up)/anti nor for other Gph conformers. As seen in Table 1 The relative energy between the most stable conformer Gph(out)/anti and the least stable conformer Gph(in)/syn of serotonin was found to be 14.71 at MP2/ aug-cc-pVDZ level (See Fig. 2a, Table 1), which is relatively more larger than the value computed at B3LYP/aug-cc-pVDZ level (Table 2). The ground state geometry optimization of eight experimentally observed serotonin conformers were also performed using CC2/TZVP and various DFT (M05,M06,Mo5-2X, M06-2X, X3LYP, B3LYP and B3PW91) methods employing the basis sets 6-31+G(d), 6-311++G(d,p) and aug-cc-pVDZ yield different stability order. The relative energies and dipole moments of these eight conformers at various levels of theory were summarized in Table 3. The corresponding structural parameters at MP2/aug-cc-pVDZ level are given in Table S1 of ESI , of the supporting information. Fig. 3 compares the MP2/aug-cc-pVDZ relative energies of the eight experimentally observed serotonin conformers with the corresponding B3LYP, X3LYP and M06-2X (employing the same basis set aug-cc-pVDZ) values, while Fig. 4 and 5 compares the MP2 and DFT-B3LYP values at different basis sets respectively. The main conclusion drawn from the comparison Table 3 and Figures 3-5 were that (i) MP2 , CC2, CCSD, M06-2X, wB97X-D and B3LYP-D3 results preferentially stabilize the Gph(out)/anti conformer relative to Gpy (out) and Gpy (up), (ii) the relative energy orders

obtained by DFT employing M06-2X and ωB97XD functionals and CC2 are found almost similar to MP2 (iii) in DFT calculations employing the X3LYP and the conventional B3LYP and B3PW91 functionals the Gpy-out/anti conformer (earlier assigned as conformer A) [5] was found most stable, and the Gph-out/anti conformer was found as the second most stable, which is in agreement with the R2PI, LIF and UVHB spectroscopy results of LeGreve et al.,[5] however later on the experimental rotational spectroscopy report [2] contradicts this result [5] (iv) since the dispersion corrected, B3LYP-D3 method supports the MP2 result, the relative stability change from MP2 to the conventional DFT functional may arise due to the inability of these functionals to describe the dispersion energy quantitatively. (v) the appropriate level of theory for tackling the study of non bonded interactions in flexible bio-molecule-serotonin was checked. It was found that DFT functionals M05-2X , M06-2X, ωB97XD and dispersion corrected B3LYP-D3 performs well for the NH...π intramolecular interactions. (vi) the conformational landscape of the experimentally observed conformers Gpy(up)/anti (B), Anti(py)/anti (D) and Anti(up)/anti (E), are found to be most sensitive to the basis set, while the B and D are also sensitive to the change in the density functional (See Fig. 3-5).

The rotational constants and zero point vibrational energy of the eighteen conformers of serotonin are listed in Table S2 and S3 of ESI. The values of rotational constants of Table S2 of ESI allow to classify the rotamers of serotonin as belonging to different families. Conformers belonging to the same family have similar mass distributions so their rotational constants are very similar. A first comparison of these predicted values of rotational constants given in Table S2 of ESI with those experimentally observed allows us to classify the observed rotamers as belonging to different families (Gph, Gpy, and Anti). The rotational constants for the conformers Gph(out)/anti and Gph(out)/syn are very similar, as well as the values of Gph(up)/anti, Gph(up)/syn and Gph(in)/syn are also almost similar to this group which belongs to a 'Gph' family. Similarly the rotational constants for the conformers Gpy(out)/anti, Gpy(out)/syn , Gpy(up)/anti, and Gpy(up)/syn indicate that it belongs to a 'Gpy' family. 'Anti' family also have similar values of rotational constants. The NBO calculations at the MP2/aug-cc-pBDZ level of theory led to negative charge densities of -0.930, -0.773, -0.663 (in serotonin C) and -0.928, -0.768 and -0.665 (in serotonin A) on amino N_2, hydroxyl O_1, and N_1 (of indole NH) atoms respectively (See Fig. S2 and Table S4 of the ESI). One can note that the negative charge densities

on the N_1 and O_1 atoms are also large but not as significant as on the amino nitrogen atom. Other serotonin conformers led to similar values (See Table S4 of the ESI).

Harmonic vibrational frequencies and IR intensities of the twenty three stable conformers of serotonin were calculated by DFT-B3LYP employing the various basis sets including 6-311++G(d,p), 6-311++G(2d,2p), aug-cc-pVDZ. Computed IR spectra of the eight experimentally observed conformers of serotonin at B3LYP/6-311++G(2d,2p) level using the S_0 optimized geometries at the MP2/aug-cc-pVDZ level of theory are displayed in Fig. 6. Sixty nine normal vibratinal modes were determined for the each conformer. However, only fundamental frequencies above 2800 cm^{-1} corresponding to hydride stretch region and selected significant frequencies below 2800 cm^{-1} were analyzed in present study. Intra-molecular interactions have a strong influence on the frequencies of vibrational stretching modes involving hydrogen. All computed hydride stretch mode frequencies and intensities of the eight experimentally observed conformers of serotonin are assigned and are listed in Table 4 along with the corresponding gas phase IR frequencies.[5] Computed vibrational fundamentals are found only slightly larger than the experimental values, excluding the hydroxyl (OH) stretching mode. This disagreement may be partly due to the anharmonicity. As we have mentioned in the earlier section that the scaling factor of 0.977 and 0.988 for the OH and the NH/CH stretch fundamentals respectively are used to scale down the vibrational mode frequencies of the monomer, however there is no scaling used for the alkyl CH stretching and bending fundamentals. The hydride stretching modes can be divided into three groups: the heterocyclic system of the indole ring (aromatic CH and indole NH stretches), the ethylamine side chain (alkyl CH and amine NH stretches), and hydroxyl group (OH stretch). Each computed fundamental mode vibrations due to the OH, indole NH, NH_2, aromatic CH, and alkyl CH stretching modes of serotonin are assigned with the aid of Gaussian view 5.0, by comparing with experimentally observed fundamentals. It is evident from theoretical and experimental IR spectra that the frequency (predicted at 3530/3531 cm^{-1}) and intensity of indole NH stretching mode in the all conformers of serotonin are almost unchanged from one conformer to other, as they were found in tryptamine.[45] The OH stretch mode frequency and intensity of each anti-OH conformer are also almost unchanged, however the corresponding mode for all OH-syn conformers is blue shifted by 4-6 cm^{-1} (Table 4) and the intensity is increased significantly (See Figure 6 and Table 4). Our theoretical calculations predict that amino NH stretching modes have very low IR intensities, which is in consonance to observed

resonant ion-dip infrared (RIDIR) and fluorescence-dip infrared (FDIR) spectra of isolated serotonin cooled in supersonic expansion.[5] It was reported[5] that the symmetric and antisymmetric NH_2 stretching modes are only observable in conformers A, C and F at 3339-3342 and 3407-3410 cm^{-1} respectively. However, Raman intensity for the NH_2 stretching modes is predicted to be high; the symmetric stretching mode has three times more high Raman intensity than the antisymmetric (See Table 4). Recently Mayorkas et al[45] observed the strong Raman lines at 3342- 3345 cm^{-1} in the Ionization Loss Stimulated Raman (ILSR) spectra of tryptamine (for the analogous conformers A, C(2) and F) [45] which can be attributed to symmetric NH_2 stretching fundamental. The corresponding ant symmetric NH_2 stretching fundamental in the ILSR spectra was observed at 3411-3413 cm^{-1} with a weak Raman intensity. Surprisingly Mukherjee et al[18] have assigned the conventional IR bands observed at 3438 and 3397 cm^{-1} as symmetric and asymmetric NH_2 stretching mode respectively. However Yang and Gao[17] and Bayari et al [16] have assigned the IR band observed at 3438 cm^{-1} as a indole NH stretching band. LeGreve et al [5] have attributed symmetric and asymmetric NH_2 stretching modes to very weak IR bands observed in the experimental gas phase IR spectra at 3342 and 3407 cm^{-1} respectively which is supported by our DFT calculations as well as by earlier reported theoretical predictions. The asymmetric amino NH stretching band is predicted at the higher frequency than the corresponding symmetric mode which is in agreement to the assignment of LeGreve et al.[5] Therefore the assignment of conventional IR band observed at 3438 cm^{-1} as a symmetric NH_2 stretching mode by Mukherjee et al[18] seems to be ambiguous. The small changes in the frequencies and patterns of NH_2 stretching modes of different experimentally observed conformers of 5-HT is predicted (See Table 4) as observed in the ILSR spectra of tryptamine.[45] The aromatic CH stretch fundamental bands are predicted to lie between 3045- 3135 cm^{-1} and the alkyl chain CH-stretches between 2848- 2977 cm^{-1}. The aromatic CH stretches are generally rather similar in the different conformers, though individual stretching modes are affected by the vicinity of the alkyl chain and OH group. As seen in Table 4 the phenyl symmetric and anti symmetric CHHC stretch frequencies are higher than the phenyl C_4H stretch mode for all OH-anti conformers, however for OH-syn conformers, the phenyl C_4H stretch mode frequency becomes higher than CHHC stretch frequencies and intensity is increased due to blue shifting CH...O hydrogen-bonding. It should be noted that the phenyl C_4H bond lies in the vicinity of the OH group (see Fig 1). So, all the OH-syn conformers located in our calculations have unconventional blue shifted hydrogen bonds. It can be inferred then that the blue shifting H-bonds may also play an important role in the conformation stability of serotonin.

The alkyl CH stretch frequencies have been claimed to be most sensitive to conformational changes in serotonin and are most useful diagnostic of the ethylamine side chain conformation.[5,11] We have assigned precisely the vibrational fundamentals of the reported gas phase IR spectra in the alkyl CH stretch region of the experimental serotonin conformers[5] by comparing the vibrational modes obtained from ab initio and density functional theory calculations invoking higher basis sets. Present work has aided the interpretation of alkyl CH stretch fundamentals of eight experimentally observed conformers more systematically and many previous incomplete assignments[11] have been analyzed and amended. Computed four alkyl CH stretch fundamental frequencies attributed to in phase and out of phase $CH_2(\alpha)$ and CH_2 (β) vibrations for each lowest energy conformer of serotonin. Predicted alkyl CH stretch frequencies for the conformers A, C, F and H are found very close to the experiment[5] and there is no need of further scaling. However in the rest four experimental conformers the most of the observed alkyl CH stretch band[5] are not found very close to the corresponding predicted values. The symmetric (low frequency) $CH_2(\alpha)$ stretch vibration predicted between 2915- 2925 cm^{-1} and 2849- 2856 cm^{-1} in the 'up' and 'non up' structures. Mourik and Emson [11] explained that in all 'up' structures (conformers B, G and E) the lone pair on the amino group is positioned between the two $CH_2(\alpha)$ atoms and in all 'not-up' conformers (A,C,F and H) the amino lone pair lies trans to one of the H(α) atom. This causes low frequency $CH_2(\alpha)$ stretch to occur at small value and with higher intensities than in the 'up' conformer.[11] This so called trans lone pair effect can shift the CH-stretch fundamentals towards the longer wavelength side by as much as 100-150 cm^{-1}. [11] However experimental FDIR and RIDIR spectra[5] reveals that the lowest frequency band observed at 2846- 2856 cm^{-1} for all the eight experimentally observed conformers, which can be attributed to low frequency $CH_2(\alpha)$ stretch in the most of the eight conformers and its intensity is observed weak for all of the conformers. However the predicted lowest alkyl CH-stretching frequency of serotonin conformers B, E and G is increased by only about 50 cm^{-1} but relative intensity is decreased which is opposite, to the above[11] interpretation. This is somewhat difficult to interpret clearly at this stage because due to coupling of symmetric and antisymmetric CH_2 stretch modes probably the anharmonicty is increased non-linearly and the harmonic calculations do not account for anharmonicity. In each of eight conformers the antisymmetric CH_2 stretch frequency is higher than the corresponding symmetric mode. Generally the CH_2 (β) stretching mode frequencies are higher than that of $CH_2(\alpha)$ in both symmetric and antisymmetric modes. The antisymmetric CH_2 (β) stretch frequency observed at 2949-2969 cm^{-1} for all eight

conformers are found in consonance to our corresponding predicted values (See Table 4). The LeGreve et al[5] observed the two peaks at 2949 and 2944 cm^{-1}, 2948 and 2939 cm^{-1} and 2949 cm^{-1} 2945 cm^{-1} for the conformers B, G and E respectively. This splitting is expected to arise due to a Fermi resonance between the antisymmetric CH_2 (β) stretch and a overtone of CH bending mode predicted at 1474 cm^{-1}. Such type of splitting was not observed in the ILSR spectra of tryptamine probably due to low Raman intensity of CH bending mode. In addition to the hydride stretch region, the frequency and the intensity of the OH bending mode of the experimentally observed conformers is also included in Table 4. The OH bending is the most intense fundamental of the stable serotonin conformers and a comparison of its predicted intensity between the different conformations could be also useful in making conformational assignments. The frequency of OH bending fundamental is only slightly increased by 4 cm^{-1} for the OH-syn conformation, however the intensity of OH-syn conformers reduced to half in comparison to corresponding OH-anti conformers (Fig. 6 & Table 4). As seen in Table 4 and Figure 6, the intensity of the OH-bending fundamental can be a useful diagnostic for the conformational changes in serotonin.

3.2. Lowest Excited Electronic States of Serotonin

The electronic absorption spectra of serotonin dissolved in ethylene glycol-water (with HCl) is composed of three broad overlapping bands located between 3.83 and 5.85 eV.[20] The electronic absorption spectrum computed at TDDFT-B3LYP/aug-cc-pVDZ level calculated on MP2-optimized S_0 geometries, is found in agreement with the experiment concerning both electronic excitation energies and oscillator strength of absorptions. Table 5 shows the TDDFT and CC2 vertical excitation energies of the low lying singlet excited states of eight experimentally observed serotonin conformations. The CC2 vertical excitation energies of each lowest energy conformer was calculated on corresponding CC2-optimized S_0 geometries. The observed UV-absorption peaks[20] are analyzed and assigned well by TD-DFT and CC2 vertical electronic excitation energies (VEE) as well as by comparing the assignments of related molecules.[45-50] B3LYP-TDDFT VEE for the two lowest singlet $\pi\pi^*$ excited states L_b and L_a predicted at 4.16- 4.22 eV (with an oscillator strength 0.048- 0.056) and at 4.48- 4.52 eV (with oscillator strength 0.062-0.102) respectively for different observed conformers, are found consistent with the experimental longer wavelength absorption peaks[20] of serotonin at

4.09 and 4.46 eV respectively (with corresponding oscillator strengths 0.054 and 0.098 respectively).[20] . We found that the TDDFT-B3LYP method computes the more accurate vertical electronic excitation energies for serotonin in comparison to CC2 method.

The electronic spectra of indole containing molecules such as tryptamine and serotonin is challenging due to the close proximity and interactions between the lowest excited states of the indole chromosphere. If one wishes to begin studying the electronic spectra of serotonin in a systematic way , one strategy is to start with a relatively simple model and then gradually incorporate additional substituent groups. Indole provides a first approximation to all three related species, 5-hydroxyindole, tryptamine and serotonin. The electronic spectroscopy of indole in the gas phase has been well studied and photo excitation in the 285-220 nm region is primarily due to two valence states of $^1\pi\pi^*$ charactor and ^{1}A' symmetry which are historically assigned ^1L_a and ^1L_b states.[46-48] Hollas [46] identified the first electronic origin of the indole (the lowest excited ^1L_b ($\pi\pi^*$) state) in the gas phase at 283.83 nm. At slightly higher excitation energies, laser induced fluorescence experiments later identified the ^1L_a, ($\pi\pi^*$) state , which was found to have a larger absorption cross-section than the ^1L_b state. The ^1L_a state of indole is only 1000–1500 cm^{-1} above than the origin of lowest excited singlet ^1L_b state.[47,48] While the ^1L_b state gives rise to a structured band and has a small dipole moment, the ^1L_a state produces a broad band and its large dipole moment makes it sensitive to polar environment. In addition, there is a third dissociative state of $^1\pi\sigma^*$ character which plays a decisive role in the photo-physics of indole. The $^1\pi\sigma^*$ has a vertical excitation of only 0.12 eV higher than that of the ^1L_a state and its dipole moment is much larger than the ^1L_a state. The S_0 - $^1\pi\sigma^*$ transition posses little or no oscillator strength and so is essentially "optically dark" to single photon absorption. At energies above ~ 220 nm, strong absorption to two higher lying $^1\pi\pi^*$ states denoted ^1B_a and ^1B_b, also becomes significant. [46-48]

5-hydroxy indole is an indole substituted at the 5-position with a hydroxyl (-OH) group. The low lying electronic excitation energies of 5-hydroxy indole also cover similar excited states : the two $\pi\pi^*$ L_b and L_a states, the dark $\pi\sigma^*$ state with dissociative character and the higher lying $^1\pi\pi^*$ state ^1B_b etc (Table S5 of ESI).[49,50] An additional dark $\pi\sigma^*$ state also arises due to 5-OH group.[49] It was observed that the 5-hydroxy substitution lowers the S_0-S_1 origin by over 2324 cm^{-1} from indole to 5-hydroxy indole (Table S5 of ESI). [49,50] The ultraviolet spectrum of 5-hydroxyindole exhibits transitions due to two isomers, with $S_1 \leftarrow S_0$ origins at 32685 and 32914 cm^{-1}, separated by 229 cm^{-1}.[50] These transitions have been tentatively assigned to the syn and anti isomers of the 5-OH group, respectively, on the basis of their relative intensities and the

calculated energy ordering. The tryptamine is a constituent of an indole moiety connected to a flexible ethylamine side chain. It also resembles structurally and chemically the neurotransmitter serotonin. It was observed that the 3-ethylamie substitution lowers the S_1-S_0 origin by over 313 cm^{-1} from indole to tryptamine.[5,15,45] The low lying electronic excitation energies of tryptamine covers the two excited singlet ππ* (L_b and L_a) states. Recently DFT/MRCI calculations on tryptamine [15] predicted that the ^1L_b state is the lowest electronic state. The optically bright ^1L_a state constitutes the second excited state in the vertical excitation spectrum, about 2000-2500 above the ^1L_b state, depending on conformer.

Therefore it can be expected that in the case of serotonin, 5-OH substitution has a similar effect on the $S_1 \leftarrow S_0$ transition energy as found in 5-HI. It was observed that the 5-hydroxy substitution in serotonin lowers the S_1-S_0 origin by over 2335 cm^{-1} from tryptamine to serotonin which is similar to energy lowering in 5-HI.[45] The lowest singlet excited state L_b of each experimentally observed serotonin conformer is of ππ* character dominated by single configuration corresponding to HOMO → LUMO (~0.67) excitation. The energy gap between HOMO and LUMO of the most stable conformer C was found to be 4.94 eV at the B3LYP-D3/TZVP level of theory. The dipole moment, 3.699 D, of the S_1 state is predicted slightly higher than that of the ground state dipole moment, 3.125 D at the same level of theory, which indicates that position of S_0-S_1 band may be red-shifted in polar solvents. The S_0-S_2 excitation energy, of the optically bright L_a (^{1}ππ*) state , is dominated by H-1→L(0.67) transition. The predicted VEE of the optically dark $^1\pi\sigma^*_{OH}$ and $^1\pi\sigma^*_{NH}$ states of serotonin is computed at 4.63 and 4.85 eV with very weak oscillator strengths 0.002 and 0.011 respectively. These two $^1\pi\sigma^*_{OH}$ and $\pi\sigma^*_{NH}$ states are dominated by H→L+2 (0.63) and H-1 →L+2 (0.53) transitions respectively. The intense absorption peak observed[20] at 5.62 ev is attributed to ^1B_b (ππ*) state. This high lying ^1B_b (ππ*) state is expected to be dominated by H-1 →L+1 and H→L+3 excitation. As seen in Table 5 the VEE of the observed conformers of serotonin for ^1B_b (ππ*) state predicted between 5.54-5.85 eV, is in remarkable agreement to experiment.[20] The ultraviolet spectrum of serotonin is also divided into two subsets due to the two distinct OH orientations, syn and anti. As earlier mentioned that the anti and syn conformations of 5-HI are separated by 229 cm^{-1}, the similar separations are reported to be 231, 235, 262 cm^{-1} between serotonin conformers A(Gpy(out)/anti) and F(Gpy(out)/syn), B (Gpy(up)/anti) and G(Gpy(up)/syn) and C(Gph(out)/anti) and H (Gph(out)/syn) respectively.[5]

We have optimized the lowest excited states of serotonin in the Gph(out)/anti conformation at the B3LYP/def.TZVP and PBEO/def.TZVP levels of theory. The optimized energy of the lowest ^1L_b state shows an excellent agreement with the corresponding gas phase S_0-S_1 electronic

transition energy. Interestingly the B3LYP value was found to be more close to the experiment in comparison to PBEO.[26] As seen in the Table S5 of ESI the relative energy between the computed origin of the ^1L_a and the ^1L_b states of serotonin for the most stable Gph(out) conformation is found to be 3841 cm^{-1}.

3.3. Optimized structures of hydrated clusters of Serotonin

Serotonin carries out its biological functions in aqueous solution, so it is important to understand how the conformational preferences of serotonin changes in the presence of water molecules. Our goal is to probe the preferred binding sites for the water molecules and to characterize the spectroscopic signatures and structural landscapes of the most stable hydrated complex of serotonin. For hydration, there are three main sites in serotonin to which a water molecule can bind strongly: (1) the NH_2 group of the ethylamine side chain (2) the 5-OH group on the indole ring as a donor/acceptor and (3) the NH bond and pi cloud of indole. Delchev and Mikosh[51] calculated the relative energies of water binding to serotonin with Anti(py) conformation at four possible binding sites and found that the water bound to the amino group was the most stable of the four hydrogen bonding possibilities.[22] We have also performed the similar calculations for the Gpy(out)/anti conformation of serotonin forming complex with a water molecule for the bonding sites: indole NH donor, OH donor/acceptor, and amino group acceptor (See Fig. S4 of ESI) at MP2/6-31+G(d) level and found the greater binding energies for the complex involving amino group acceptor binding site. The binding energies with BSSE corrections of $Sero_1$-$(H_2O)_1$ complex involving water at (indole) NH donor, OH donor, OH acceptor and amino group acceptor sites are found to be , 25.16, 19.33, 38.3 KJ/Mol respectively .Therefore for computing the optimized structures of $Sero_1$-$(H_2O)_1$ complex, the input geometry of the experimentally observed [5] stable conformers A, B, C, D, E, F, G and H of bare serotonin were used for the complexation with a single water at amino group site. These $Sero_1$-$(H_2O)_1$ complexes, in which the water molecule is hydrogen bonded donor to the lone pair of electrons on the amino group of the ethylamine side chain. are assigned as A-W_1, B- W_1, C- W_1 , D-W_1, E- W_1, F- W_1, G- W_1 and H- W_1, in view of the assignment of the corresponding monomer.[5] Optimized structures of these monohydrated complexes (of the observed serotonin monomers) along with their relative energies are shown in Fig. S3 of ESI. The hydrogen bonding energies with BSSE corrections of these complexes from A

to H are found to be 38.3 (A-W_1), 30.9 (B- W_1) , 51.7 (C- W_1), 31.8 (D-W_1) , 28.8 (E- W_1), 38.8 (F- W_1), 30.5 (G- W_1) and 34.0 (H- W_1) KJ/Mole respectively. Thus the complex involving Gph(out)/anti conformation is predicted to be the most stable, the complex involving Gpy(out) ethylamine side chain orientation with syn and anti 5-OH conformations are predicted to be the second and the third most stable and the complex involving Anti(up)/anti conformation is the least stable. The only one conformer Gph(out)/anti (C), is capable of bringing NH_2 and 5-OH groups into close enough proximity to form hydrogen bonded bridge with a single water. It should be noted here that in the bare serotonin eight different conformers with five different ethylamine side chain orientations were experimentally observed.[5] With the addition of a water the complex involving just three conformations Gph(out)/anti, Gpy(out)/syn and Gpy(out)/anti are only observed in the R2PI and UV-UV hole- burning spectra.[22] The relative energies (See Figure S 3 of supporting information) and binding energies (with BSSE corrections) computed in the present work indicates that the experimentally observed complexes are the three lowest energy structures of $Sero_1$-$(H_2O)_1$. We re-optimized these three lowest energy structures of $Sero_1$-$(H_2O)_1$ complex involving Gph(out)/anti, Gpy(out)/syn and Gpy(out)/anti conformations at the M06/6-311++G(d,p) and MP2/6-311++G(d,p) levels of theory which is shown in Fig. 7. As seen in Figure 7, the most stable $Sero_1$-$(H_2O)_1$ complex has serotonin in the same Gph(out)/anti (C) conformation with the water molecule forming a hydrogen-bonded bridge linking the lone pair of electrons of the amino group nitrogen and the 5-hydroxy group. The water molecule serving as donor to the amino group and acceptor to the 5-hydroxy group. This most stable monohydrated serotonin contains two traditional H-bonds, involving OH..N and OH...O linkages. LeGreve et al,[22] however noted that the both H-bonds in the bridge have heavy-atom distances that are elongated relative to their values in optimal single H-bonds and the H-bond angles are bent away. Thus the single water bridge is straining to span the gap between amino and hydroxyl groups. Further the addition of a second water molecule to serotonin completes the conformational locking process for both the 5-OH and ethylamine side chains.[22] Therefore optimization of the ground state structure of the only observed $Sero_1$-$(H2O)_2$ cluster involving Gph-out/anti conformation in which the water dimer bridge forms a set of three strong H-bonds linking the NH_2 and OH groups (see Fig. 7), was performed at the M06/6-311++G(d,p) and MP2/6-311++G(d,p) levels of theory. The hydrogen bond geometries and dipole moments of the three lowest energy structures of $Sero_1$-$(H_2O)_1$ complex and the lowest energy structure of $Sero_1$-$(H_2O)_2$ complex, obtained from MP2 and M06 methods are listed in Table 6. The computed hydrogen bond geometries are found in excellent agreement with the experiment. Further it is now worthwhile to discuss briefly

the IR spectroscopic signatures accompanying these $Sero_1$-$(H_2O)_{1,2}$ complexes. Harmonic wave numbers and IR intensities of the most stable isomers of $Sero_1$-$(H_2O)_1$ and $Sero_1$-$(H_2O)_2$ complexes were calculated by DFT-B3LYP employing the basis sets 6-311++G(d,p) and 6-311++G(2d,2p), using the S_0 optimized geometries at the MP2/6-311++G(d,p) level of theory. The IR spectral shift in the OH and the NH stretch fundamentals of the complexes were determined by comparing their frequencies with corresponding monomer fundamental at the same level of theory. A large spectral shift of 332 cm^{-1} was predicted for the symmetric OH stretch fundamental of water molecule in $Sero_1$-$(H_2O)_1$ involving Gph(Out)/anti. The free (asymmetric) OH stretch mode is predicted at 3729 cm^{-1} which is in consonance to the experimental value [22] of 3720 cm^{-1}. The spectral shift of 131 cm^{-1} was predicted for 5-OH stretch, which is close to the reported experimental shift[21] of 117 cm^{-1}. Spectral shift of 37 cm^{-1} is predicted for the antisymmetric NH_2 stretch fundamentals of $Sero_1$-$(H_2O)_1$. In $Sero_1$-$(H2O)_2$ cluster involving the same Gph-out/anti conformation, the water dimer bridge has highly strengthened hydrogen bonds due to the cooperative strengthening in hydrogen bond bridges. The strengthening of the hydrogen bonds in $Sero_1$-$(H2O)_2$ complex reflects from its high binding energy of 104.94 KJ/mol and reduction in hydrogen bond distances of the 5-OH...O and OH...N relative to their values in $Sero_1$-$(H2O)_1$. The lowest frequency symmetric OH stretch, which is mainly localized on the OH group of a water molecule bound to NH_2, is strongly red shifted, more than 660 cm^{-1} below its free value, with greatly enhanced intensity. This OH (OH... NH_2) stretch fundamental of the water dimer, was predicted, at 3104 cm^{-1} (with a high IR intensity of 1109) which is slightly different to the experimental value of 3029 cm^{-1}. [22] This small discrepancy in part may be due to a large anharmonicity involved in this strongly H-bonded OH stretching vibration. The other two OH groups of the $Sero_1$-$(H2O)_2$ bridge (the 5-OH and its neighboring water OH) have OH stretch modes which are strongly coupled, producing in-phase and out-of-phase stretching modes. These two in-phase and out-of-phase OH stretching modes predicted at 3357 and 3422 cm^{-1} respectively, which is in consonance to the experiment.[22] The NBO calculations show that the OH bond of water (in $Sero_1$-$(H2O)_2$ complex), which points toward the lone pair electron of amino nitrogen, resulting in a withdrawal of electron density located at nitrogen atom. The electron redistribution is promoted by the formation of intermolecular interactions between the OH bond of the water and amino nitrogen (See Table S5 of ESI). This is supported by the existence of several physical criteria that are used to appreciate whether a hydrogen bond is established between a hydrogen bond donor and a lone pair of a hydrogen bond acceptor. (i) the elongation of the OH bond by more than 0.03 A^0 due to the increase of the electron population of the OH orbital, (ii) a large red

shift of the OH frequency with enhanced intensity, and (iii) the very large spectral shift of more than 660 cm^{-1} shows a strong H-bonding between OH bond of a water molecule and lone pair of NH_2.

3.4. Vertical Excitation Energies of Serotonin-water complexes

We have performed calculations for the lowest lying excited state of all the experimentally observed isomers of monohydrated serotonin by TDDFT method in order to estimate the spectral shift caused by micro-hydration as well as by macro-hydration. The TDDFT (B3LYP) vertical excitation energies (VEE) to the lowest S_1 ($^1\pi\pi^*$) state, followed by S_2 ($^1n\pi^*$) state for $Sero_1$-$(H2O)_1$ complexes are listed in Table 7. The CC2 vertical excitation energies of the two lowest energy $Sero_1$-$(H2O)_1$ complexes (involving monomers A and C) calculated at corresponding CC2-optimized S_0 geometry are also given in Table 7. As seen in Table 7 that the VEE to the lowest L_b ($^1\pi\pi^*$) state for the $Sero_1$-$(H_2O)_1$ complex involving conformer Gpy(out)/anti (A) is blue shifted by 140 cm^{-1} in comparison to the corresponding monomer. The VEE to the same L_b ($^1\pi\pi^*$) state for $Sero_1$-$(H_2O)_1$ involving conformer Gph(out)/anti (C) is red shifted by 314 cm^{-1} and for the strongly H-bonded $Sero_1$-$(H2O)_2$ complex involving the same conformer C, it (VEE to the L_b ($^1\pi\pi^*$) state) was significantly red shifted by 1287 cm^{-1} in comparison to monomer. The electronic frequency shift measures the difference in binding energies of the complex in the S_0, S_1 and S_2 states.[22] Thus the blue shift in L_b ($^1\pi\pi^*$) state of $Sero_1$-$(H_2O)_1$ (See Figure 7-II) from their corresponding monomer state reflects a decrease in the binding energy upon electronic excitation. A blue shift for a π-π^* transition is also attributed to the reduction in the π-electron conjugation length of the molecule due to the hydrogen bond formation.[52] The corresponding red shift (in L_b ($^1\pi\pi^*$) state) for $Sero_1$-$(H_2O)_{1,2}$ involving C conformation shows that S_0-S_1 electronic transition increases the strength of the hydrogen bonds to water. Interestingly the VEE to the optically bright state ^1L_a ($^1\pi\pi^*$) of $Sero_1$-$(H_2O)_1$ (See Figure 7-II) is red shifted by 223 cm^{-1} and conversely the VEE to the same L_a ($^1\pi\pi^*$) state for $Sero_1$-$(H_2O)_1$ (See Figure 7-I) is blue shifted significantly by 352 cm^{-1}. Relatively a lower red shift of 282 cm^{-1} to the same L_a ($^1\pi\pi^*$) state is predicted for $Sero_1$-$(H_2O)_2$ involving C. Thus the blue shift in L_a- state of $Sero_1$-$(H_2O)_1$ (See Fig. 7-I) from their corresponding monomer state reflects a decrease in the binding energy and the red shift in L_a - state of $Sero_1$+$(H_2O)_1$ (See Figure 7-II) reflects an increase in the binding energy, upon electronic excitation to the second lowest excited state. One striking feature is the coexistence of the blue and red shift of the vertical excitation energies of the lowest lying states

^1L_b ($^1\pi\pi^*$) and ^1L_a ($^1\pi\pi^*$) respectively upon forming complex with a water at amino group site in the two most stable serotonin conformations which is predicted in just opposite order for $Sero_1$-$(H_2O)_1$ involving A and C.

The effect of hydration on ^1L_b ($\pi\pi^*$) and ^1L_a ($\pi\pi^*$) excited states due to bulk water environment was also performed by a combination of polarizable continuum solvent model (PCM)[39-41] and the conductor like screening model (COSMO)[42] which shows a red shift for each state of the experimentally observed conformers. The TD-B3LYP/aug-cc-pVDZ VEE for ^1L_b ($\pi\pi^*$) and ^1L_a ($\pi\pi^*$) excited states in bulk water environment are given in Table S6 of ESI. The TD-B3LYP/TZVP VEE for the ^1L_b ($\pi\pi^*$) and ^1L_a ($\pi\pi^*$) excited states of bare serotonin C are computed at 4.39 (with an oscillator strength 0.068) and 4.72 eV (with an oscillator strength 0.095) respectively, while in COSMO bulk water environment the corresponding VEE are computed at 4.34 (with an oscillator strength 0.074) and 4.66 eV (with an oscillator strength 0.121) respectively. Thus a red shift of 0.05 and 0.06 eV for the transition to ^1L_b and ^1L_a excited states respectively is predicted. Similar red shift in COSMO bulk water environment are also predicted for Serotonin A. This indicates the strengthening of the hydrogen bonding, in general, in the bulk water environment due to electronic excitations to the low lying excited states ^1L_b and ^1L_a. We have earlier mentioned that the red shift in the lowest lying L_b ($^1\pi\pi^*$) state for $Sero_1$-$(H_2O)_1$ involving Gph(out)/anti (C) conformation shows that S_0-S_1 electronic transition increases the strength of the hydrogen bonds to water. LeGreve et al[22] anticipated that a major consequence of electronic excitation is the strengthening of the 5-OH...water H-bond, which should shift the water molecule toward the 5-OH group. We have optimized the lowest excited structure of $Sero_1$-$(H_2O)_1$ involving conformer C at the B3LYP/def.TZVP level of the theory. Interestingly the optimized lowest excited state (^1L_b) structure of $Sero_1$-$(H2O)_1$ complex involving the Gph(out)/anti (C) conformation shows a major shift of 1.15 A^0 of water molecule towards the 5-OH group due to S_0-S_1 electronic excitation and a significant lengthening of the OH...N distance by 0.12 A^0 in the S_1 excited state (See Figure 8). However earlier calculation [22] was performed at the CIS/6-31+G(d) level of theory, predicted a decrease in the 5-OH...O heavy atom distance of 0.32 Å and a minor lengthening of the OH..N distance by 0.02 in the excited S_1 state. [22] Our result is significantly consistent with the argument [22] that a single water molecule cannot span the gap between 5-OH and NH_2 groups in serotonin and so has two non optimal hydrogen bonds whose strength is changed upon electronic excitation.

4. Conclusion

The ground state optimized structures, harmonic vibrational wave numbers and intensities of the twenty three lowest energy conformers of neutral serotonin including the eight experimentally observed were computed using MP2 and DFT methods employing the basis sets 6-311++G(d,p) and aug-cc-pVDZ for the first time. The weak NH.... π intramolecular interactions between an ethylamine group and aromatic system leading to stabilizing the various structures in serotonin. The eight experimentally observed stable structures were characterized by CC2, CCSD, MP2 and by employing the various density functional B3LYP, B3PW91, X3LYP, TPSSh, M05, M06, M05-2X, M06-2X of the density functional theory including the dispersion corrected ωB97XD and DFT-D3 methods. The present work concluded that the Gph(out)/anti (C) is the most stable conformer of the neutral serotonin in the gas phase which is in agreement with recent experimental rotational spectroscopy results.[2] The most striking change induced by 5-OH substitution in serotonin is expected to stabilize the Gph(out)/anti conformation more relative to all other conformations. The appropriate level of theory for tackling the study of non bonded interactions in flexible bio-molecule-serotonin was checked. It was found that DFT functionals M05-2X , M06-2X, ωB97XD and B3LYP-D3 performs well for the NH...π intramolecular interactions. Computed vibrational wave-numbers and intensities of eight experimentally observed conformers are found in agreement with experiment.[5] Vibrational assignments of the lowest energy conformers of serotonin have been made and the predicted intensity of OH bending fundamental provides one of the most useful diagnostic for the 5-OH anti and syn conformations. The infrared alkyl CH stretch fundamentals are assigned which are found to be the most sensitive to conformational changes due to ethylamine side chain in serotonin. The splitting in the high frequency alkyl CH stretch band of conformers B, G and E is expected to arise due to a Fermi resonance between CH_2 (β) stretch at ~ 2949 cm^{-1} and a overtone of CH bending mode predicted at 1474 cm^{-1}. The assignment of conventional IR band at 3438 cm^{-1} to NH_2 symmetric stretching vibration by Mukherjee et al [18] seems to be ambiguous. The fundamental frequencies calculated by DFT method with B3LYP parameterization and 6-311++G(2d,2p) basis set using the MP2 optimized geometries are significantly more close to experiment than those produced by using the DFT optimized geometries. We investigated the low-lying excited states of bare serotonin by means of coupled cluster singles and approximate doubles (CC2) and TDDFT methods and a satisfactory interpretation of the electronic absorption spectra is obtained. B3LYP-TDDFT approach gives the more accurate VEE for serotonin in comparison to CC2. The relative energy between the computed origin of the ^1L_a and the ^1L_b states

of serotonin in the most stable Gph(out) conformation is found to be 3841 cm^{-1}. The amino group (acceptor) site seems to be the most favorable binding site for a water molecule in neutral serotonin. The most stable $Sero_1$-$(H2O)_1$ complex has serotonin in the same Gph(out)/anti (C) conformation with the water molecule forming a hydrogen-bonded bridge linking the lone pair of electrons of the amino nitrogen and the 5-OH in which the water molecule serving as donor to the amino group and acceptor to the 5-hydroxy group. The most striking feature is the coexistence of blue and red shift of the vertical excitation energy of the lowest lying states ^1L_b ($^1\pi\pi^*$) and ^1L_a ($^1\pi\pi^*$) respectively upon forming complex with a water at amino group site of serotonin. The optimized lowest excited state (^1L_b) structure of $Sero_1$-$(H2O)_1$ complex involving the Gph(out)/anti (C) conformation shows a major shift of 1.15 Å of water molecule towards the 5-OH group due to S_0-S_1 electronic excitation and a significant lengthening of the OH...N distance by 0.12 Å in the S_1 excited state. This result is consistent with the argument [22] that a single water molecule cannot span the gap between 5-OH and NH_2 groups in serotonin and so has the non optimal hydrogen bond whose strength is changed upon electronic excitation. Addition of second water molecule to serotonin C forming a water dimer bridge which spans the gap between the amino and 5-OH groups. The strengthening of the hydrogen bonds in $Sero_1$-$(H2O)_2$ complex reflects from its high binding energy of 104.43 KJ/Mol and reduction in hydrogen bond distances of the 5-OH...O and OH...N relative to their values in $Sero_1$-$(H2O)_1$. Theoretical electronic spectra (VEE) of free and hydrated complexes of neutral serotonin also provides most useful diagnostic of their different conformations. They provide characteristic 'spectroscopic signatures' which can reflect differences in the nature of hydrogen-bonded interactions in different stable conformations at amino group site. .

Acknowledgement

Authors are grateful to Research Projects [41-880/2012 (SR)] of University Grants Commission, Government of India, New Delhi and [SR/S2/LOP-16/2008] of Department of Science and Technology Government of India, New Delhi for support.

Supporting Information Available

The computed structural parameters, NBO charges, rotational constants, vertical excitation energies in bulk water environment etc of different conformers of serotonin are submitted here as a electronic supplementary information which is available in the online version of the paper.

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Table 1 Relative zero-point corrected [a] energies (kJ/mol) and dipole moments (D) in parenthesis of the *anti*/*syn* conformational pairs of serotonin at the MP2/6-311++G(d,p) and MP2/aug-cc-pVDZ levels of theory.

S. No.	description of the ethylamine side chain	tryptamine,[b] MP2/6-311++G(d,p)	serotonin, MP2/6-311++G(d,p)		serotonin, MP2/aug-cc-pVDZ	
			anti-5-OH	*syn*-5-OH	*anti*-5-OH	*syn*-5-OH
1	Gph (out)	1.63	0.00 (3.5547)	4.49 (3.9880)	0.00 (3.4172)	3.73 (3.9979)
2	Gpy (out)	0.00	0.10 (1.4270)	1.47 (2.8797)	0.48 (1.3292)	1.95 (2.8762)
3	Gpy (up)	1.50	1.12 (3.1864)	2.91 (4.2897)	2.73 (3.1398)	4.36 (3.9313)
4	Gph (up)	3.64	4.22 (2.7530)	5.77 (3.8973)	4.84 (2.7505)	5.86 (3.7228)
5	Anti (ph)	5.68	5.51 (3.5572)	8.32 (3.9954)	6.80 (3.4409)	8.86 (3.9532)
6	Anti (py)	5.77	6.27 (1.4570)	7.53 (2.8894)	7.58 (1.4012)	8.61 (2.8561)
7	Anti (up)	5.98	6.31 (3.1457)	7.85 (4.0994)	7.51 (3.0790)	8.78 (3.7923)
8	Gpy (in)	8.28	9.14 (3.5410)	10.60 (2.5610)	9.95 (3.5410)	11.22 (2.5610)
9	Gph (in)	13.04	11.21 (3.7612)	14.25 (2.5228)	12.61 (3.7612)	14.71 (2.5228)
10	Conf. 9[c]	-	11.99 (1.1419)	13.45 (2.9182)	-	-
11	Conf. 10[c]	-	4.20 (2.7525)	5.77 (3.8963)	-	-
12	Conf. 11[c]	-	12.38 (3.1694)	14.11 (3.5774)	-	-

[a] Zero-point corrections use the DFT B3LYP/6-311++G(d,p) and aug-cc-pVDZ harmonic frequencies respectively.
[b] From ref 44. [c] From ref 11.

Table 2 Relative zero-point corrected energies (kJ/mol) and dipole moments (D) in parenthesis of the *anti*/*syn* conformational pairs of serotonin at the B3LYP/6-311++G(d,p) and B3LYP/aug-cc-pVDZ levels of theory.

S. No.	description of the ethylamine side chain	tryptamine,[a] B3LYP/6-311++G(d,p)	serotonin, B3LYP/6-311++G(d,p)		serotonin, B3LYP/aug-cc-pVDZ	
			anti-5-OH	*syn*-5-OH	*anti*-5-OH	*syn*-5-OH
1	Gph (out)	2.38	1.07 (3.2219)	4.07 (3.7671)	1.03 (3.0694)	3.67 (3.6118)
2	Gpy (out)	0.00	0.00 (1.2503)	0.76 (2.7944)	0.00 (1.2191)	0.70 (2.6650)
3	Gpy (up)	0.88	0.57 (2.9144)	1.49 (3.8737)	0.77 (2.7854)	1.60 (3.6585)
4	Gph (up)	3.55	3.56 (2.4814)	3.97 (3.6515)	3.84 (2.4160)	4.17 (3.4901)
5	Anti (ph)	2.63	1.60 (3.2526)	2.84 (3.7773)	1.05 (3.1181)	2.33 (3.6081)
6	Anti (py)	2.63	2.21 (1.3352)	2.55 (2.7712)	1.76 (1.2749)	2.11 (2.6138)
7	Anti (up)	2.34	1.51 (2.8270)	2.13 (3.6515)	1.35 (2.7072)	2.01 (3.4670)
8	Gpy (in)	4.89	5.11 (3.2144)	5.37 (2.2588)	4.53 (3.0299)	5.05 (2.1336)
9	Gph (in)	10.62	8.37 (3.4308)	11.22 (2.4161)	8.51 (3.2628)	10.68 (2.2810)
10	Conf. 9[b]	-	4.22 (0.9963)	4.63 (2.7356)	3.23 (0.9674)	3.99 (2.5717)
11	Conf. 10[b]	-	5.39 (1.1272)	3.96 (3.6512)	4.78 (0.9475)	4.17 (3.4900)
12	Conf. 11[b]	-	4.65 (3.2005)	5.38 (3.6449)	3.28 (3.0464)	4.31 (3.4675)

[a] From ref 44. [b] From ref 11.

Table 3 Relative zero-point corrected energies [a] [E_{rel} (kJ/mol)] and dipole moments (Debye) in parenthesis of the eight experimentally observed conformers of serotonin computed at aug-cc-pVDZ levels.

conformer	E_{rel}^{CCSD} [b]	E_{rel}^{MP2}	$E_{rel}^{M06\text{-}2X}$	$E_{rel}^{M05\text{-}2X}$	$E_{rel}^{\omega B97X\text{-}D}$	E_{rel}^{M06}	E_{rel}^{X3LYP}	$E_{rel}^{B3LYP\text{-}D3}$ [c]	E_{rel}^{B3LYP}	E_{rel}^{B3PW91}	E_{rel}^{TPSSh}	E_{rel}^{CC2} [c]
A	1.58 *(1.3828)*	0.48 *(1.3292)*	1.00 *(1.1703)*	0.13 *(1.1853)*	0.23 *(1.1134)*	**0.00** *(1.1449)*	**0.00** *(1.2174)*	0.72 *(1.2230)*	**0.00** *(1.2191)*	**0.00** *(1.1963)*	**0.00** *(1.1967)*	0.81
B	3.26 *(3.3745)*	2.73 *(3.1398)*	2.33 *(2.8046)*	1.31 *(2.8565)*	0.57 *(2.7993)*	1.21 *(2.6799)*	0.87 *(2.7929)*		0.77 *(2.7854)*	0.41 *(2.7545)*	0.46 *(2.7693)*	1.62
C	**0.00** *(3.6153)*	**0.00** *(3.4172)*	**0.00** *(3.0793)*	**0.00** *(3.1865)*	**0.00** *(3.0695)*	0.36 *(2.9575)*	0.83 *(3.0659)*	**0.00** *(3.1533)*	1.03 *(3.0694)*	1.14 *(3.0445)*	0.92 *(3.0897)*	**0.00**
D	9.52 *(1.4006)*	7.58 *(1.4012)*	6.09 *(1.1898)*	5.09 *(1.2560)*	4.68 *(1.1721)*	4.10 *(1.1323)*	1.99 *(1.2673)*		1.76 *(1.2749)*	2.30 *(1.2482)*	2.76 *(1.2654)*	9.38
E	7.45 *(3.2999)*	7.51 *(3.0790)*	5.88 *(2.7939)*	4.74 *(2.8431)*	4.00 *(2.7595)*	4.37 *(2.6568)*	1.63 *(2.7250)*		1.35 *(2.7072)*	1.64 *(2.6713)*	2.01 *(2.7169)*	8.50
F	3.78 *(3.2202)*	1.95 *(2.8762)*	2.09 *(2.7296)*	1.14 *(2.7704)*	1.19 *(2.7195)*	0.97 *(2.6796)*	0.72 *(2.6748)*		0.70 *(2.6650)*	0.78 *(2.6930)*	0.76 *(2.6842)*	3.09
G	4.47 *(4.3264)*	4.36 *(3.9313)*	3.54 *(3.6910)*	2.46 *(3.7323)*	1.68 *(3.6935)*	2.30 *(3.5939)*	1.71 *(3.6662)*		1.60 *(3.6585)*	1.30 *(3.6460)*	1.33 *(3.6410)*	4.14
H	5.77 *(4.3486)*	3.73 *(3.9979)*	3.41 *(3.6508)*	3.34 *(3.7958)*	3.20 *(3.6410)*	3.37 *(3.5405)*	3.52 *(3.6116)*		3.67 *(3.6118)*	3.86 *(3.6287)*	3.64 *(3.6571)*	5.38

[a] Zero-point corrected energies at the MP2, M06-2X, M05-2X, ωB97X-D, M06, X3LYP, B3LYP, B3PW91 and TPSSh optimized geometries as well as for CCSD single-point energies were obtained by including the zero-point corrections from B3LYP/aug-cc-pVDZ and the CC2 and B3LYP-D3 optimized geometries were corrected from DFT B3LYP/def-TZVP harmonic frequencies. [b] Single-point energy calculation with basis set 6-31+G(d). [c] Optimized using the basis set def-TZVP.

Table 4 Computed harmonic infrared frequencies, intensities and raman activities for the eight experimentally observed serotonin conformers at B3LYP/6-311++G(2d,2p) level.

approximate descriptions of vibrations	Conformer A				Conformer C				Conformer F				Conformer H			
	expt.[a]	freq	int	raman activity	expt.[a]	freq	int	raman activity	expt.[a]	freq	int	raman activity	expt.[a]	freq	int	raman activity
ν (OH)	3667	3667	48	102	3666	3664	50	98	3671	3674	65	141	3671	3674	63	142
ν (indole NH)	3530	3530	78	143	3530	3530	78	147	3531	3531	77	143	3530	3531	77	147
ν $(NH_2)_a$	3410	3430	3	67	3407	3426	3	70	3409	3429	2	67	-	3429	3	69
ν $(NH_2)_s$	3339	3358	4	145	3342	3356	7	160	3338	3358	4	146	-	3359	7	165
ν (CH) pyrrole	-	3096	0	109	-	3090	0	117	-	3095	0	110	-	3089	0	117
ν (HCCH) (+) in phenol ring	-	3051	5	193	-	3051	6	195	-	3035	13	160	-	3034	12	172
ν (HCCH) (-) in phenol ring	-	3029	7	98	-	3029	7	108	-	3009	12	105	-	3008	12	106
ν (C4-H) phenyl	-	3010	13	64	-	3022	6	47	-	3044	3	89	-	3053	1	71
ν $[CH_2(\beta)]_a$	2960 (w)	2973	29	81	2955 (m)	2966	24	65	2963 (w)	2973	29	78	2955 (m)	2968	22	61
ν $[CH_2(\alpha)]_a$	2947 (w)	2956	63	218	2941 (m)	2952	82	293	2945 (w)	2957	63	221	2942 (m)	2951	85	302
ν $[CH_2(\beta)]_s$	2919 (m)	2925	28	120	2923 (m)	2928	20	96	2922 (m)	2928	26	119	2927 (m)	2929	19	94
ν $[CH_2(\alpha)]_s$	2851 (w)	2871	51	87	2849 (w)	2867	61	113	2854 (w)	2878	46	87	2846 (w)	2863	64	111
Overtone of δ (CH)					2912 (w)	-	-	-					2915 (m)	-	-	-
δ $(CH)_{ring}$	-	1474	66	5	-	1474	69	4	-	1472	42	5	-	1472	44	6
δ (OH)	-	1181	182	8	-	1182	152	5	-	1180	75	10	-	1175	84	8

Table 4 Continued

approximate descriptions of vibrations	Conformer B				Conformer G				Conformer D				Conformer E			
	expt.[a]	freq	int	raman activity	expt.[a]	freq	int	raman activity	expt.[a]	freq	int	raman activity	expt.[a]	freq	int	raman activity
ν (OH)	3668	3666	49	102	3672	3674	66	142	3668	3667	47	100	3667	3667	48	99
ν (indole NH)	3531	3530	79	143	3530	3531	78	143	3531	3532	79	147	3530	3531	80	149
ν $(NH_2)_a$	-	3414	3	35	-	3414	2	35	-	3434	0	87	-	3428	0	52
ν $(NH_2)_s$	-	3348	1	111	-	3347	1	112	-	3365	1	165	-	3361	2	133
ν (CH) pyrrole	-	3091	0	109	-	3091	0	110	-	3092	0	119	-	3091	0	119
ν (HCCH) (+) in phenol ring	-	3052	5	193	-	3035	12	160	-	3052	5	195	-	3052	5	195
ν (HCCH) (-) in phenol ring	-	3030	7	98	-	3010	12	105	-	3029	7	100	-	3030	7	100
ν (C4-H) phenyl	-	3011	13	64	-	3045	2	89	-	3007	16	69	-	3007	15	68
ν $[CH_2(\beta)]_a$	2944 (m)	*2947*	47	134	2948 (m)	*2948*	47	145	2969 (w)	*2975*	32	54	2925 (m)	*2938*	10	114
ν $[CH_2(\alpha)]_a$	2949 (s)	2973	54	75	2939(w)	2976	50	68	2949 (m)	*2957*	21	64	2949 (s)	*2979*	33	13
ν $[CH_2(\beta)]_s$	2851 (w)	2908	38	145	2849 (w)	2911	37	145	2937 (m)	2905	31	151	2852 (s)	2905	39	166
ν $[CH_2(\alpha)]_s$	2883 (w)	*2934*	18	181	2882 (w)	*2936*	19	174	2856 (s)	2882	55	98	2884 (m)	2943	37	55
						-	-	-					2854 (w)	-	-	-
Overtone of δ (CH)	2909 (w)	-	-	-	2911 (w)	-	-	-					2912 (m)	-	-	-
	2921 (w)	-	-	-	2919 (w)								2945 (s)	-	-	-
$\delta (CH)_{ring}$	-	1475	66	5	-	1472	43	5	-	1474	68	5	-	1474	69	6
δ (OH)	-	1183	218	7	-	1195	89	25	-	1182	230	6	-	1182	229	7

[a]Taken from ref 5. * vibrational mode wave numbers in italic fonts exhibits mixed vibrations

Table 5 Vertical excitation energies (eV) and oscillator strengths (in bracket) for the low-lying excited states of serotonin.

conformational state	experimental gas phase value (0-0 excitations) for ^1L_b, [a]	calculated values at TD-DFT-B3LYP/aug-cc-pVDZ level					calculated values at CC2/def-TZVP level	
		^1L_b	^1L_a	$^1\pi\sigma^*_{(O-H)}$	$^1\pi\sigma^*_{(N-H)}$	^1B_b	^1L_b	^1L_a
Gph(out)/*anti*	4.0350	4.218	4.497	4.632	4.850	5.585	4.488	5.045
		(0.056)	(0.097)	(0.002)	(0.011)	(0.256)	(0.072)	(0.113)
		4.091 [b]	*4.463* [b]	-	-	*5.616* [b]	-	-
		(0.054)	*(0.098)*			*(0.56)*		
Gpy(out)/*anti*	4.0399	4.222	4.482	4.541	4.828	5.588	4.495	4.999
		(0.048)	(0.098)	(0.002)	(0.005)	(0.133)	(0.069)	(0.105)
Gpy(up)/*anti*	4.0354	4.219	4.501	4.598	4.886	5.594	4.489	5.032
		(0.053)	(0.092)	(0.004)	(0.012)	(0.035)	(0.072)	(0.108)
Anti(py)/*anti*	4.0369	4.218	4.464	4.504	4.712	5.648	4.493	4.995
		(0.050)	(0.102)	(0.001)	(0.002)	(0.249)	(0.070)	(0.113)
Anti(up)/*anti*	4.0340	4.197	4.451	4.493	4.701	5.544	4.489	5.000
		(0.053)	(0.062)	(0.012)	(0.003)	(0.137)	(0.072)	(0.112)
Gph(out)/*syn*	4.0024	4.162	4.507	4.572	4.900	5.560	4.422	5.022
		(0.056)	(0.084)	(0.001)	(0.001)	(0.260)	(0.071)	(0.100)
Gpy(out)/*syn*	4.0112	4.170	4.502	4.589	4.873	5.641	4.430	4.985
		(0.049)	(0.084)	(0.000)	(0.012)	(0.254)	(0.067)	(0.095)
Gpy(up)/*syn*	4.0063	4.166	4.517	4.615	4.952	5.541	4.424	5.016
		(0.053)	(0.092)	(0.000)	(0.001)	(0.234)	(0.070)	(0.098)

[a] From ref 5. [b] Corresponding experimental value taken from the ref 20.

Table 6 Computed hydrogen-bond geometries and dipole moments for the experimentally observed conformers of SERO ─ $(H_2O)_1$ and SERO ─ $(H_2O)_2$ clusters at 6-311++G(d,p) level.

conformer	methods	bond lengths (Å)			angles (°)			dipole moments (D)
		N – O	O – O	O – O-5	N ••• HO	O ••• HO	O ••• HO-5	
				SERO ─ $(H_2O)_1$				
	exptl.[a]	2.97		2.96	167		157	-
I	M06-2X	2.97		2.94	168		156	5.3632
	MP2	2.98		3.01	168		157	5.6732
	exptl.[a]	2.87			165			-
II	M06-2X	2.86			164			2.6267
	MP2	2.87			166			2.8289
	exptl.[a]	2.87			165			-
III	M06-2X	2.86			164			2.4535
	MP2	2.87			166			2.4994
				SERO ─ $(H_2O)_2$				
	exptl.[a]	2.79	2.75	2.77	179	176	176	-
I	M06-2X	2.77	2.73	2.75	178	174	177	5.9775
	MP2	2.79	2.75	2.77	178	175	176	6.3200

[a] From ref 22.

Table 7 Vertical excitation energies (eV) and oscillator strengths (in bracket) of SERO – $(H_2O)_{n=1}$, n =1, 2 clusters.

conformer	experimental gas phase value of 0-0 excitations [a]	calculated values at TD-DFT-B3LYP/6-311++G(d,p) level		calculated values at CC2/def-TZVP level	
		^1L_b	^1L_a	^1L_b	^1L_a
		Bare serotonin			
A Gpy(out)/anti	4.0399	4.2924 (0.051)	4.5582 (0.046)	4.4952 (0.069)	4.9994 (0.105)
C Gph(out)/anti	4.0350	4.2895 (0.061)	4.5710 (0.095)	4.4876 (0.072)	5.0454 (0.113)
F Gpy(out)/syn	4.0112	4.2411 (0.052)	4.5701 (0.078)	-	-
		SERO – $(H_2O)_1$			
A Gpy(out)/anti	4.0507	4.3078 (0.040)	4.5306 (0.088)	4.5051 (0.066)	4.9482 (0.107)
C Gph(out)/anti	3.9908	4.2506 (0.068)	4.6146 (0.085)	4.4294 (0.077)	5.1141 (0.109)
F Gpy(out)/syn	4.0237	4.2637 (0.044)	4.5469 (0.079)	-	-
		SERO – $(H_2O)_2$			
C Gph(out)/anti	3.9363	4.1299 (0.044)	4.6055 (0.074)	-	-

[a] From ref 5, 22.

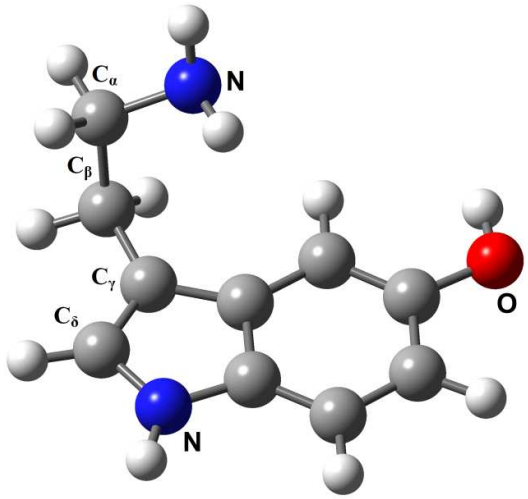

Fig.1 Picture of the serotonin molecule.

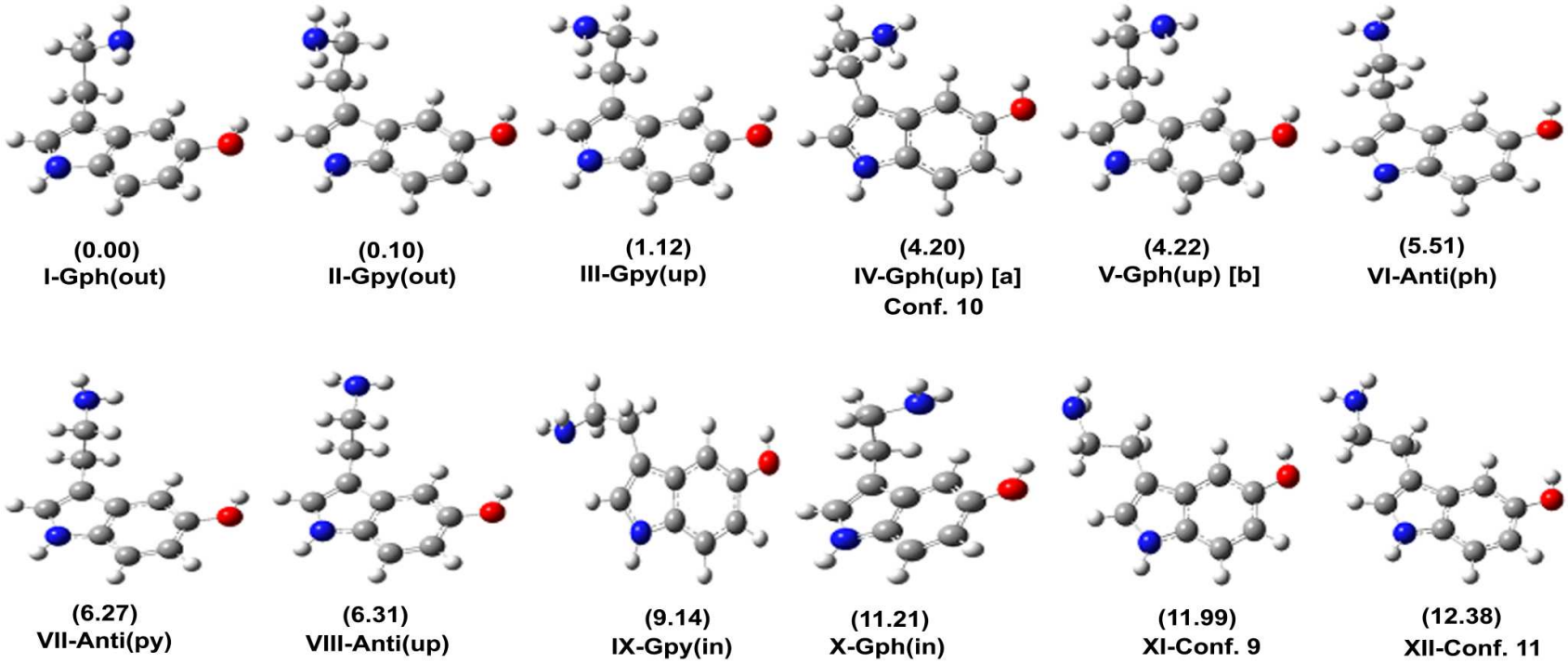

Fig. 2a Optimized structures and zero-point corrected relative energies (in kJ/mol) of twelve (OH-*anti*) serotonin conformers at MP2/6-311++G(d,p) level. The structures are labeled according to decreasing stability.

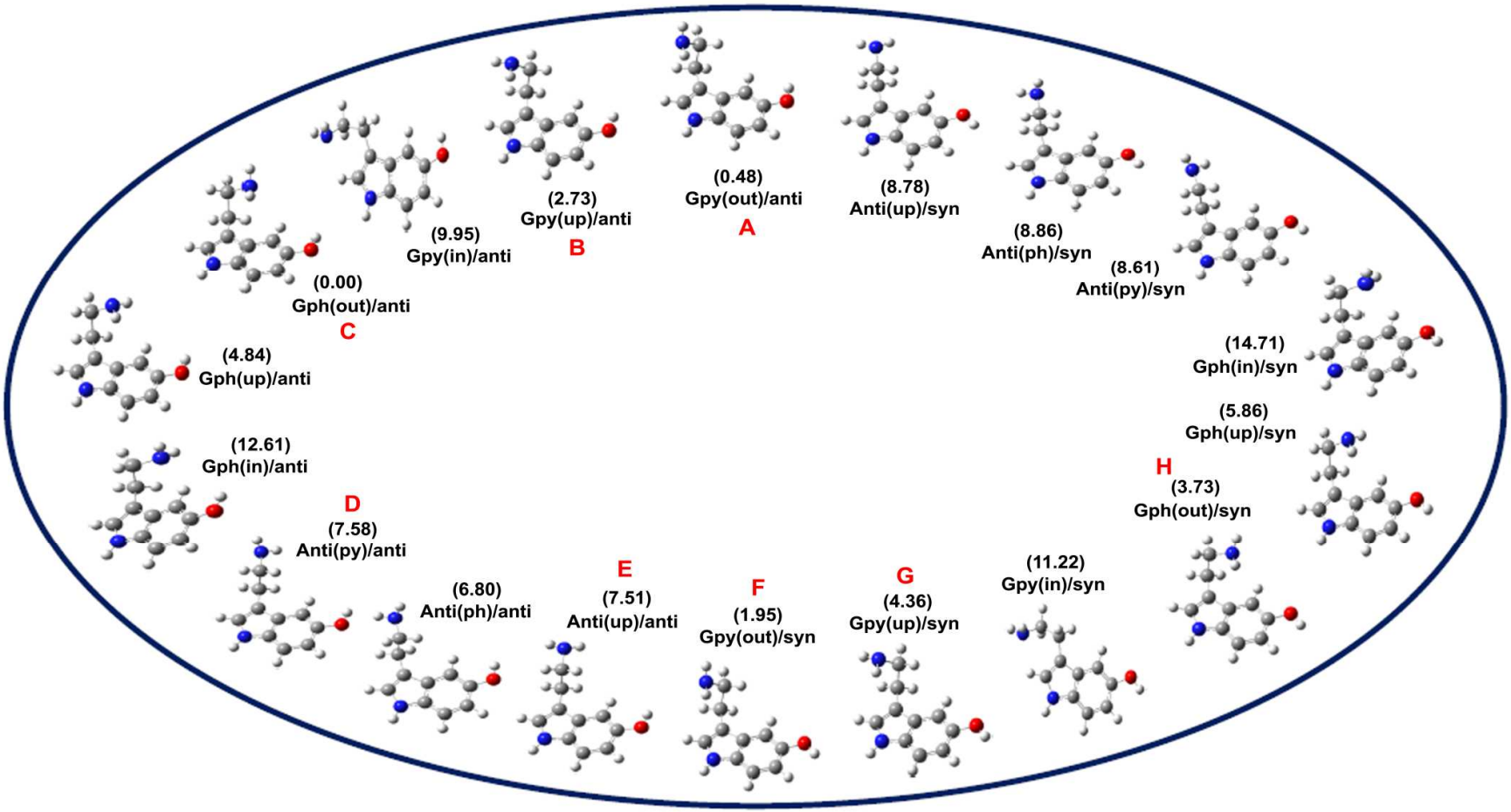

Fig. 2b Optimized structures (with ZPE-corrected relative energies) of the eighteen lowest-energy conformers of serotonin at the MP2/aug-cc-pVDZ level of theory.

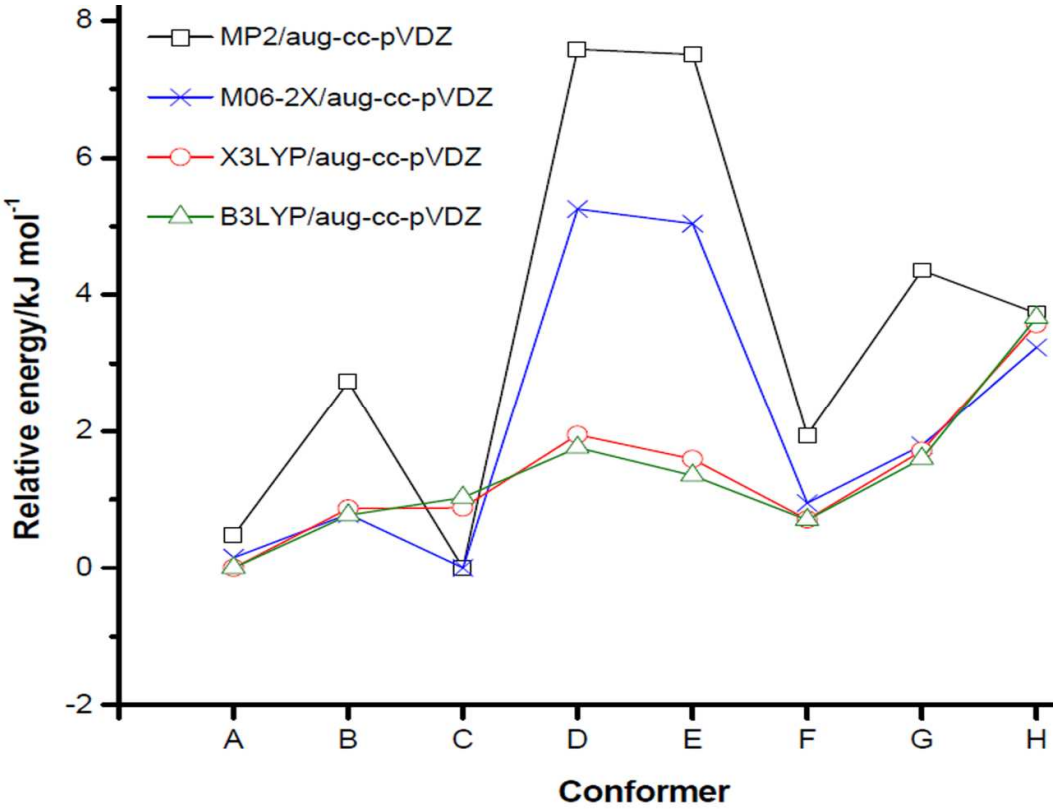

Fig. 3 Comparison of the MP2, M06-2X, X3LYP and B3LYP relative energies of serotonin conformers.

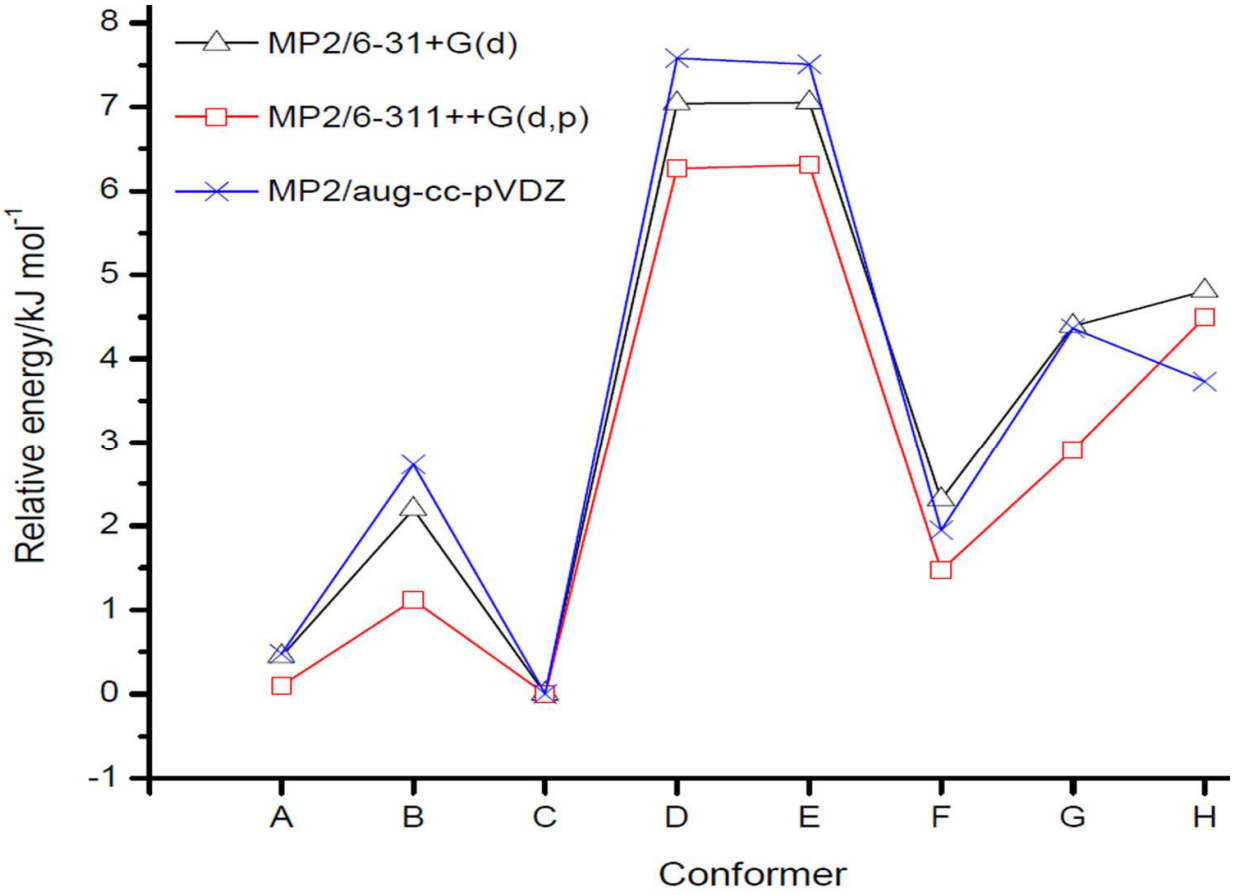

Fig. 4 Comparison of the relative stabilities of the serotonin conformers at MP2 level employing 6-31+G(d), 6-311++G(d,p) and aug-cc-pVDZ basis sets.

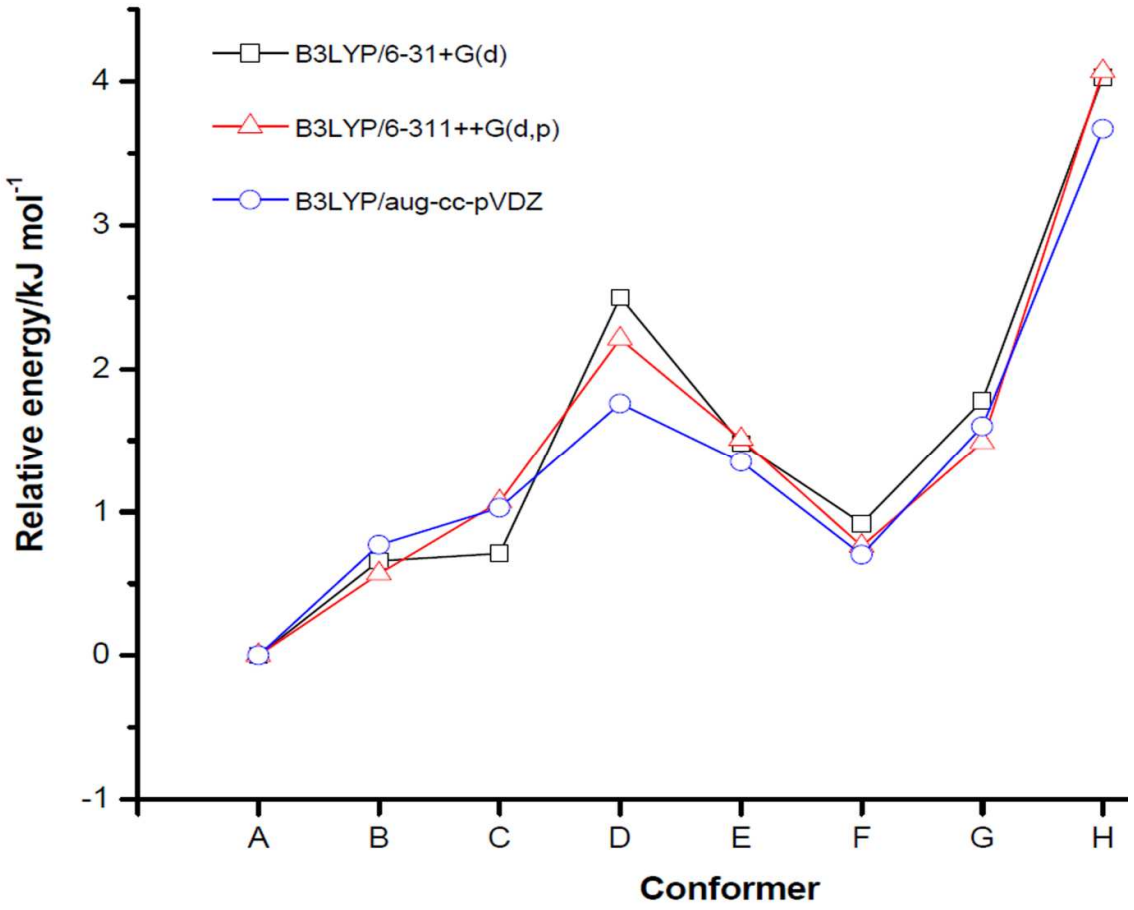

Fig. 5 Comparison of the relative stabilities of the serotonin conformers at B3LYP level employing 6-31+G(d), 6-311++G(d,p) and aug-cc-pVDZ basis sets.

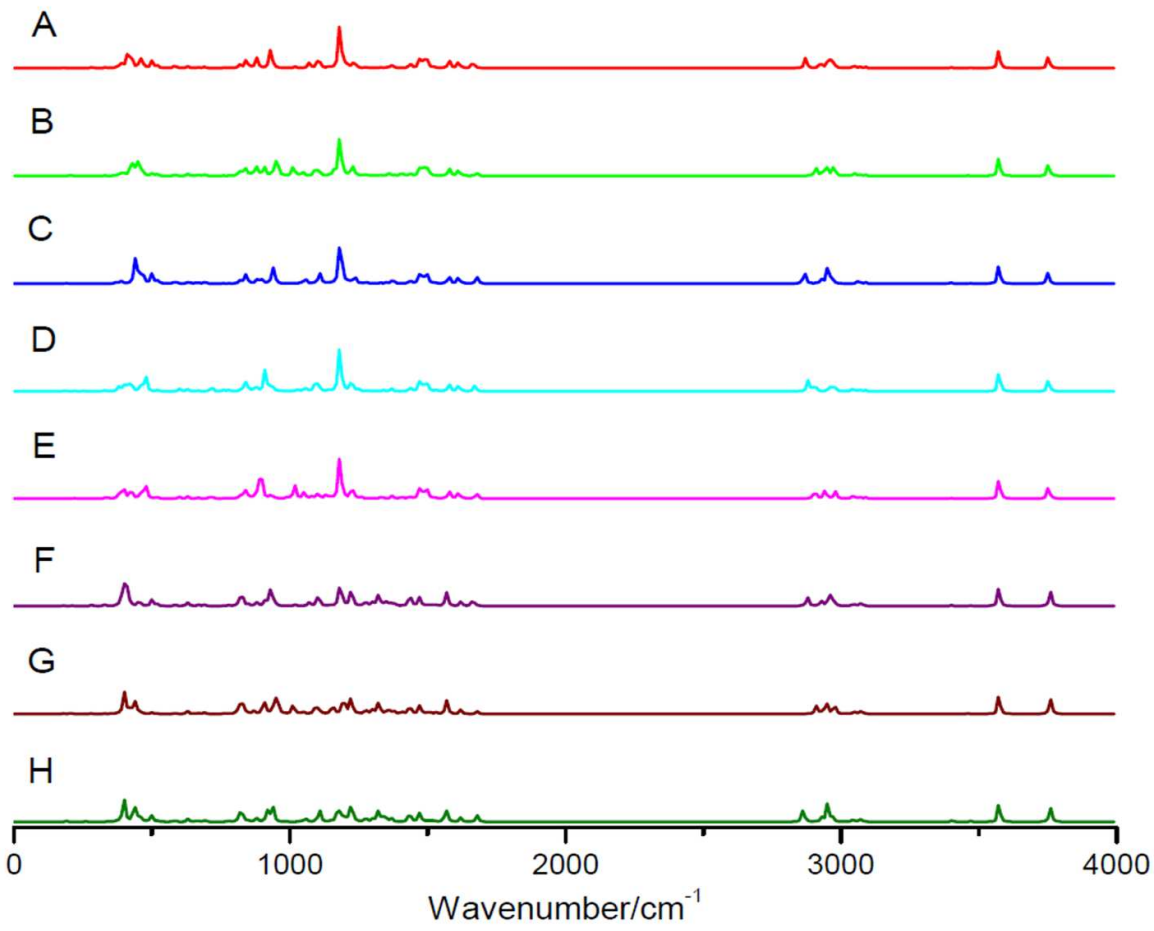

Fig. 6 . Calculated IR spectra of the eight experimentally observed conformers of serotonin at the B3LYP/6-311++G(2d,2p) level of theory.

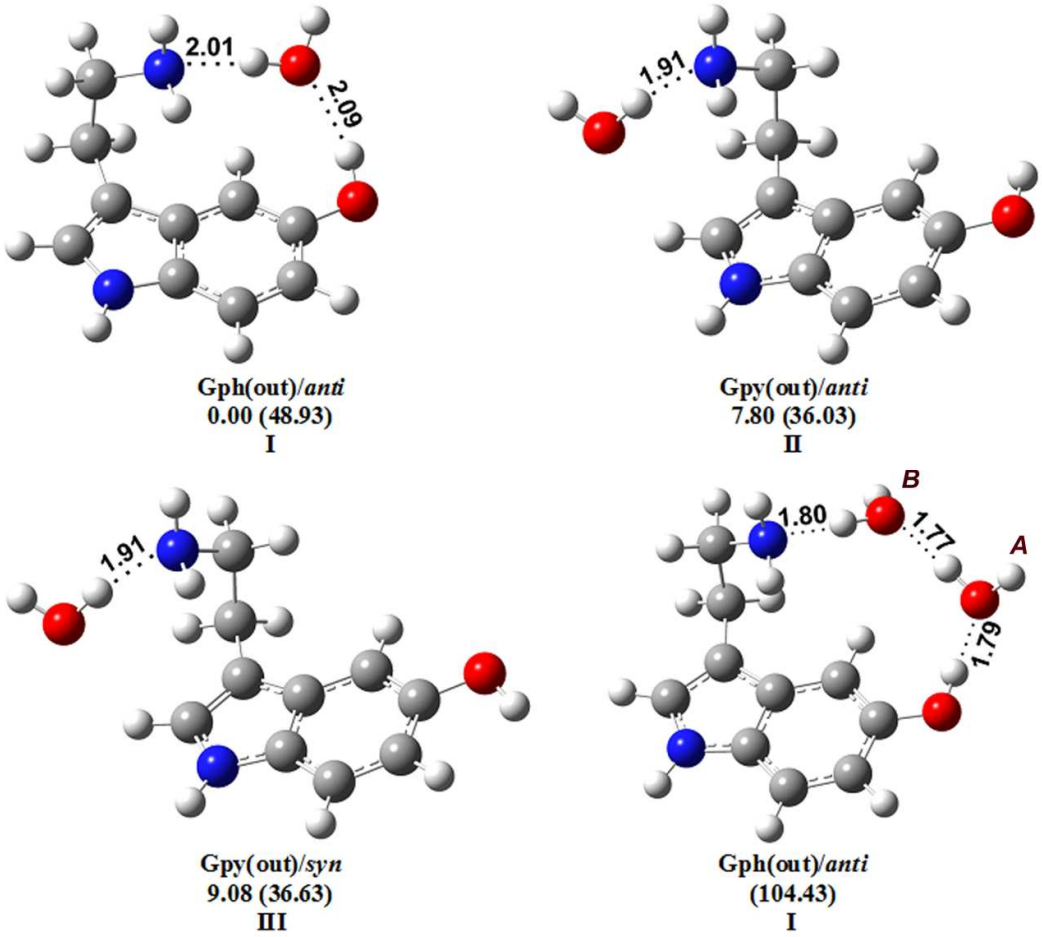

Fig. 7 Computed optimized structures, relative energies and binding energies (in parenthesis) (in kJ/mol) for the observed SERO-$(H_2O)_1$ [**I-III**] and SERO-$(H_2O)_2$ [**I**] clusters at MP2/6-311++G(d,p) level.

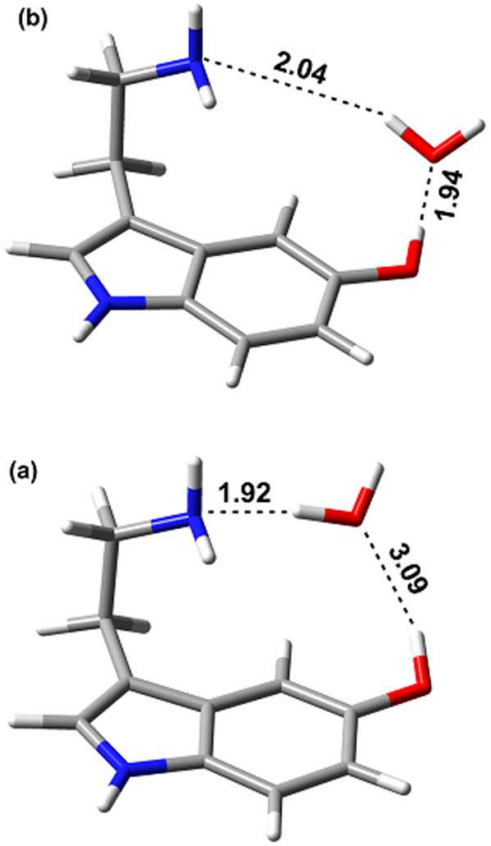

Fig. 8 Optimized (a) ground and (b) lowest excited state structures of SERO-$(H_2O)_1$ complex involving Gph(out)/*anti* conformation at B3LYP/def-TZVP level.