สัญญาเลขที่ MRG5180084

รายงานวิจัยฉบับสมบูรณ์

การวิเคราะห์ทางคอนฟอร์เมชันของสารเชิงซ้อนเซอโรโตนิน – น้ำและการเปรียบเทียบกับคอนฟอร์เมชันในสภาวะคอนตินัม: การคำนวณทางกลศาสตร์ควอนตัมเพื่อความเข้าใจในคุณสมบัติทางอิเล็กตรอนและสเปรกโทรสโคปีของสารสื่อประสาท

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(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ. และ สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

Contents

Executive summary	I
Abstract	II
บทคัดย่อ	III
Introduction	1
Objective	5
Material and Methods	6
Results and Discussion	8
Summary	23
References	24
Output of Research	26

EXECUTIVE SUMMARY

Serotonin (5-hydroxytryptamine; 5-HT) a monoamine neurotransmitter plays a critical role in a wide variety of physiological and behavioral processes. At physiological pH, 5-HT is “protonated”. The flexibility of its ethylamine side chain and 5-OH group renders conformational isomers of 5-HT. Because 5-HT carries out its biological functions in aqueous solution, it is important to understand how the conformational preferences of 5-HT change in the presence of water molecules. Until now, there are few publications available, only for mono- and di-hydrated complexes of “neutral” 5-HT.To sustain the importance of water environment on 5-HT molecule, the aim of this study is to investigate effect of the first hydration shell on stability and conformation of 5-HT-water cluster using theoretical chemistry calculations.Based on the 5-HT conformational analysis in gas-phase, the three most stable conformers *i.e.* Gp, Gm, and At in which their ethylamine side chain oriented in +*gauche*, -*gauche*, and *anti*, respectively, while their 5-hydroxyl groups are in the *anti*-position were used as starting structures. The 5-HT-water cluster were constructed by saturating all possible H-bond centres with water molecules in the vicinity of 5-HT polar groups, leading to a first stable hydration shell of 5-HT-$(H_2O)_7$. The structure of three conformers of 5-HT-$(H_2O)_7$ (Gp7W, Gm7W and At7W)were optimized using the B3LYP/6-311+G(2d,2p) and the PBE1W/6-311+G(2df,2p) levels of theory. Vibrational mode analysis shows no imaginary frequency at each level of optimization confirming those hydrate complexes are the real minima.The interaction energy between 5-HT and each water molecule was calculated in order to extract the role of water on geometrical stability of 5-HT. The identified key-stone water may play an important role for stabilization of the hydration shell in cage-like structural cluster. The 5-HT-$(H_2O)_7$ was embedded in explicit water system *i.e.* the Polarized Continuum Model (PCM), in order to evaluate the bulk solvent effect covering the first solvation shell on the stability and conformation of hydrated 5-HT.

The strength of H-bonds in terms of molecular structures, stabilization energies and cooperative effects deduced from 5-HT-$(H_2O)_7$could be evaluated using several theoretical calculations like the Atom in Molecules (AIM) and the Natural Bond Orbital (NBO) analyses. The results showed that the stability of the hydrogen bonds depends tightly on the arrangement of water molecules. The OH and NH stretch infrared absorptions indicate the influence of water molecules on bridge structure linking the NH_2 and OH groups of 5-HT. The hydrogen bond energy could be estimated between H-donor and H-acceptor using the NBO approach. The AIM method confirms the existence of real H-bonds bridging water and 5-HT molecule. Our results suggest that first hydration shell and ligand sculpt each other in order to form strongly stable supermolecules. Incorporating water in the *in silico* drug design becomes thus mandatory and will lead to the development of the next generation of computational tools in drug discovery.

ABSTRACT

Conformational analysis of serotonin-water complex and comparison with conformation in continuum condition: a quantum mechanical calculation for understanding electronic and spectroscopic properties of neurotransmitter

Protonated serotonin (5-HT)-water clusters have been studied using theoretical chemistry calculations. The a first stable hydration shell of seven water molecules around three 5-HT conformers were constructed and optimized using the B3LYP/6-311+G(2d,2p) and PBE1W/6-311+G(2df,2p) levels of theory leading to stable Gp7W, Gm7W and At7W conformers which the ethylamine side chain of 5-HT are in +*gauche*, -*gauche*, and anti-conformations, respectively. The energetic stabilities of 5-HT$(H_2O)_7$ conformers decrease in the order of At7W > Gp7W > Gm7W for both levels of calculations. The structural parameters of 5-HT, for instance the lengthening of amino-NH bonds were observed when they are located in the ring-like structure of water clusters indicating the effect of hydrogen bond forming. The calculated total binding energies and stepwise binding energies of water molecules exhibit the contribution of water molecule for cooperative effects on stability of 5-HT$(H_2O)_7$ conformers. The key-stone molecule of water within tetramer-like (for Gp7W and Gm7W) and pentamer-like (for At7W) forming cluster can be identified. The shifting of vibrational spectra for OH, NH, and CH stretch fundamentals reflect the presence of hydrogen bond formation linking between water and 5-HT (indole-NH, and 5-OH). The Bader's Atoms in Molecule (AIM) analyses show the value of electron density (ρ) and Laplacian density $\nabla^2\rho$ at Bond Critical Point (BCP) of three conformers are in the range of ideal hydrogen bond by Popelier's definition. Moreover, the Ring Critical Point (RCP) and Cage Critical Point (CCP) can be identified confirming the topological evidence of intramolecular hydrogen bond interactions. The second-order energy of electron transfer between donor-acceptor H-bond interactions *i.e.* Lone-pair O$\rightarrow\sigma$*H-O or σ *H-N within 5-HT$(H_2O)_7$ conformers were computed by the Natural Bond Orbital (NBO) analyses showing high magnitude of stabilizing energy for hydrogen bond. The bulky solvent effects on the conformers of 5-HT$(H_2O)_7$ were also investigated. The results show that the relative energetic stabilities obtained for the 5-HT$(H_2O)_7$ under Polarizable Continuum Model (PCM) are consistent with those of corresponding isolated cluster conformers in gas phase calculations.

Keywords: serotonin, water, hydrogen bond, AIM, NBO, PCM

บทคัดย่อ

โครงการการวิเคราะห์ทางคอนฟอร์เมชันของสารเชิงซ้อนเซอโรโตนิน-น้ำและการเปรียบเทียบกับคอนฟอร์เมชันในสภาวะคอนตินัม: การคำนวณทางกลศาสตร์ควอนตัมเพื่อความเข้าใจในคุณสมบัติทางอิเล็คตรอนและสเปรกโตรสโคปีของสารสื่อประสาท

การศึกษาโมเลกุลคลัสเตอร์ของโปรโตเนทเซอโรโตนิน – น้ำ โดยวิธีคำนวณทางเคมีทฤษฎีในการเกิดชั้นแรกของไฮเดรชันรอบเซอโรโตนิน 3 โครงรูป โดยการเติมน้ำ 7 โมเลกุลและการหาค่าที่เหมาะสมที่สุดทางโครงสร้างด้วยการคำนวณทางคอมพิวเตอร์ที่ระดับทฤษฎี B3LYP/6-311+G(2d,2p) และ PBE1W/6-311+G(2df,2p) พบว่ามีโครงรูปที่เสถียร 3 โครงรูป ได้แก่ Gp7W Gm7W และ At7W ซึ่งโซ่ข้างเอททิลเอมีนอยู่ในตำแหน่ง *+gauche -gauche* และ *anti* ตามลำดับ ความเสถียรทางพลังงานของโครงรูป 5-HT$(H_2O)_7$ ลดลงตามลำดับ กล่าวคือ At7W > Gp7W > Gm7W ในทั้งสองระดับทฤษฎี พารามิเตอร์ทางโครงสร้างของเซอโรโตนิน เช่น การยืดออกของพันธะอะมิโน NH พบได้เมื่ออยู่ในตำแหน่งที่มีลักษณะแบบ ring-like ของคลัสเตอร์น้ำแสดงถึงผลของการเกิดพันธะไฮโดรเจน ค่าพลังงานยึดเกาะทั้งหมดและค่าพลังงานยึดเกาะของแต่ละโมเลกุลน้ำ แสดงถึงผลร่วมกันต่อเสถียรภาพทางโครงสร้างของโครงรูป 5-HT-$(H_2O)_7$ ในที่นี้สามารถระบุโมเลกุลน้ำที่เป็นกุญแจหลักซึ่งอยู่ในโครงสร้างที่มีลักษณะแบบ tetramer-like (สำหรับ Gp7W และ Gm7W) และ pentamer-like (สำหรับ At7W) การเกิด shift ของ สเปกทราการสั่นของพันธะ OH NH และ CH สะท้อนถึงพันธะไฮโดรเจนซึ่งเชื่อมระหว่างโมเลกุลน้ำและเซอโรโตนิน (indole-NH และ 5-OH) การวิเคราะห์โดยวิธี Atoms in Molecule (AIM) ของ Bader แสดงถึงค่าความหนาแน่นอิเล็กตรอน (ρ) และค่าความหนาแน่น Laplacian ($\nabla^2\rho$) ที่ตำแหน่ง Bond Critical Point (BCP) ของทั้ง 3 โครงรูปเป็นไปตามข้อกำหนดของ Popelier สำหรับการเกิดพันธะไฮโดรเจนในอุดมคติ นอกจากนี้ยังสามารถระบุตำแหน่งของ Ring Critical Point (RCP) และ Cage Critical Point (CTP) ซึ่งยืนยันถึงข้อพิสูจน์ทอพอโลยีในการเกิดพันธะไฮโดรเจนระหว่างโมเลกุล ค่าพลังงานอันดับสองของการส่งผ่านอิเล็กตรอนระหว่างตัวให้และตัวรับในพันธะไฮโดรเจน กล่าวคือ Lone-pair O$\rightarrow\sigma$*H-O หรือ σ *H-N ภายในโครงรูป 5-HT-$(H_2O)_7$ คำนวณได้โดยวิธีวิเคราะห์ Natural Bond Orbital (NBO) ซึ่งแสดงถึงความสำคัญของค่าพลังงานดังกล่าวต่อเสถียรภาพของพันธะไฮโดรเจน การศึกษาผลของตัวทำละลายปริมาณมากต่อโครงรูปของ 5-HT-$(H_2O)_7$ พบว่าความเสถียรทางพลังงานสัมพัทธ์ของ 5-HT$(H_2O)_7$ ภายใต้สภาวะ Polarizable Continuum Model (PCM) สอดคล้องกับค่าที่ได้จากการคำนวณในสภาวะก๊าซของโมเลกุลคลัสเตอร์ โปรโตเนทเซอโรโตนิน–น้ำแต่ละโครงรูป

คำสำคัญ: เซโรโตนิน น้ำ พันธะไฮโดรเจน AIM NBO PCM

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter of the central nervous system (CNS) and peripheral play a critical role in a wide variety of physiological and behavioral processes. The important physiological roles of 5-HT and its implications in many of pathological states have stimulated an intensive research on the design of drugs targeting the serotonergic system. At physiological pH, 5-HT is protonated with two pK_a. Rudnick *et al*, have demonstrated that the ionization forms of 5-HT are important for its transport mechanism [Rudnick *et al*., 1989]. The neutral form is transported into secretory vesicles through vesicular monoamine transporters (VMATs) [Keyes *et al.*, 1982], whilst the cationic one is transported by cytoplasmic membrane transporter SERT [Kanner *et al.*, 1982]. For cationic 5-HT, it exists in several conformations due to the flexibility of its ethylamine side chain. 5-HT can adopt at least two local minimum energy conformations in aqueous solvent, evidenced by NMR [Ison*et al*., 1972] that was previously confirmed by theoretical calculations in gas phase [Pratuangdejkul et al., 2006]. Our team has published the precisely conformational analyses of cationic 5-HT in gas phase in term of electronic distribution and energy decomposition analysis in order to indicate the importance of cation-π interaction on the stability of cationic 5-HT. A specific geometry of 5-HT is recognized at 5-HT receptor or transporter sites and knowledge of this interaction is important in drug design. The conformer of 5-HT influences its transport properties and plays an important role in recognition processes in serotonin transporter (SERT) interactions has been indicated by our previous 3D-QSAR analyses [Pratuangdejkul *et al*., 2005]. We are success to publish for the first time, the “definition of an uptake pharmacophore of SERT through this 3D-QSAR model. Our finding suggests that the conformational energy of compounds transported by SERT does not represent a key factor for SERT uptake. Nevertheless, we cannot exclude that such conformers in biological fluids are recognized by SERT and that 5-HT conformation changes along the translocation process inside SERT. The identification of the 5-HT anti conformation as the biological active form toward SERT transport has strong implications for the design of new generation of SERT uptake inhibitors [Pratuangdejkul *et al*., 2008].

For the pharmacological activity of serotonin, however, it is very important to have information about the conformation in solution. As commonly known, intramolecular and intermolecular interaction, as well as interactions with its chemical environment (for instance with water) play important role in the structural stabilization of molecule in biological systems. Accordingly, the question about stable conformations of 5-HT in aqueous solution is still the

subject of interest. To this reason, the water molecules have to incorporate into the system in order to evaluate the effects of specific hydrogen-bonding interactions around the various sites in the distinct stable conformers. As shown in our previous calculations, the application of the cluster-continuum model to solve the problem of 5-HT p*K*a's calculation shows that it constitutes a reliable theoretical methodology for modeling chemical reaction of 5-HT in aqueous phase [Pratuangdejkul *et al.*, 2006]. The results pinpoint the importance of explicit waters molecules in the determination of solution phase reaction free energy and p*K*a prediction.

Indeed, proper treatment of the solvent was found necessary, for example, in the prediction of the Raman optical activity spectra of zwitterionic peptides that do not exist in vacuum [Bour *et al.*, 2001; Jalkanen *et al.*, 2001], or in the assessment of accurate vibrational frequencies of the amide group [Kubelka and Keiderling, 2001]. Hydrogen bonds significantly influence protein IR spectral intensities. Interactions with solute/solvent also influence the energy and the conformational of solute molecules [Elstner *et al.*, 2000]. Indole derivatives have received much attention from researchers for its spectral properties. For instance, tryptophan, one of the essential amino acids, its UV spectroscopic activity due to conformation change, is used as an indicator of protein structure and environmental. Also the neurotransmitter serotonin (5-HT), and consequently many hallucinogens that target serotonin sensitive neurons, like psilocybin and lysergic acid diethylamide, have indole as a component. The interaction of indole with water is crucial for understanding the stability of indole-containing compounds in a natural environment, and therefore, a large variety of experiments and a number of theoretical studies have been reported on the indole-water complex [van Mourik *et al.*, 2000, Jalbout and Hall, 2003]. Both experimental and theory show that the global minimum of indole-water complex contains (i) water molecule interacting with N-H in indole and (ii) water molecule interacting withπ-electron cloud of the membered ring [Carney and Zwier, 1999; Mons *et al.*,1999].

However, it might be different for 5-HT in water, since the flexibility of its ethylamine side chain can affects the structure of the first hydratation shell, that in turn should influence the energy and conformation of 5-HT itself. Indeed, the formation of hydrogen-bonded system between 5-HT and water molecules involves the amine and 5-hydroxyl functional groups that can be hydrogen bonded with water molecules. The π-electron cloud of indole ring also contributes to the structure of the network of hydrogen bond within the solvent. The FT-IR spectroscopy of 5-HT in a solid form and in water, D_2O, and ethanol have been obtained in the range of 4000-400 cm-1 [Bayari *et al.*, 2005]. The authors have assigned the observed bands corresponding to 5-HT on the basis of theoretical calculations and the comparison with related

molecule like tryptamine, indole, and 5-methoxytryptamine. Nevertheless, they used a weak basis set (B3LYP/6-31G(d)) in gas-phase without any consideration of solvent effects, neither continuum nor explicit water on calculations. The assignment of IR spectra of 5-HT in solution were then compared to the solid one. In general, two approaches have been carried out tograsp water solvent influence on 5-HT conformation, *i.e.* the implicit (continuum) and the explicit (water cluster) aqueous solvent models. A solvatation of protonated 5-HT was carried out with 5-HT conformers using four implicit solvents including chloroform, dimethylsulfoxide, ethanol and water in a polarized continuum model (PCM) [Pisterzi *et al.*, 2002]. The authors established that the relative conformational stability of 5-HT conformers increased with solvent polarity. Conformation analysis of protonated 5-HT has also been performed using an implicit aqueous solvent at the IEF-PCM/HF/6-31G*, PCM/MP2/6-31G*, IEF-PCM/B3LYP/6-31G* and IEF-PCM/MP2/6-31G*//IEF-PCM/MP2/6-31G*levels of theory [Alagona *et al*., 2005; Alagona *et al*., 2006]. Two *gauche* and one *trans* conformations of5-HT were obtained corresponding to local energy minimum structures, with side chain dihedral angles close to those obtained in gas phase. However, the most difference concerns the energetic stability among 5-HT conformations *i.e.-gauche* and *trans* conformations were the most stable in aqueous continuum, whereas + *gauche* was the most stable in gas phase. To define the role of solvent of 5-HT conformation, theoretical calculations of hydrogen-bonded complexes of 5-HT with explicit water molecule have been performed. However, the limit of those calculations relies on the use of a single mono-hydration with either neutral [Delchev*et al.*, 2006] or protonated 5-HT [Alagona *et al.*, 2006].Recently, LeGreve et al succeeded to assign the infrared and ultraviolet spectra of a unique conformation of neutral 5-HT interacting with two waters molecules, with the help of quantum chemistry calculations at the B3LYP and M0-52X levels of theory and by using the 6-31+G(d) basis set [LeGreve *et al.*, 2009]. Those results confirm that an appreciation of water molecules contributes to the 5-HTstability and geometry putting forward the non-negligible effects of solvent on the super molecule structure. Since water molecules can solvate 5-HT polar groups, calculations performed on hydrated 5-HT should take into account cluster of water molecules around ammonium and hydroxyl chemical groups as well as indole nitrogen.

To sustain the importance of water environment on 5-HT molecule, our ultimate goal of this research is to study effect of the first hydration layer of water molecules on stability and conformation of 5-HT at atomic level. The electronic distribution and interaction energies between 5-HT and water was calculated in order to extract the role of water on geometrical stability of 5-HT. Furthermore, the hydrated 5-HT was embedded in the continuum solvent, the dielectric model, in order to evaluate the bulk solvent effect covering the first hydration shell on

the stability and conformation of hydrated 5-HT. Our results can be used to explain characteristics of serotonin in condensed phase, and to characterize how and how much structure and stability change with respect to 5-HT molecule in gas phase. The forces supporting the hydrogen-bonded complexes formed between 5-HT and water are valid to explain the ability of 5-HT to form heavily hydrated clusters with water in living organisms. Finally, our studies will allow to obtain an insight into the physicochemical properties and chemical reactions of 5-HT under physiological condition. To our knowledge of this study, we can conclude that hydration, ionization, conformation, physical and biological properties are thus factors intimately linked together.

OBJECTIVES

This study focuses on effect of aqueous environment on conformational analysis of serotonin (5-HT). The theoretical chemistry was used for calculation of 5-HT in explicit and implicit water using electron correlation methods with highly extended basis set. Various methods were carried out in order to gain the accurate conformation analyses and the contribution of water molecules on the geometry and stability of 5-HT. The network of hydrogen bond system for stabilizing the 5-HT-water cluster was also analyzed. Thus, the aims of this research are as follows.

1. To investigate the stable conformations of 5-HT in the aqueous condition using theoretical chemistry calculations.
2. To study the theoretical spectroscopic properties 5-HT-water cluster.
3. To interpret the network of hydrogen bonds and co-operative effect of water molecules for stabilizing of 5-HT-water cluster using the Atoms in Molecule (AIM) and Natural Bond Orbital (NBO) analyses.
4. To investigate the influence of the continuum solvent on conformational stability of 5-HT-water cluster

MATERIAL AND METHODS

Computational Methods

The conformational analysis of 5-HT-water cluster was performed on the three most stable 5-HT conformers (*i.e. +gauche, -gauche* and *anti*-conformation of ethylamine side chain in which their 5-hydroxyl group in *anti*-position) by saturating all possible H-bond centers with water molecules in the vicinity of 5-HT polar groups, leading to a first stable hydration shell of seven water molecules (5-HT$(H_2O)_7$). The geometries of 5-HT$(H_2O)_7$ conformer were initially optimized using the Density Function Theory (DFT) with the B3LYP/6-311+G(2d,2p) level of theory. The optimized 5-HT$(H_2O)_7$ were further refined by performing a full optimization with the Generalized Gradient Approximation (GGA) method at thePBE1W/6-311+G(2df,2p)level of theory. A vibrational frequency analysis was performed on all optimized geometries at each level of calculations in order to reassert their reality of local minimum.

Infrared (IR) stretch spectra, *i.e.* wave numbers (ν) in $cm^{-1}$and intensities (*I*) in km/mol, of 5-HT$(H_2O)_7$conformers were obtained using vibrational frequencies calculated at the B3LYP/6-311+G(2d,2p) and PBE1W/6-311+G(2df,2p) levels of theory with a scaling factor of 0.98and 1.04, respectively, to achieve optimal agreement with experimental spectra obtained for the stretch of $NH\cdots OH_2$ (indole-water) of 3436 cm^{-1} [Dian *et al.*, 2003].

The intermolecular H-bonding between water and serotonin leads to the formation of the supersystem. The cooperativity in hydrogen-bonded interactions that stabilized the hydrated 5-HT conformers was precisely determined using several criterions including geometrical parameters, hydrogen bond energy and the decomposition of the interaction energy, topological parameters and natural bond orbital analyses.

To study the nature of hydrogen bond interactions in 5-HT-$(H_2O)_7$conformers, the topological analysis was performed in order to calculate the charge density (ρ) and its second Laplacian derivative of charge density ($\nabla^2\rho$) for the bond critical points (BCP) using the Bader's Atoms in Molecules (AIM) theory [Bader *et al.*, 1995]. A set of well-established cases of hydrogen bonding was studied computationally employing the Natural Bond Orbital (NBO) analysis.

To analyze the cooperative effect of hydration shell on the stability of 5-HT$(H_2O)_7$, the total binding energies (BE as Eq.1) and stepwise binding energy ($BE_{W(n)}$ as Eq. 2) of each water molecule (W1 to W7) were calculated together with the basis set superposition errors

(BSSE) by means of the counterpoise method. The ΔE_{hyd} of shell of seven-water and $\Delta E_{(Wn)}$of individual water on 5-HT molecule were investigated in order to extract the critical water molecule that contribute the highest reaction energy for solvation and hydrogen bonding stabilization of hydrated 5-HT.

$$BE = E[5\text{-}HT(H_2O)_7] - E[5\text{-}HT] - E[(H_2O)_7] \quad \text{(Eq.1)}$$

$$BE_{(Wn)} = E[5\text{-}HT(H_2O)_7] - E[5\text{-}HT(H_2O)_6] - E[(H_2O)]_{Wn} \quad \text{(Eq.2)}$$

when E [5-HT$(H_2O)_7$] is energy of 5-HT/seven water cluster, E [5-HT$(H_2O)_6$] is energy of 5-HT/six water cluster (that remove water number n), E $[(H_2O)]_{Wn}$ is energy of water number n, and n is water number 1 to 7.

To investigate the influence of the continuum solvent on conformational stability of 5-HT-water cluster, the calculations performing under the bulk of cluster-continuum *i.e.* polarizable continuum model (PCM) model were employed using single point calculations at the B3LYP/6-311+G(2d,2p) and the PBE1W/6-311+G(2df,2p) levels of theory.

Software and Hardware

Almost theoretical calculations were performed using the Gaussian 03W program. For Bader's atom in molecule (AIM) analysis of electron density, theAIM2000 software was used. The natural atomic orbital and natural bond orbital (NBO) analysis was calculated using NBO Version 3.1 incorporated in the Gaussian 03W package. Molecular structures and their analyses were visualized using the ChemCraft 1.6 and Gabedit 2.36 softwares.

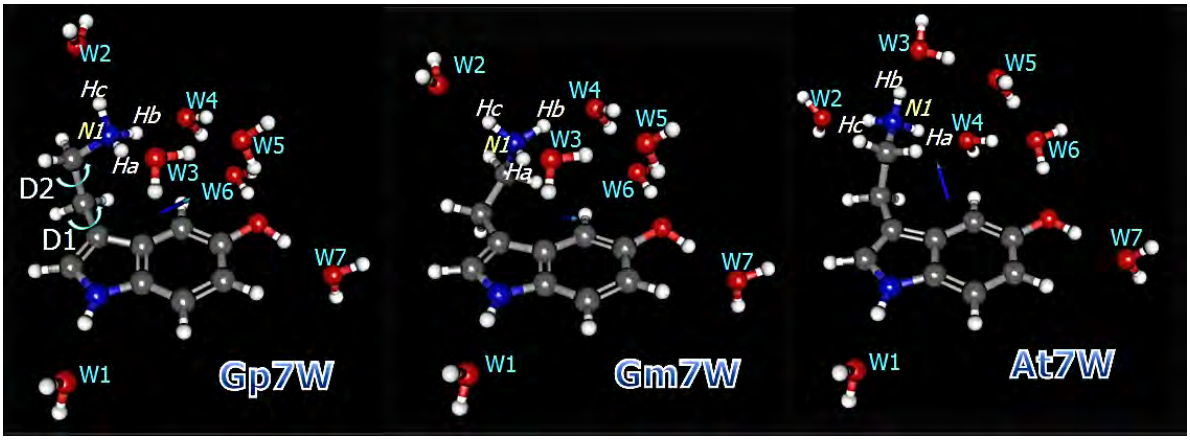


Fig.3. The structure of three stable conformers of 5-HT-$(H_2O)_7$ optimized at the PBE1W/6-311+G(2df,2p).

Three 5-HT$(H_2O)_7$ conformers show the 5-hydroxyl group in *anti*-orientations. Upon the position of the ethylamine side chain, 5-HT$(H_2O)_7$ conformers can be (i) +*gauche* (Gp7W) when it folds on the phenol ring side, (ii) -*gauche* (Gm7W) when it folds on the pyrole ring side and (iii) *anti* (At7W) when it extends from the indole ring. Energetically, the stability of 5-HT-$(H_2O)_7$ conformer decrease in the order of At7W > Gp7W > Gm7W when optimized at the B3LYP/6-311+G(2d,2p) level of calculation. Reoptimization of all the conformers at the PBE1W/6-311+G(2df,2p) level did not modify significantly their internal coordinates value or their relative energies. However, the GGA method has given the 5-HT$(H_2O)_7$ clusters energetically more stable than the hybrid DFT method. The elongation of N–H bond distance can be observed resulting from the contribution of network of hydrogen bond formation between NH···OH_2. The result shows that bonds distance of N1–Ha and N2–Hb of three 5-HT$(H_2O)_7$ conformers are longer than that of N1–Hc at all levels of calculations. This may imply some interactions between N–H and water molecule. Since the N1–Ha and N1–Hb bonds locate in the ring-like network of 5-HT-water cluster, thus degree of hydrogen bond interactionofN1–Ha···OH_2 and N1–Hb···OH_2 is higher than that of N1–Hc···OH_2. The amine N–H bond lengths of the three conformers are slightly shortened when calculated at the B3LYP/6-311+G(2d,2p) level compared to those obtained at the PBE1W/6-311+G(2df,2p) level. Due to many reasons including (i) the PBE1W functional has been optimized specifically for water and ice, (ii) PBE1W/MG3S is the most accurate for predicting the binding energies of the water clusters and (iii) the MG3S basis set is identical to 6-311+G(2df,2p) for water [Dahlke *et al*., 2005; Dahlke *et al*., 2006], the structures of 5-HT$(H_2O)_7$ conformers obtained from the optimization at the PBE1W/6-311+G(2df,2p) were used for further calculations and analyses.

Table 1 Geometric Parameters (see Fig.1 for definition) and relative Energies (ΔE_{rel}) of the three 5-HT-$(H_2O)_7$ conformers obtained from optimization at the B3LYP/6-311+G(2d,2p)andPBE1W/6-311+G(2df,2p) levels of theory.

Geometrical parameter	B3LYP/6-311+G(2d,2p)			PBE1W/6-311+G(2df,2p)		
	Gp7W	Gm7W	At7W	Gp7W	Gm7W	At7W
D1	-92.533	-35.764	-69.274	-92.055	-35.170	-68.91
D2	61.859	-53.124	156.772	61.707	-52.880	155.61
D4	-162.095	-155.233	-175.630	-163.380	-157.229	-176.910
N1–Ha	1.038	1.035	1.033	1.049	1.046	1.042
N1–Hb	1.042	1.046	1.042	1.053	1.059	1.054
N1–Hc	1.031	1.029	1.028	1.039	1.037	1.036
N2–Hd	1.014	1.014	1.012	1.023	1.023	1.021
O–H	0.978	0.978	0.975	0.989	0.989	0.985
ΔE_{rel} (kcal/mol)	0.870	3.564	0.000	0.628	3.138	0.000

The energetically stable 5-HT$(H_2O)_7$ conformers have 5-HT in *gauche*- and *anti*-conformation of protonated ethylamine side chain with four water molecules forming a H-bonding bridge linking the protonate amine group (NH_3^+) and the 5-hydroxy (5-OH) group on the indole ring. The network of H-bonds contains two traditional H-bonds, involving NH···O and OH···O linkages. The water molecule acts as H-bond acceptor to the protonated amine group and H-bond donor to the 5-OH group. Two types of bridges are formed *i.e.* ring-like in At7W and book-like in Gp7W and Gm7W (Fig. 4).Structural assignment for the observed 5-HT$(H_2O)_7$ conformers, with theoretical H-bond distances in angstroms taken from the PBE1W/6-311+G(2df,2p) calculations are shown in Fig. 5. The intermolecular H-bonding between water and serotonin leads to the formation of the serotonin-water supersystem. Here, some longer intermolecular H-bonds are formed between water and serotonin in Gp7W and Gm7W indicating weak hydrogen bonds (longer than 1.9 Å) [Delchev *et al*., 2006]. A shorter, stronger intermolecular H-bond is found in the At7W supersystem than in the Gp7W and Gm7W.The energy of the H-bond depends on the length of H-bonds, which is the reason why At7W is more stable than Gp7W and Gm7W.

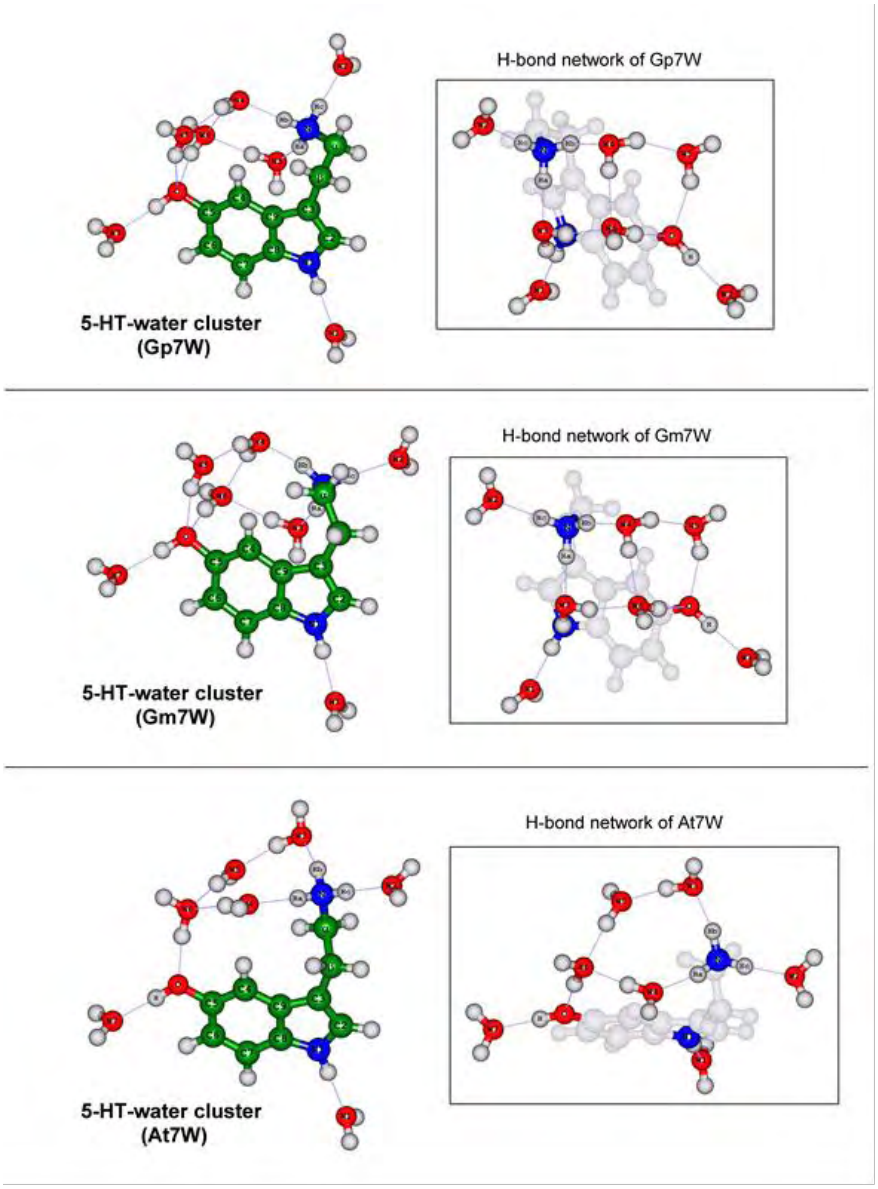


Fig.4. Structure of 5-HT$(H_2O)_7$ conformers (Gp7W, Gm7W, and At7W) and their network of hydrogen bond.

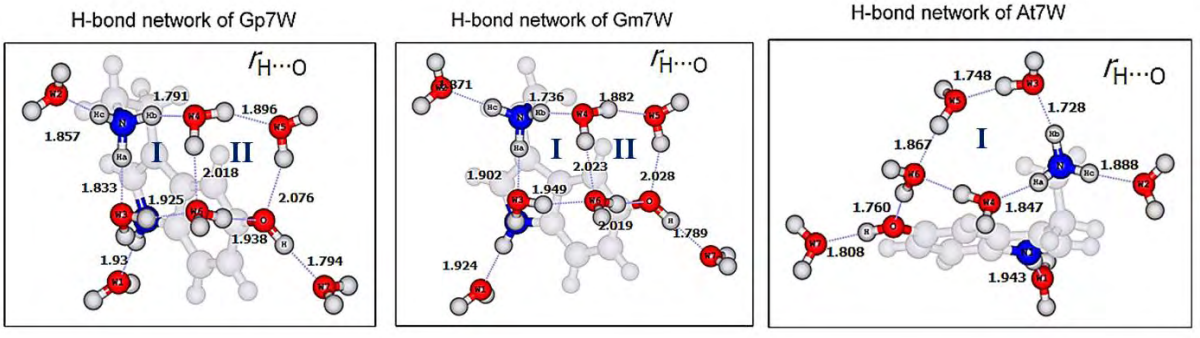


Fig.5. Schematic representation of H-bond clusters between 5-HT and water molecules and bond distance (Å) of H-bond ($r_{H...O}$) calculated at the PBE1W/6-311+G(2df,2p).

2. Theoretical Binding Energy for Stabilizing 5-HT$(H_2O)_7$ conformers

To analyze the cooperative effect of hydration shell on the stability of 5-HT$(H_2O)_7$ conformers, binding energies were computed. The presence of water molecules can result in existing conformation of the 5-HT conformer due to the flexibility of the ethylamine side chain. Difference forms of the binding energy can be calculated. In the conventional calculations, the total binding energies for cluster structures were calculated as BE = $E_{cluster} - E_{monomer} - E_{water}$, that is

$$BE = E_{[5\text{-}HT(H2O)7]} - E_{[5\text{-}HT]} - E_{[(H2O)7]} \quad \text{(Eq.1)},$$

and the stepwise binding energy ($BE_{W(n)}$) of each water molecule (W1 to W7) were further calculated as

$$BE_{(Wn)} = E_{[5\text{-}HT(H2O)7]} - E_{[5\text{-}HT(H2O)6]} - E_{[(H2O)]Wn} \quad \text{(Eq.2)},$$

where $E_{[5\text{-}HT(H2O)7]}$ is energy of 5-HT/seven water cluster, $E_{[5\text{-}HT(H2O)6]}$ is energy of 5-HT/six water cluster (that remove water number n), $E_{[(H2O)]Wn}$ is energy of water number n, and n is water number 1 to 7.

At each of binding energies calculations, the basis set superposition errors (BSSE) were determinedby the counterpoise method. All types of binding energies were computed using single-point energy at the PBE1W/6-311+G(2df,2p) level of theory.

The results show that the heptahydration forms a stable pentamer-like complex with the ethylamine side chains locked in an *anti*-conformations for At7W and book-like with the ethylamine side chains locked in an *gauche*-conformations for Gp7W and Gm7W complex with the ethylamine side chains locked in a gauche conformations (Fig. 4 and 5). The total binding energy (BE) of the system is quite large (∼80 kcal/mol) showing a strong stabilization of 5-HT by water (Table 2). It was found that the actual BE value (BSSE subtracted) of At7W is higher than that of Gp7W and Gm7W around 2 kcal/mol. The BE are consistent with the overall structural energy of 5-HT-$(H_2O)_7$ conformer in which the At7W is the lowest energy minimum at the PBE1W/6-311+G(2df,2p) level of optimization. However, the energy of 5-HT itself after removing water molecules has shown the value of 3.46 and 2.32 kcal/mol for At7W and Gm7W, respectively, relative to the global minima of 5-HT in Gp7W. Thus, the geometries of water for hydrated 5-HT are important for the stabilization of the clusters.

Table 2 Total binding energy (BE, in kcal/mol) of 5-HT$(H_2O)_7$ supermolecules, the BSSE by means of the counterpoise method, and relative energy of 5-HT monomer and seven water-cluster calculated single-point at the PBE1W/6-311+G(2df,2p) level of theory.

Clusters	BE	BSSE	Relative energy	
			5-HT monomer	7 water-cluster
Gp7W	-80.73	-3.58	0.00	2.82
Gm7W	-80.51	-3.66	2.32	2.81
At7W	-81.97	-3.05	3.46	0.00

The results demonstrate the cooperative effects taken into account of hydrogen-bonds and water cluster in such bounded system of 5-HT$(H_2O)_7$ conformers. To estimate the contribution of water molecule for cooperative effects, the stepwise binding energy (BE_{Wn}) of each water molecule were computed and results are shown in Table 3.

Table 3 Stepwise binding energy (BE_{Wn}in kcal/mol) of 5-HT$(H_2O)_7$ supermolecules together with the BSSE by means of the counterpoise method calculatedat the PBE1W/6-311+G(2df,2p) level of theory.

	Gp7W		Gm7W		At7W	
	BE_{Wn}	BSSE	BE_{Wn}	BSSE	BE_{Wn}	BSSE
W1	-8.41	-0.46	-8.53	-0.46	-7.72	-0.44
W2	-10.73	-0.49	-10.59	-0.50	-10.52	-0.43
W3	-15.02	-1.09	-13.62	-1.11	-19.72	-0.85
W4	-20.13	-1.15	-20.57	-1.18	-16.61	-0.88
W5	-11.27	-0.76	-12.62	-0.82	-16.54	-0.91
W6	-17.29	-1.09	-16.29	-1.08	-24.12	-1.11
W7	-10.96	-0.58	-11.06	-0.58	-10.10	-0.56

The cyclic water cluster mimics that of previously described for NH_4^+/water systems and render the experimental IR spectra [Karthikeyan *et al.,* 2008]. Water molecules, the cationic head and the 5-OH group form together an H-bond network. Two cyclic clusters imbricate in

which water W3, W4, W5, and W6 individually participate to the total stabilization energy (Fig. 4 and 5). For Gp7W and Gm7W, a “book-line” forming tetramer-like ring I and II known for $NH_3^+(H_2O)_4$Oinvolving water W3, W4, W5 and W6, the cationic head and the 5-OH group. While At7W, a “pentamer-like” ring I known for $NH_3^+(H_2O)_4$, involving water W3, W4, W5 and W6, the cationic head were found. From the stepwise binding energy (BE_{Wn}) of each watermolecule, the key-stone water in the system can be identified. For Gp7W and Gm7w, two cyclic clusters imbricate in which water W3, W4, W5, and W6 individually participate for 15.02, 20.13, 11.27 and 17.29 kcal/mol, respectively for Gp7Wand 13.62, 20.57, 12.62, 16.29 kcal/mol, respectively for Gm7W. While the At7W, one pentamer-like forming by water W3, W4, W5, and W6 individually contribute for 19.72, 16.61, 16.54 and 24.12 kcal/mol, respectively. According to the BE_{Wn} values, the key-stone water of Gp7W and Gm7W is W4 whereas that of At7W is W6. This arrangement is an outline of Gp7W and Gm7W clusters observed in protonated water and consists of square faces forming irregular cuboids that are known to evolve intoclathrate-like cages as the number of molecules reaches the dozen.

3. Computed Vibrational Spectra of 5-HT$(H_2O)_7$

Based on three stable conformers of 5-HT-water clusters *i.e.* Gp7W, Gm7W, and At7W,the vibrational frequency analysis enables us to simulate infra-red (IR) spectra of 5-HT in the explicit water environment (Fig. 6). From experimental fluorescence-dip infrared (FDIR) spectra of indole-water, the indole NH acts as a H-bond donor to the water molecule ($NH\cdots OH_2$), producing a shift in the indole NH stretch from 3525 cm^{-1}(gas phase) to 3436 cm^{-1} which is signature of an $NH\cdots OH_2$H-bond [Dian *et al.,* 2003]. Accordingly, the scaling factor of 0.98 and 1.01 were adjusted for vibrational spectra computed at the B3LYP/6-311+G(2d,2p) and PBE1W/6-311+G(2df,2p), respectively, to bring the experimental and calculated indole NH stretch frequencies into agreement with one another (*i.e.* 3436 cm^{-1}). The calculated IR spectra of three conformers of 5-HT-$(H_2O)_7$ in the 2800-3900 cm^{-1} region are shown in Fig. 7 and 8 for the B3LYP/6-311+G(2d,2p) and PBE1W/6-311+G(2df,2p), respectively. This wavenumber range spans the OH, NH, and CH stretch fundamentals.

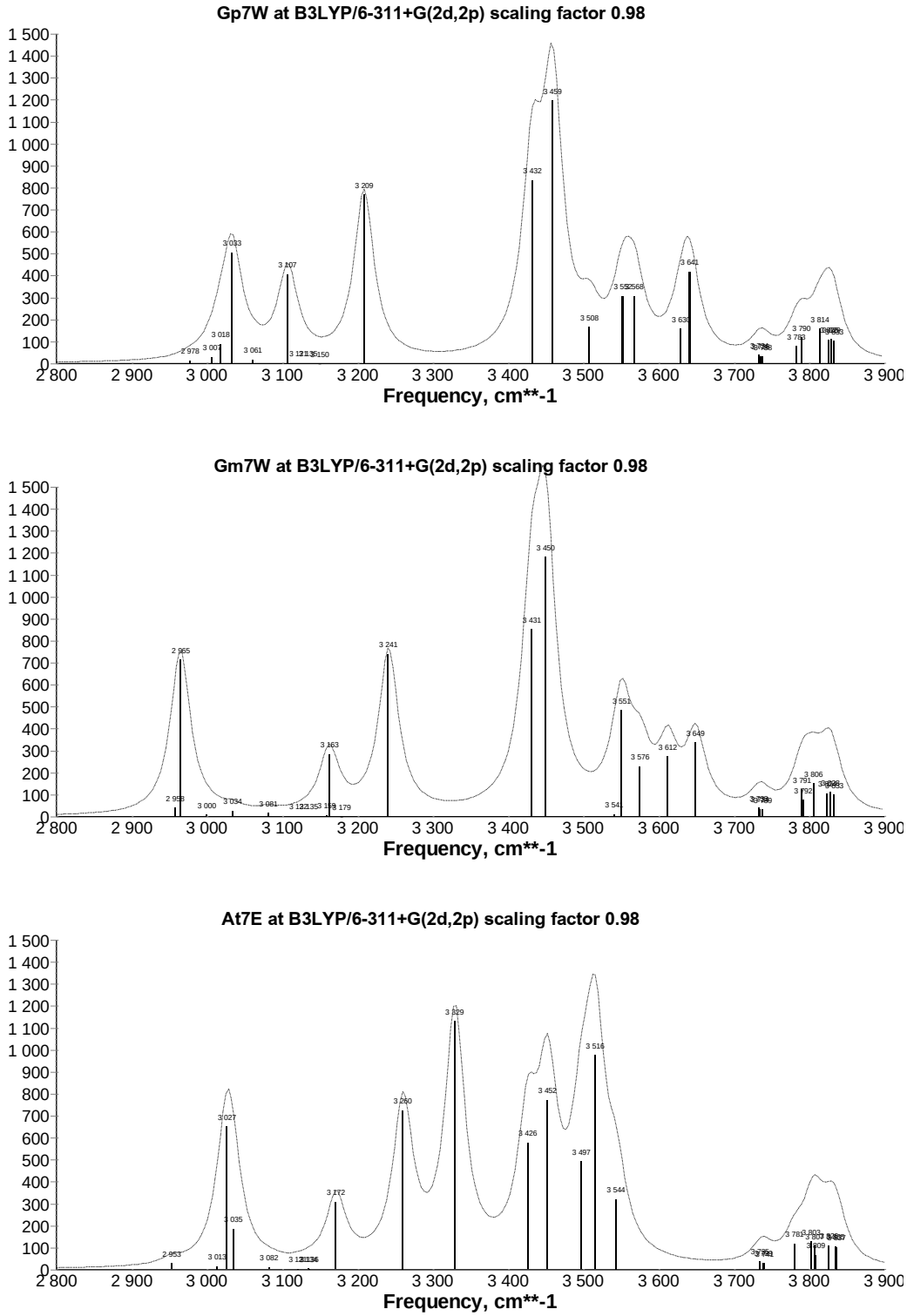


Fig.7. Calculated harmonic vibrational frequencies and IR intensities for 5-HT/$(H_2O)_7$ at the B3LYP/6-311+G(2d,2p) level (scaling factor of 0.98)

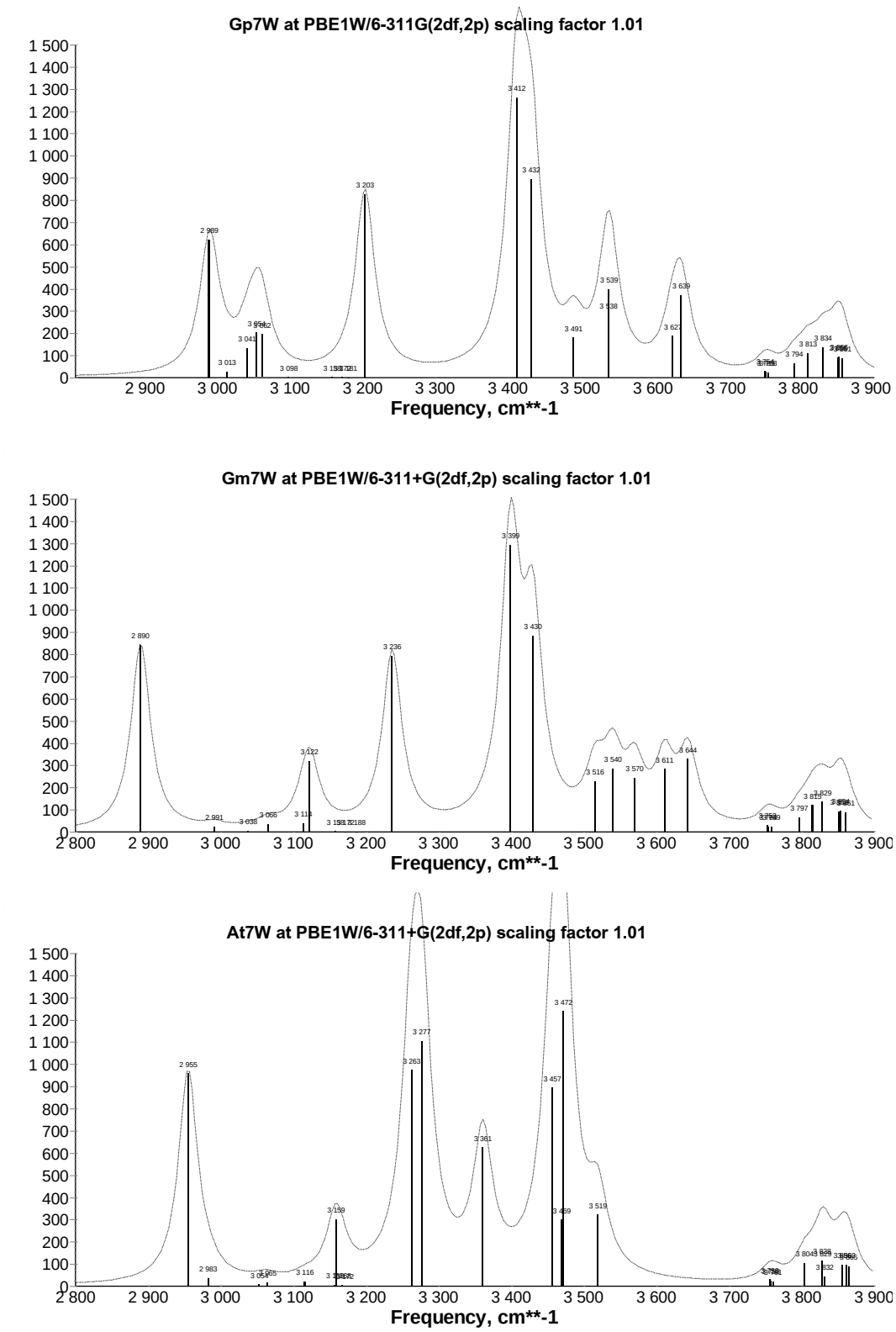


Fig.8. Calculated harmonic vibrational frequencies and IR intensities for 5-HT/$(H_2O)_7$ at the PBE1W/6-311+G(2df,2p) level (scaling factor of 1.01)

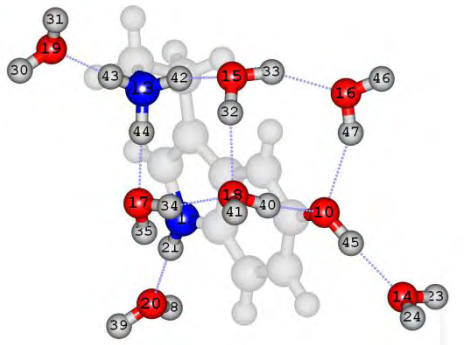
4. Atoms in Molecule Topological Analysis of 5-HT$(H_2O)_7$

To characterize the formation of hydrogen bond(s) network in three 5-HT$(H_2O)_7$conformers (Gp7W, Gm7W and At7W), topological analysis of the electron density using the Bader's atoms in molecules (AIM) theory was applied. We used this method to detect bond paths inside the 5-HT$(H_2O)_7$conformers and critical points associated to them. The value of the electronic density at the BCP for a given bond, ρ, can be correlated to the concept of bond order with higher values of ρ corresponding to stronger bonds. The positive sign of the Laplacian of the charge density at the BCP, $\nabla^2\rho$ corresponds to a closed shell interaction that is responsible for the bonding where the electronic charge is concentrated around each nucleus. This is the typical case for ionic or hydrogen bonds. Based on the Popelier's theory of atoms in molecules used to characterize a D–H···A hydrogen bond, the electron density ρ, at the bond critical point lies between 0.002 and 0.035 a.u. and the Laplacian of the electron density $\nabla^2\rho$ at the bond critical point lies between 0.024 and 0.139 a.u. The value of ρ (a.u.) and $\nabla^2\rho$ (a.u.) at BCP are listed in Table 5. The results show that all interactions were classified as real hydrogen bond interaction using definition by Popelier.

Beside the location of bond critical point (BCP), the ring and electron cage critical points (RCP and CCP, respectively) including bond paths are shown in Fig. 9. The example of electron density contour maps for atoms through the plane of the tetramer-like (found in Gp7w and Gm7W) and pentamer-like (found in At7W) H-bonds structures are shown in Fig. 10. There is topological evidence of electron density concentration around the internuclear axis and thus evidence of an intramolecular H-bond interaction in usually accepted sense. Water molecules, the cationic head and the 5-hydroxyl group form together an H-bond network. For Gp7w and Gm7W, two cyclic clusters imbricate in which water W3, W4, W5, and W6 individually participate (i) a "tight" tetramer-like ring known for $NH_4^+(H_2O)_4$ involving water W3, W4, and W6, stabilized by cooperative topological effects; (ii) a cage-like structural cluster with longer O-O distances, known for $NH_4^+(H_2O)_6$, involving water W3, W4, W5 and W6, the cationic head and the 5-hydroxyl group. This arrangement in 5-HT is an outline of clusters observed in protonated water and consists of square faces forming irregular cuboids that are known to evolve into clathrate-like cages as the number of molecules reaches the dozen.

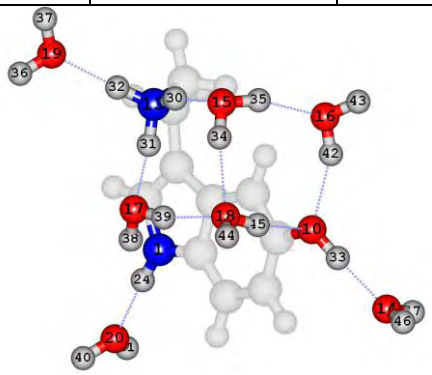
Table 5 Bond critical point analysis of O···H bond in three 5-HT$(H_2O)_7$ clusters calculated at the PBE1W/6-311+G(2df,2p) level of theory.

Gp7W



line	Atom1	Atom2	ρ	$\nabla^2\rho$
1	10	40	0.0255	0.0797
2	10	47	0.0185	0.0614
3	14	45	0.0344	0.1022
4	15	42	0.0386	0.1048
5	16	33	0.0276	0.0887
6	17	44	0.0354	0.0972
7	18	32	0.0215	0.0701
8	18	34	0.0259	0.0819
9	19	43	0.0308	0.0960
10	20	21	0.0252	0.0844

Gm7W



line	Atom1	Atom2	ρ	$\nabla^2\rho$
1	10	42	0.0204	0.0676
2	10	45	0.0212	0.0678
3	14	33	0.0351	0.1029
4	15	30	0.0445	0.1099
5	16	35	0.0287	0.0902
6	17	31	0.0304	0.0883
7	18	34	0.0214	0.0703
8	18	39	0.0244	0.0783
9	19	32	0.0299	0.0932
10	20	24	0.0254	0.0849

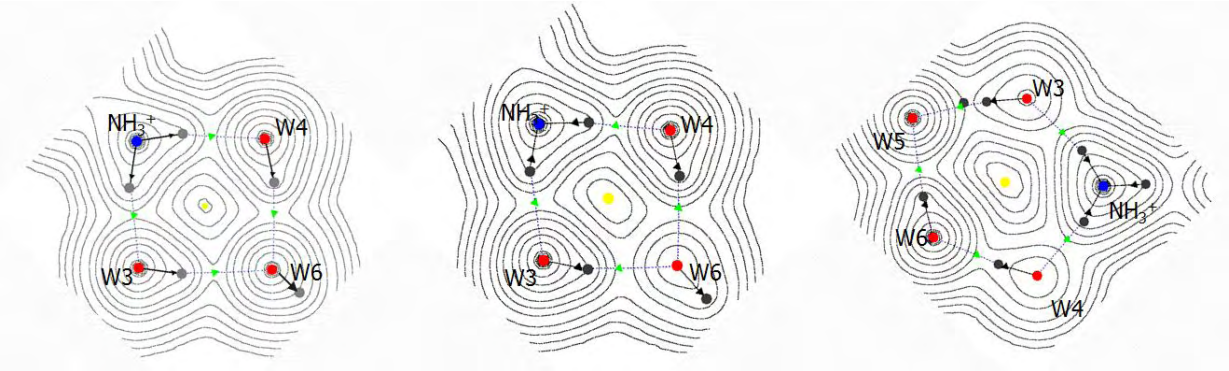


Fig.10. Electronic density surface for atoms in tetramer-like ring I and pentamer-like ring I (see Fig.5) in 5-HT$(H_2O)_7$ are shown along with their respective critical points and bond paths.

5. Natural Bond Analysis of 5-HT$(H_2O)_7$

The natural bond orbital (NBO) approach, a hydrogen bond is viewed as an interaction between an occupied non-bonded natural orbital of the acceptor atom n_A and the unoccupied antibonding orbital of the DH bond σ^*_{DH}. The NBO second-order interaction energy (E_2 kcal/mol) for the corresponding donor-acceptor H-bond interactions *i.e.* Lone-pair O$\rightarrow\sigma$*H-O or σ *H-N within 5-HT-$(H_2O)_7$ conformers are illustrated in Fig. 11. This E_2 were found to be 1 or 2 orders of magnitude greater for hydrogen bonds than for any other single nA$\rightarrow$ σ *DH contribution in hydrogen-bonded complexes

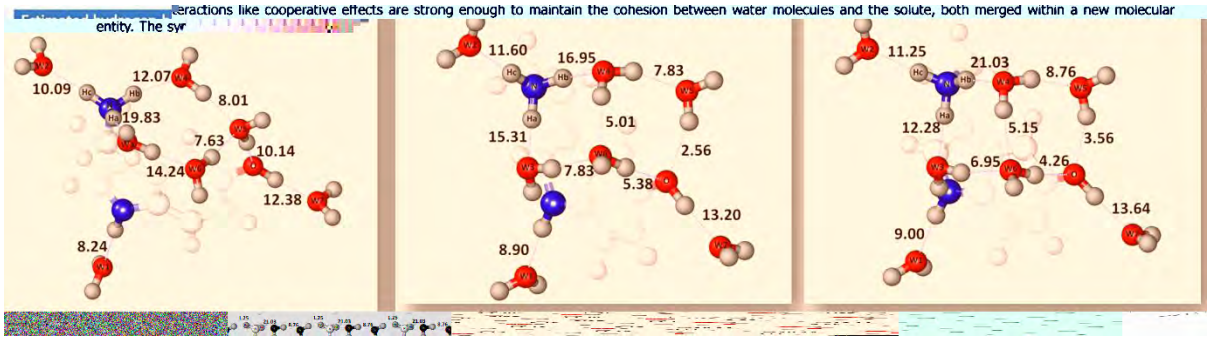


Fig.11. The NBO second-order interaction energy (E_2 kcal/mol) for the corresponding donor-acceptor H-bond interactions in 5-HT-$(H_2O)_7$ conformers.

6. Effect of The Continuum Solvent on Conformational Stability of 5-HT$(H_2O)_7$ Clusters

To investigate the influence of the continuum solvent on conformational stability of 5-HT-water cluster, the calculations performing under the bulk of cluster-continuum *i.e.* polarizable continuum model(PCM) model were carried out on the relevant optimized geometries using single point calculations at the PBE1W/6-311+G(2df,2p)levels of theory. The results show in Table 6. When the most stable 5-HT-$(H_2O)_7$ conformers in the gas phase are rigidly embedded in the continuum solvent to account for bulk solvent effects, Gp7W and Gm7W clusters are stabilized with respect to the At7W, becoming 3.7 and 6.4 kcal/mol, respectively, less stable than At7W. Consequently, the solute polarization, total electrostatic and ΔG of salvation can be deduced to confirm the effect of polarizable continuum on the ordered stability of clusters. The results show that the relative stabilities obtained for the heptahydrated 5-HT under bulky solvent are consistent with those of corresponding isolated conformers in gas phase calculations.

Table 6 Single point energy of 5-HT$(H_2O)_7$ clusters calculated under PCM at the PBE1W/6-311+G(2df,2p) levels of theory.

Clusters	Gp7W-PCM	Gm7W-PCM	At7W-PCM
Gas phase	-1109.01834 (a.u.) (ΔE_{rel} 0.628 kcal/mol)	-1109.014332 (a.u.) (ΔE_{rel} 3.138 kcal/mol)	-1109.019327 (a.u.) (ΔE_{rel} 0.000 kcal/mol)
PCM	-1109.117917 (a.u.) (ΔE_{rel} 3.702 kcal/mol)	-1109.113682 (a.u.) (ΔE_{rel} 6.401 kcal/mol)	-1109.123885 (a.u.) (ΔE_{rel} 0.000 kcal/mol)
Solute polarization (kcal/mol)	3.25	3.13	3.85
Total electrostatic (kcal/mol)	-62.48	-62.34	-65.61
ΔG (solv) (kcal/mol)	-41.38	-41.18	-44.08

SUMMARY

The 5-HT-$(H_2O)_7$ exhibit clear evidence of cooperative effects of hydrogen bonding system. The results showed that the stability of the hydrogen bonds depends tightly on the arrangement of water molecules. The OH and NH stretch infrared absorptions indicate the influence of water molecules on bridge structure linking the NH2 and OH groups of 5-HT. The hydrogen bond energy could be estimated between H-donor and H-acceptor using the NBO approach. The AIM method confirms the existence of real H-bonds bridging water and 5-HT molecule. Our results suggest that first hydration shell and ligand sculpt each other in order to form strongly stable supermolecules. Incorporating water in the *in silico* drug design becomes thus mandatory and will lead to the development of the next generation of computational tools in drug discovery.

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OUTPUT OF RESEARCH

Poster presentation:

1. Water and Serotonin: Unity in Duality.
   Pratuangdejkul J, Chongrujiroj S, Ungwitayatorn J, Launay J-M, Manivet P.
   การประชุม นักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 9 วันที่ 15-17 ตุลาคม 2552 ณ โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ จังหวัดเพชรบุรี
2. Quantum Calculations on Hydrogen Bonded Cluster of Water and Serotonin: AIM and NBO Analyses.
   Pratuangdejkul J, Chongrujiroj S, Ungwitayatorn J, Launay J-M, Manivet P.
   การประชุม นักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 10 วันที่ 14–16 ตุลาคม พ.ศ. 2553 ณ โรงแรมฮอลิเดย์ อินน์ รีสอร์ท รีเจ้นท์ บีช ชะอำ จังหวัดเพชรบุรี

Oral presentation:

1. Theoretical study of hydrogen bond network in hydration shell of serotonin-water cluster: implication of aqueous environment on biological conformation.
   Pratuangdejkul J.
   Invited Speaker, the 1st Universiti Kebangsaan Malaysia – Mahidol University Joint Scientific Conference (UKM-MU JSC). 12-13th October, 2010, Puri Pujangga, UKM Bangi, Selangor, Malaysia.

Publication:

1. Pratuangdejkul J, Manivet P. Water and Serotonin: Unity in Duality. (Manuscript on preparation).
2. Pratuangdejkul J, Manivet, P. The DFT and TD-DFT Investigation of IR and UV Spectra of Hydrated Serotonin Molecules: Comparison of Gas-phase, Water Cluster and Water-Cluster-Continuum Models. (Manuscript on preparation).

Water and Serotonin: Unity in Duality

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Solute and water molecules sculpt each other within a stable new molecular entity

Pure and charged water solvents, solute molecules in water and forces driving their interactions have been intensively studied since many years ago by experimental and theoretical methods, leading to an amazing number of publications. Two major reasons explain this success: i) water is the "solvent of the life", driving all biochemical reactions in cells, molecules phase transitions, macromolecules stability, chemical synthesis routes, etc; ii) with an apparent simplicity, water is a medium where forces are extremely complex to understand and still remain partly elucidated.

In pharmacology it is well known that before interacting with its target, a hydrophilic ligand is shielded by water molecules. In the vicinity of its binding site, ligand-receptor interactions are achieved, thanks to the widely admitted entropy-enthalpy compensation phenomenon established between the solvent and the protein. The water molecules solvating the ligand and the surface of the receptor are "washed out" upon binding. Water always appears as a "vehicle" undergoing passively the influence of the ligand and the receptor, accommodating favorably the thermodynamic reaction. In pure and protonated water, cyclic water clusters $(H_2O)_n$ are formed and remain stably associated until the bulk solvent dynamics induced a dissociation until reformation, or an exchange of water molecules with the bulk. The rate of exchange with the bulk depends on the stability of the cyclic cluster and its size. Hydrogen bond (H-bond) cooperative effects participate to the stability of the clusters through many-body interactions due to an increase of charge density at critical points of H-bond.

Only a few theoretical studies have been published on small ligand/first hydration shell interactions as compared to pure or protonated water models. The size of the systems is preventing sophisticated theoretical calculations with extended basis sets that are obviously needed for weak interactions description like charge transfers and many-body cooperative effects. The first, and in a lesser extend the second hydration water layer, are well known for influencing the conformation of small molecules and consequently their binding to specific receptors. As an example, serotonin (5-hydroxytryptamine, 5-HT), is a monoamine neurotransmitter, involved in a wide variety of physiological and behavioral processes [1]. At

physiological pH, 5-HT is protonated with two pK_a. Rudnick *et al*, have demonstrated that the ionization forms of 5-HT are important for its transport mechanism [2]. The neutral form is transported into secretory vesicles through vesicular monoamine transporters (VMATs) [3], whilst the cationic one is transported by cytoplasmic membrane transporter SERT [4]. 5-HT ionizations occur in the cell aqueous medium by following a specific conformational route between acid/base pair of 5-HT corresponding to stable conformations localized along the potential energy surface [5]. 5-HT can adopt at least two local minimum energy conformations in aqueous solvent, evidenced by NMR [6] that was confirmed by theoretical calculations in gas phase [7]. Hydration, ionization, conformation, physical and biological properties are thus factors intimately linked together.

Recently, LeGreve*et al*succeeded to assign the infrared and ultraviolet spectra of a unique conformation of neutral 5-HT interacting with two waters molecules, with the help of quantum chemistry calculations at the B3LYP and M0-52X levels of theory and by using the 6-31+G(d) basis set [8]. However, monohydration does not allow a clear appreciation of water molecules contribution to the stability, conformational and physical properties of 5-HT.

We thus constructed a supramolecule by saturating all possible H-bond centers with water molecules in the vicinity of 5-HT polar groups, leading to a first stable hydration shell of seven water molecules (see figure part a). The system was then embedded inside a continuum solvent model. The quantum chemical calculations show that the heptahydration forms a stable ice-like complex with the ethylamine side chains locked in a *gauche* conformations (Gp7W, lowest energy minimum) at the B3LYP//MP2/6-311G+(2d,2p) level of theory. The cyclic water cluster mimics that of previously described for NH_4^+/water systems and render the experimental IR spectra [9, 10]. The binding energy of the system is quite large (70 kcal/mol) showing a strong stabilization of 5-HT by water. Water molecules, the cationic head and the 5-hydroxyl group form together an H-bond network. Two cyclic clusters imbricate in which water W3, W4, W5, and W6 individually participate for 18, 16, 16 and 24 kcal/mol respectively, to the total stabilization energy: i) a “tight” tetramer-like ring known for $NH_4^+(H_2O)_4$ involving water W3, W4, and W6, stabilized by cooperative topological effects. W6 strongly stabilize the hydration shell and carries an acceptor-acceptor-donor-type dangling hydrogen; ii) a cage-like structural cluster with longer O-O distances, known for $NH_4^+(H_2O)_6$, involving water W3, W4, W5 and W6, the cationic head and the 5-hydroxyl group. This arrangement in 5-HT is an outline of clusters observed in protonated water and consists of square faces forming irregular cuboids that are known to evolve intoclathrate-like cages as the number of molecules reaches the dozen. The existence of H-bond within the cyclic water cluster was confirmed by Bader analysis [11] that

indicated the presence of bond, ring and electron cage critical points, evidence of a strong stabilization of hydrated 5-HT by a stable H-bond network (see figure part b).

If water and 5-HT are so stuck together, how dehydration can occur? W6 is the key-stone of the 5-HT water cluster. After H-bond breaking pulling apart W6 from the resting cyclic cluster, the ideal water scaffold is broken. If the rate of reformation of the initial cluster is slower than the exchange rate with bulk solvent or evolves toward a least stable one, then, 5-HT binding to its protein target becomes possible after dehydration. This observation suggest that: i) the first hydration shell and the ligand associate in an unique and stable molecular entity; ii) this entity exits thanks to key-stone water molecules; iii) prior to binding, specialized amino acids in receptors could promote desolvation by interacting with key-stone water molecules.

These preliminary results suggest that first hydration shell and ligand sculpt each other in order to form strongly stable supramolecules. The supramolecules are able to influence physicochemical properties of the ligand like phase transitions, chemical reactivity, biological activity, etc. Incorporating water in the *in silico* drug design becomes thus mandatory and will lead to the development of the next generation of computational tools in drug discovery.

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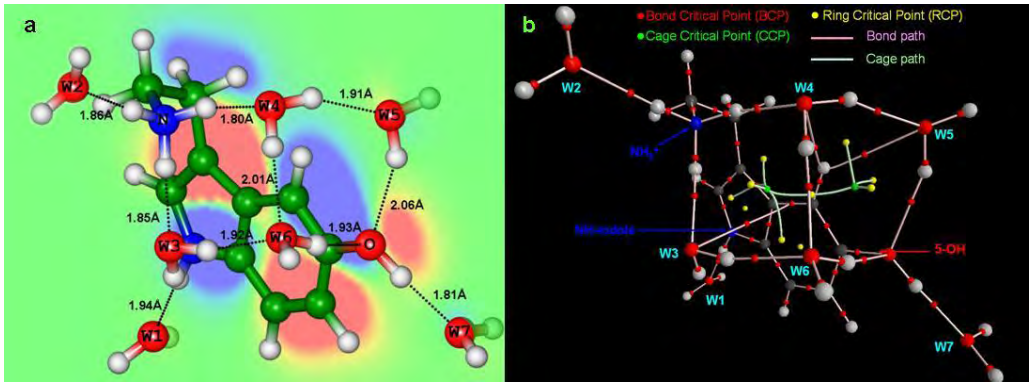


Hydrated serotonin.a) Cyclic water cluster of the first hydration shell of serotonin. Fully energy optimized structure at the B3LYP//MP2 6-31+G(2d,2p) level of theory. b) Electronic charge densities with critical points.

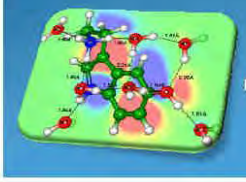
INVITED SPEAKER 5:

THEORETICAL STUDY OF HYDROGEN BOND NETWORK IN HYDRATION SHELL OF SEROTONIN-WATER CLUSTER: IMPLICATION OF AQUEOUS ENVIRONMENT ON BIOLOGICAL CONFORMATION

Dr. Jaturong Pratuangdejkul
Department of Microbiology, Mahidol University, Thailand

The serotonin-$(H_2O)_7$ cluster formed via hydrogen bond (H-bonds) interactions have been studied by density functional theory (DFT). Structures showing a clustering of water molecules were found to be preferred over structures with water distributed around three conformers of serotonin in the clustered complexes. The results plausibly indicate the first hydration shell structure of serotonin. The relevant geometries, energies, and IR characteristics of the intermolecular H-bonds have been investigated. The quantum theory of atoms in molecule (AIM) and natural bond orbital (NBO) analysis have also been applied to understand the nature of the hydrogen bonding interactions in clusters. The results show that both the strength of H-bonds and the deformation are important factors for the stability of serotonin-$(H_2O)_7$ clusters. The strengths of H-bonds in term of molecular structure could be deduced from a comparison of three conformers. Cooperative effect (CE) in terms of stabilization energy of clusters are calculated and discussed as well. The linear relationships between the electron density of BCP (ρb) and the H···O bond length of H-bonds as well as the second-perturbation energies E(2) have also been investigated to study the nature of H-bonds by using AIM and NBO analyses, respectively. The network of H-bonded complexes formed between serotonin and water shed light on the stability of serotonin conformers to form heavily hydrated cluster in living organisms.

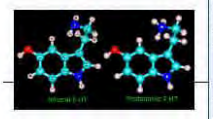
THEORETICAL STUDY OF HYDROGEN BOND NETWORK IN HYDRATION SHELL OF SEROTONIN-WATER CLUSTER: IMPLICATION OF AQUEOUS ENVIRONMENT ON BIOLOGICAL CONFORMATION



Jaturong Pratuangdejkul Ph.D.
Department of Microbiology
Faculty of Pharmacy Mahidol University

MAHIDOL UNIVERSITY

Serotonin (5-hydroxytryptamine; 5-HT)

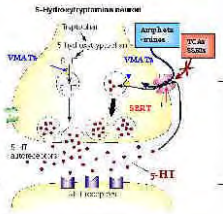


- $C_{10}H_{12}N_2O$
- IUPAC name: 3-(2-aminoethyl)-1*H*-indol-5-ol
- MW 176.22 g/mol
- Complex with creatinine sulfate monohydrate, and other salts *e.g.* hydrogen oxalate, picrate monohydrate, hydrochloride
- pK_{a1} = 9.97; pK_{a2} = 10.73
- Symmetric or achiral molecule, no optical isomers

•Serotonin (5-hydroxytryptamine; 5-HT) a monoamine neurotransmitter plays a critical role in a wide variety of physiological and behavioral processes.

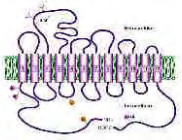
•At physiological pH, 5-HT is "protonated".

Storage, Release & Re-uptake

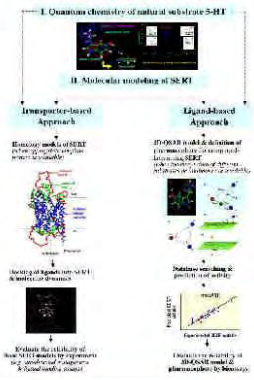


5-HT TRANSPORTER (SERT)

- Na^+/Cl^--dependent transporter family
- Co-transport of external Na^+ and Cl^- and counter-transport of K^+ (electroneutral)
- 12 TMDs, large EL between TMD_3 & TMD_4 with N-linked glycosylation sites
- N- and C-termini in cytoplasm with phosphorylation sites
- hSERT spans 37.8 kb on chromosome 17q11.2, encodes 630 amino acids.



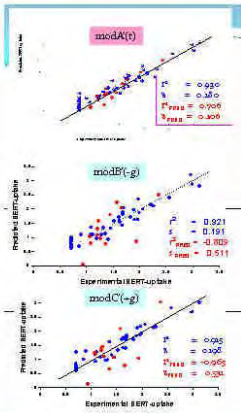
In Silico Approaches for Molecular Modeling of 5-HT and SERT



My research on 5-HT and SERT

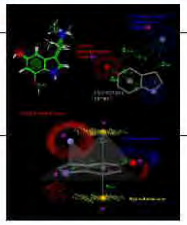
Computational approaches for the study of serotonin and its membrane transporter SERT: implications for drug design in neurological science.

Pratuangdejkul J, Schneider B, Launay J-M, Kellermann O, Manivet P. *Curr. Med. Chem.* 2008; 15: 3214-27. 8



Definition of an uptake pharmacophore of the serotonin transporter through 3D-QSAR analysis.

Pratuangdejkul J, Schneider B, Jaudon P, Rosillo V, Baudoin E, Loric S, Conti M, Launay J-M, Manivet P. *Curr. Med. Chem.* 2005; 12: 2393-410.



Cation-π interactions in serotonin: conformational, electronic distribution and energy decomposition analysis.

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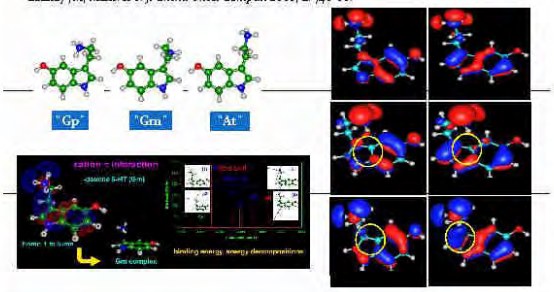


Conformational dependence for theoretical pK_a calculation of serotonin..

Pratuangdejkul J, Nosoongnoen W, Guerin GA, Loric S, Conti M, Launay J-M, Manivet P. Chem. Phys. Lett. 2006; 420: 538-44

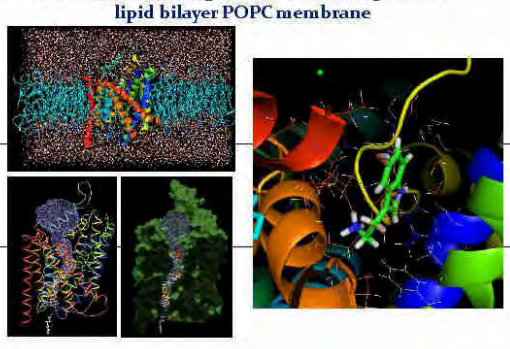
$AH\cdot(H_2O)_{n\ gas} + H_2O_{gas} \xleftrightarrow{\Delta G_{gas}} A^-\cdot(H_2O)_{n\ gas} + H_3O^+_{gas}$ ← (Gas-phase)

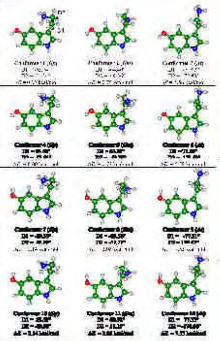
$\uparrow -\Delta G_{solv}(AH\cdot(H_2O)_n)$ $\uparrow -\Delta G_{solv}(H_2O)$ $\downarrow \Delta G_{solv}(A^-\cdot(H_2O)_n)$ $\downarrow \Delta G_{solv}(H^+)$

$AH\cdot(H_2O)_{n\ aq} + H_2O_{aq} \xleftrightarrow{\Delta G_{aq}} A^-\cdot(H_2O)_{n\ aq} + H_3O^+_{aq}$ (Cluster-PCM-UAHF)

Calculated pK_{a2} (experimental pK_{a2} = 10.73)

Molecular modeling of serotonin transporter in lipid bilayer POPC membrane





Because 5-HT carries out its biological functions in aqueous solution, it is important to understand how the conformational preferences of 5-HT change in the presence of water molecules.

- Water molecules may play a role in the way in which 5-HT interacts with the receptor or transporter.
- The explicit water is important for the determination of reaction free energy and pK_a prediction of 5-HT.
- There are few publications available, only for mono- and di-hydrated complexes of "neutral" 5-HT.

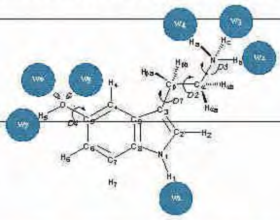
Acknowledgements

The Thailand Research Fund:
Grant number MRG5180084

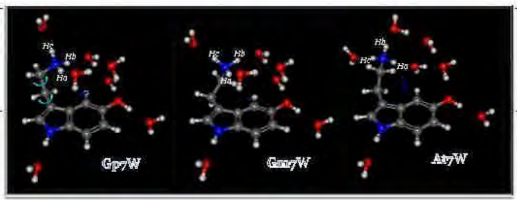
In this study, the strength of H-bonds in terms of molecular structures, stabilization energies and cooperative effects could be deduced from 5-HT-$(H_2O)_7$ using theoretical quantum chemistry, the atom in molecules (AIM) and the natural bond orbital (NBO) analyses

Geometries and Stabilization Energies of 5-HT-$(H_2O)_7$

Based on the three most stable conformers of 5-HT in gas-phase *i.e.*, Gp, Gm and At, the seven water molecules are distributed over the H-bond donor (5-OH, NH-indole) and H-bond acceptor (NH_2) sites, labeled W1 - W7



The 5-HT$(H_2O)_7$ clusters were fully optimized using the generalized gradient approximation (GGA) PBE1W/6-311+G(2df,2p) levels of theory. The conformational analysis revealed the existence of three stable clusters of 5-HT$(H_2O)_7$ *i.e.*, Gp7W, Gm7W and At7W. Frequency analysis revealed the absence of any imaginary frequency, confirming they are real stable minima.



The relative energies and some geometrical parameters

Stability of clusters are decrease in this order: At7W > Gp7W > Gm7W.

The binding energy is ~80 kcal/mol) showing a strong stabilization of 5-HT by water.

The elongation of N_1-H_a and N_2-H_b bonds results from the contribution of H-bond

	PBE1W/6-311+G(2df,2p)		
	Gp7W	Gm7W	At7W
D1	-92.055	-35.170	-68.91
D2	61.707	-52.880	155.61
N_1-H_a	1.049	1.046	1.042
N_1-H_b	1.053	1.059	1.054
N_1-H_c	1.039	1.037	1.036
N_2-H_d	1.023	1.023	1.021
O−H	0.989	0.989	0.985
ΔE_{rel}	0.628	3.138	0.00
BE^*	80.732	80.508	81.973
BSSE	3.577	3.656	3.054

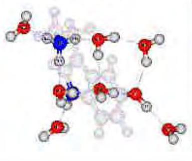
$^*BE = E[5\text{-HT}(H_2O)_7] - E[5\text{-HT}] - E[(H_2O)_7]$

Effect of hydration shell on the stability of 5-HT$(H_2O)_7$

Stepwise hydration energy ($DE_{W(n)}$) of each water molecule (W1 to W7) in 5-HT-$(H_2O)_7$ were calculated together with the basis set superposition errors (BSSE) by means of the counterpoise method at the PBE1W/6-311+G(2df,2p) level.

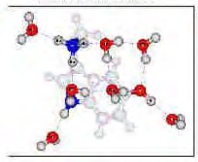
$DE_{(Wn)} = E[5\text{-HT-}(H_2O)_7] - E[5\text{-HT-}(H_2O)_6] - E[(H_2O)]_{Wn}$

H-bond network of Gp7W



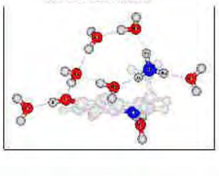
Gp7W	DE_{Wn}	BSSE
W1	-8.41	-0.46
W2	-10.73	-0.49
W3	-15.02	-1.09
W4	-20.13*	-1.15
W5	-11.27	-0.76
W6	-17.29	-1.09
W7	-10.96	-0.58

H-bond network of Gm7W



Gm7W	DE_{Wn}	BSSE
W1	-8.53	-0.46
W2	-10.59	-0.50
W3	-13.62	-1.11
W4	-20.57*	-1.18
W5	-12.62	-0.82
W6	-16.29	-1.08
W7	-11.06	-0.58

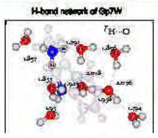
H-bond network of At7W

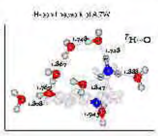


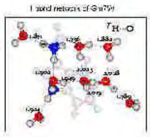
At7W	DE_{Wn}	BSSE
W1	-7.72	-0.44
W2	-10.52	-0.43
W3	-19.72	-0.85
W4	-16.61	-0.88
W5	-16.54	-0.91
W6	-24.12*	-1.11
W7	-10.10	-0.56

In 5-HT-$(H_2O)_7$ conformers, water molecules form H-bonded bridges. It was found that four water molecules form cyclic water bridge linking the NH3+ of the ethylamine side chain and the 5-OH group of 5-HT.

Two types of bridges are formed *i.e.* ring-like in At7W and book-like in Gp7W and Gm7W.

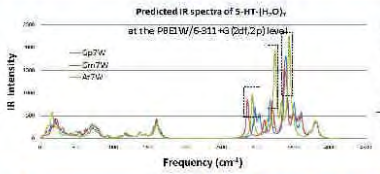






Schematic representation of H-bond clusters between 5-HT and water molecules optimized at PBE1W/6-311+G(2df,2p), bond lengths (Å) of H-bond ($r_{H\cdots O}$) have been illustrated.

Calculated harmonic vibrational frequencies and IR intensities for 5-HT/(H2O)7



The shifting of marked frequencies reflect the presence of H-bonded OH and NH stretch transitions.

	Gp7W	Gm7W	At7W
5-OH	3378.68	3365.60	3437.52
NH-indole	3398.33	3395.79	3422.34
NH-amin (point-out)	3171.42	3204.02	3230.52
NH-amin (point in ring)	2959.55	2861.21	2925.76

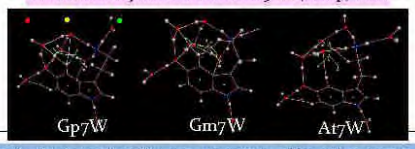
Atoms in Molecule Topological Analysis of 5-HT-(H2O)7

The existence of H-bonded clusters of 5-HT(H2O)7 was confirmed using Bader's AIM (Atom in Molecule) topological analysis of the electron density.

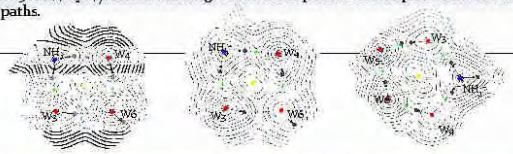
- Electron density (ρ)
- Laplacian $\nabla_{2\rho(r)}$
  - $\nabla_{2\rho(r)} < 0$, covalent bonds
  - $\nabla_{2\rho(r)} > 0$, ionic or H-bonds.
- critical points (CP)
  - Bond (BCP)
  - Ring (RCP)
  - Electron cage critical points (CCP)

The AIM analyses of 5-HT(H2O)7 clusters show their fulfilment to criteria of H-bond proposed by Popelier et al. implies the existence of real H-bonds in the supersystem.

Molecular graphs of 5-HT/(H2O)7 clusters obtained from AIM analysis at the PBE1W/6-311+G(2df,2p) level.



Electronic density surface for atoms in tetramer-like and pentamer-like ring in 5-HT/$(H_2O)_7$ are shown along with their respective critical points and bond paths.



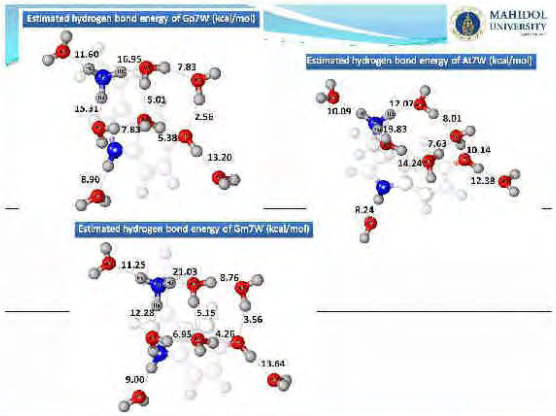
Natural Bond Analysis of 5-HT-(H2O)7

The natural bond orbital (NBO) approach,

Hydrogen bond is viewed as an interaction between an occupied nonbonded natural orbital of the acceptor atom n_A and the unoccupied antibonding orbital of the DH bond σ^*_{DH}

The NBO second-order interaction energy (E_2)

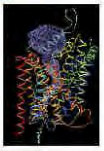
- the corresponding donor-acceptor H-bond interactions *i.e.* Lone-paire O→σ*H-O or σ*H-N is the major within 5-HT-$(H_2O)_7$.
- found to be 1 or 2 orders of magnitude greater for hydrogen bonds than for any other single nA→σ*DH contribution in hydrogen-bonded complexes



Conclusion

- The cooperative effects of H-bond system in 5-HT-$(H_2O)_7$
- The stability of H-bond depends on the arrangement of H_2O molecules.
- The OH and NH stretch IR absorptions indicate the influence of water molecules on 'bridge' structure linking the NH_2 and OH groups of 5-HT.
- The AIM method confirms the existence of real H-bonds bridging water and 5-HT molecule.
  The hydrogen bond energy could be estimated using the NBO approach.

If water and 5-HT are so stuck together, how dehydration can occur? The key-stone water of the cluster is a good answer. 5-HT binding to its protein target becomes possible after dehydration.



This observation suggest that:

- The first hydration shell and the ligand associate in an unique and stable molecular entity;
- This entity exist thanks to key-stone water molecules;
- Prior to binding, specialized amino acids in receptors/transporter could promote desolvation by interacting with key-stone water molecules.

Acknowledgements

This work is supported by
The Thailand Research Fund:
Grant number MRG5180084