# Cation-π Interactions in Serotonin: Conformational, Electronic Distribution and Energy Decomposition Analysis

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## 5-HT (local minima)



Conformer 10 (GpGp) Conformer 11 ( GpGm )

OH-syn

Conformer 12 (GpAt)

Figure S1. Structures of the twelve energy minima of 5-HT obtained from optimization at the B3LYP/6-31+G(d,p) level of theory.

**Table S1.** Gas-phase Energies (in Hartree) of 1) Twelve Local Minima of 5-HT at the B3LYP/6-31G(d) and B3LYP/6-31+G(d,p) Levels of Theory and 2) Eight Transition States of 5-HT at the B3LYP/6-31+G(d,p) Level of Theory .

conform	ners	B3LYP/6-31G(d)	B3LYP/6-31+G(d,p)
	1 ( <b>GmGp</b> )	-573.3892903	-573.4363585
OH-anti	2 (GmGm)	-573.3879278	-573.4351901
	3 (GmAt)	-573.379786	-573.4275189
	4 (GmGp)	-573.3858371	-573.4329678
OH-syn	5 (GmGm)	-573.3861057	-573.4330615
	6 ( <b>GmAt</b> )	-573.3768034	-573.4243785
	7 (GpGp)	-573.3892935	-573.4363826
OH-anti	8 (GpGm)	-573.3879321	-573.4352121
orr unit	9 ( <b>GpAt</b> )	-573.3797761	-573.4274849
	10 ( <b>GpGp</b> )	-573.3858371	-573.4329678
OH-syn	11 ( <b>GpGm</b> )	-573.3861057	-573.4330615
	12 ( <b>GpAt</b> )	-573.3768033	-573.4243785
	Ts1	-	-573.4331611
	Ts2	-	-573.4241054
	Ts3	-	-573.4216518
Transition states	Ts4	-	-573.4221211
Transition states	Ts5	-	-573.4192445
	Ts6	-	-573.4216055
	Ts7	-	-573.4240613
	Ts8	-	-573.4331611

conformers			B3LYP/6	6-31G(d)			B3LYP/6-	31+G(d,p)	
		$\mathbf{\phi}_1$	$\phi_2$	$\phi_4$	$\Delta E$	$\phi_1$	$\phi_2$	$\phi_4$	$\Delta E$
	1 ( <b>GmGp</b> )	-81.59	57.34	-174.86	0.00	-83.74	54.34	-174.78	0.02
OH-anti	2 (GmGm)	-68.64	-52.25	-177.14	0.86	-64.40	-49.36	-176.25	0.75
	3 (GmAt)	-70.66	173.07	-177.97	5.97	-71.93	172.83	-177.78	5.56
	4 (GmGp)	-86.67	52.36	-9.53	2.17	-89.59	49.88	-9.15	2.14
OH-syn	5 (GmGm)	-71.81	-52.94	-4.17	2.00	-69.58	-51.13	-4.71	2.08
	6 (GmAt)	-76.04	176.30	-4.02	7.84	-77.31	176.63	-4.27	7.53
	7 ( <b>GpGp</b> )	81.11	-57.80	174.88	0.00	84.49	-53.44	174.68	0.00
OH-anti	8 (GpGm)	68.65	52.33	177.22	0.86	64.63	49.38	176.28	0.73
	9 (GpAt)	71.22	-173.34	177.85	5.97	71.99	173.43	177.70	5.58
	10 ( <b>GpGp</b> )	86.67	-52.36	9.52	2.17	89.59	-49.88	9.15	2.14
OH-syn	11 ( <b>GpGm</b> )	71.81	52.94	4.16	2.00	69.58	51.13	4.71	2.08
	12 (GpAt)	76.03	-176.26	3.97	7.84	77.33	-176.60	4.27	7.53

**Table S2**. Dihedral Angles ( $\phi_1$ ,  $\phi_2$ , and  $\phi_4$ ) and Relative Energies ( $\Delta E$ ) of 5-HT Conformers Calculated Using the B3LYP Method with the 6-31G(d) and the 6-31+G(d,p) Basis Sets in the Gas-Phase.<sup>*a*</sup>

<sup>*a*</sup> The dihedral angles in degree, relative energies in kcal/mol, with respect to the lowest energy conformer (OH-*anti*, **GpGp**) in each level of calculation.

**Table S3.** Geometric Parameters of the Three Most Stable Conformers of 5-HT (Conformers 1, 2 and 3From Table S2) Obtained from the B3LYP Method Using 6-31G(d) and 6-31+G(d,p) Basis Sets. <sup>a</sup>

geometric	E	33LYP/6-31G(d	l)	В3	B3LYP/6-31+G(d,p)			
parameters	GmGp	GmGm	GmAt	GmGp	GmGm	GmAt		
$\theta_1$	112.39	111.55	109.42	112.61	112.03	110.04		
$\theta_2$	109.96	107.99	111.18	109.95	108.54	110.95		
$\phi_1$	-81.59	-68.64	-70.66	-83.74	-64.40	-71.93		
$\phi_2$	57.34	-52.25	173.07	54.34	-49.36	172.83		
$\tilde{\rm NH}_a$	1.0389	1.0433	1.0274	1.0355	1.0388	1.0258		
$\tilde{N}H_{b}$	1.0262	1.0260	1.0271	1.0246	1.0244	1.0254		
$\tilde{N}H_{\text{c}}$	1.0258	1.0252	1.0272	1.0240	1.0236	1.0256		

<sup>*a*</sup>Angles  $\theta_1$  (C<sub>9</sub>-C<sub>3</sub>-C<sub> $\beta$ </sub>),  $\theta_2$  (C<sub>3</sub>-C<sub> $\beta$ </sub>-N) and dihedral angles  $\phi_1$ ,  $\phi_2$  in degrees, and bond lengths of amine N–H bonds in angstroms.

#### Molecular Dynamics Simulations and Trajectory Analysis of 5-HT

### Method:

Molecular dynamics: Based on the conformational analysis of 5-HT performed using quantum chemistry calculations, accurate empirical parameters were generated for the CHARMM force field. The set of partial charges and force field parameters were adjusted in order to bring the total dipole moments and the relative conformational energies, in good agreement with those obtained from quantum chemistry calculations at the B3LYP/6-31+G(d,p) level. The Langevin molecular dynamics (MD) simulations were then performed using our modified CHARMM force field. The 5-HT molecule was submitted to MD simulations at 900 K. The non-bonded list was cut off at 10 Å. Simulations were performed with the following protocol: energy minimizations were carried out with the Steepest Descent and Adopted Basis set Newton-Raphson routines to a r.m.s gradient  $< 10^{-4}$  kcal mol<sup>-1</sup> Å<sup>-1</sup>. The energy-minimized systems were heated at 900 K during 6 ps and equilibrated during 50 ps. Production dynamics was performed in the micro-canonical ensemble for 1000 ps. The bond lengths were constrained with the SHAKE algorithm and the integration time step was taken equal to 1 fs. From the overall trajectory data set, the geometries of 20,000 conformations were saved and further used to analyze the dynamics process of ethylamine side-chain rotation. Dihedral angles  $\phi_1$  and  $\phi_2$  of the ethylamine side chain were used to define the conformations of 5-HT in the data set. All MD calculations were performed using CHARMM 30 running on Silicon Graphics O<sub>2</sub> R12000 workstation under the IRIX 6.5 operating system.

MD simulations were used to identify the low energy conformers of 5-HT and to find transition state connecting two neighboring minima. All conformations used for quantum chemistry calculations were obtained form the analysis of the time-dependent plots of two variables, dihedral angles  $\phi_1$  and  $\phi_2$ , extracted from MD dynamics trajectories. Figure S2 shows an example of time-dependent evolution (11000-15000 time steps) of the dihedral angles  $\phi_1$  and  $\phi_2$ . The time-dependent evolution of  $\phi_1$  shows a variation in the sign of the dihedral angle which is due to the switching of ethylamine side chain from one side of indole ring (mirror image) to the other. The time-dependent evolution of  $\phi_2$  defines the position of cationic head related to indole ring. The fluctuations around  $\phi_2$  led to three marked groups of redundant energy minima conformations along the MD trajectories: **Gp**, **Gm**, and **At**. From the plot of time-dependent evolution of dihedral angles  $\phi_1$  and  $\phi_2$ , we extracted transition state (Ts) structures located between two neighboring minima (pointed in Figure S2) that were used as guess transition states for the QST3 calculations at the B3LYP/6-31+G(d,p) theory level. Finally, we checked the accuracy of our first set of CHARMM force field parameters by performing a second CHARMM PES including in the analysis the transition state structures.



Time evolution of dihedral angle  $\phi_1$ 



HT molecule (**Gp** and **Gm**) to switch between two mirror images. The lower series shows the selected  $\phi_2$  defining different 5-HT conformations (**Gp**, **Gm**, and **At**). The guess transition state (Guess **Ts**) between two stable conformers are pointed.

**Table S4**. Harmonic Stretching Frequencies (v) and IR Intensities (I) of the Three Stable 5-HT Conformers (OH-*anti*, **GmGp**, **GmGm**, and **GmAt**) Calculated Using the B3LYP/6-31+G(d,p) Theory Level.<sup>*a*</sup>

Stretch	Gn	nGp	Gn	nGm	GmAt		
Stretten	ν	Ι	ν	Ι	ν	Ι	
alkyl CH	2923	14.12	2926	16.21	2881	45.78	
	2971	4.94	2965	3.96	2946	13.36	
	2979	9.44	2989	6.64	2985	1.88	
	3035	5.74	3049	4.08	3048	0.38	
aromatic CH	3050	7.12	3059	4.47	3056	5.46	
	3061	3.75	3072	3.92	3060	14.35	
	3080	1.79	3080	2.44	3078	3.15	
	3131	1.76	3125	1.89	3129	0.34	
amine NH	3154	257.60	3104	294.88	3268	57.71	
	3321	119.99	3321	139.70	3358	81.42	
	3377	86.00	3382	84.61	3362	92.32	
indole NH	3523	125.90	3523	122.14	3528	119.11	
OH	3674	114.25	3675	109.18	3677	100.30	

<sup>*a*</sup> Frequencies (v) in  $\overline{\text{cm}^{-1}}$  and IR Intensities (I) in km/mol by scaling factor of 0.9605.



**Figure S3**. IR spectra showing **a**) CH and **b**) NH Stretching vibrational modes of the three most stable conformers of 5-HT (**GmGp**, **GmGm**, and **GmAt**) calculated at the B3LYP/6-31+G(d,p) theory level .

**Table S5**. Absolute Isotropic NMR Shielding Tensors (σ in ppm) Calculated Using the GIAO Method of C and H Atoms in the Three Most Stable 5-HT Conformers (OH *anti*, **GmGp**, **GmGm**, **GmAt**) at the B3LYP/6-311+G(2d,2p) Theory Level of.

nosition	absolut	te shielding 1	tensors
position	GmGp	GmGm	GmAt
C <sub>2</sub>	52.34	51.06	53.88
C <sub>3</sub>	73.57	77.87	78.36
$C_4$	82.40	77.69	80.16
C <sub>5</sub>	19.27	19.78	20.68
C <sub>6</sub>	63.46	62.34	64.38
$C_7$	61.72	63.34	63.57
$C_8$	45.82	44.68	45.60
C <sub>9</sub>	48.55	51.11	49.75
H <sub>1</sub>	23.35	23.49	23.69
$H_2$	24.19	24.22	24.35
$H_4$	24.84	24.50	24.72
$H_5$	27.01	27.08	27.24
$H_6$	24.54	24.53	24.65
$H_7$	23.88	23.94	24.02
$H_{\beta a}$	28.31	27.76	28.09
$H_{\beta b}$	28.22	28.59	28.35
$H_{\alpha a}$	28.43	27.56	27.48
$H_{\alpha b}$	27.63	27.98	28.18
Ha	25.19	25.28	27.03
H <sub>b</sub>	27.36	27.47	27.24
H <sub>c</sub>	27.53	28.11	26.99

Atom	GmGp	GmGm	GmAt	Atom	GmGp	GmGm	GmAt
N1	-0.3796	-0.3907	-0.4272	H1	0.3634	0.3641	0.3733
C2	-0.0770	-0.0900	-0.0783	H2	0.1636	0.1504	0.1603
C3	-0.1634	-0.0462	-0.1241	H4	0.2161	0.2143	0.2062
C4	-0.4434	-0.3980	-0.3805	Н5	0.4448	0.4409	0.4382
C5	0.4312	0.4515	0.4113	Н6	0.1385	0.1447	0.1369
C6	-0.2557	-0.2832	-0.2561	H7	0.1480	0.1369	0.1394
C7	-0.1431	-0.1269	-0.1603	На	0.1124	0.1742	0.2872
C8	0.1163	0.1391	0.1845	Hb	0.2642	0.3465	0.2875
C9	0.2133	0.0988	0.1059	Hc	0.3018	0.3296	0.3234
Са	0.0559	0.1308	0.1781	Hα(a)	0.0549	0.0606	0.0261
Сβ	0.0895	0.0187	0.0251	Ha(b)	0.0610	0.0406	0.0124
Ν	-0.1475	-0.3581	-0.2925	Hβ(a)	0.0027	0.0318	0.0365
0	-0.6237	-0.6255	-0.6271	Hβ(b)	0.0560	0.0451	0.0140

**Table S6.** Electrostatic Potential Derived Charges of Atoms in Three Most Stable 5-HT Conformers (OH *anti*, **GmGp**, **GmGm**, **GmAt**) Calculated at the B3LYP/6-311+G(2d,2p) Using ChelpG Scheme.

#### The Calculation of Shielding Increments of Amine Hydrogen

In a strong magnetic field, hydrogen nuclei located over an aromatic ring experience a reduced magnetic fields as a result of the induced magnetic field associated with the delocalized  $\pi$  electrons. To support this hypothesis, the proton shielding increment ( $\Delta\delta H$ ) for amine group of the three most stable conformers of 5-HT (**GmGp**, **GmGm**, and **GmAt**), was calculated. The H isotropic shielding tensor data for each of the protons of the cationic head of 5-HT were calculated and transformed into shielding increments by subtracting from each data point the H isotropic shielding tensor obtained from the fully optimized ammonium molecule alone, using the same level of theory and basis set. The results are listed in Table S6.

**Table S7**. The Shielding Increment of Amine Hydrogen ( $\Delta\delta$ H) Calculated Using The B3LYP/6-311+G(2d,2p) Level of Theory.

	GmGp	GmGm	GmAt
H <sub>a</sub>	1.52	1.42	-0.26
H <sub>b</sub>	-0.65	-0.76	-0.34
H <sub>c</sub>	-0.82	-1.40	-0.54

	SD				FC			PSO	
	GmGp	GmGm	GmAt	GmGp	GmGm	GmAt	GmGp	GmGm	GmAt
(N <sub>1</sub> ,C <sub>2</sub> )	0.0795	0.0831	0.0659	12.5497	12.6390	12.8178	-2.4181	-2.4207	-2.3805
$(C_2, C_3)$	1.8861	1.6691	1.9078	80.0795	77.6666	82.2894	-7.1644	-6.9825	-7.1130
$(C_3, C_9)$	0.5082	0.4988	0.5296	57.3338	58.0008	60.5757	-4.4020	-4.3071	-4.4084
$(C_8, C_9)$	0.8862	0.9397	0.9356	62.1575	62.4787	62.8608	-5.8819	-6.0070	-5.9846
(N <sub>1</sub> ,C <sub>8</sub> )	0.1310	0.1243	0.1255	12.7253	12.3075	12.5190	-1.9415	-1.8801	-1.8752
(C <sub>4</sub> ,C <sub>9</sub> )	0.7277	0.8443	0.8489	69.9704	71.7311	72.4981	-5.2305	-5.4616	-5.4632
(C <sub>4</sub> ,C <sub>5</sub> )	1.6642	1.7066	1.7137	82.1155	81.8353	82.4277	-6.6980	-6.7922	-6.7378
$(C_5, C_6)$	1.0924	1.0913	1.1125	70.7711	70.6113	71.3639	-5.7878	-5.7848	-5.7988
(C <sub>6</sub> ,C <sub>7</sub> )	1.5725	1.5818	1.5564	71.1415	71.1942	71.4274	-7.1517	-7.1654	-7.0938
(C <sub>7</sub> ,C <sub>8</sub> )	1.0358	1.0379	1.0495	74.8846	74.6892	75.2013	-5.6943	-5.6541	-5.6894
$(C_{5}, O)$	0.0659	0.0648	0.0070	20.9146	21.0123	21.0559	3.4921	3.5019	3.2430
(N,H <sub>a</sub> )	-0.0305	-0.0313	-0.0469	49.3747	48.3278	49.2428	0.8992	0.9200	1.1949
$(N,H_b)$	-0.0550	-0.0304	-0.0404	48.8458	50.0898	48.7639	0.1191	1.2211	1.2170
(N,H <sub>c</sub> )	-0.0414	-0.0467	-0.0469	49.0299	48.8078	49.1465	0.1228	1.2357	1.2062

**Table S8.** Spin Dipolar (SD), Fermi contact (FC) and paramagnetic spin-orbit (PSO) Contributions of Coupling Constant (*J*) in Hz. Using GIAO method at B3LYP/6-311+G(2d,2p) Level.

**Table S9.** Calculated Major Orbital Contribution, Wavelength ( $\lambda$  in nm), Oscillator Strength (f) and Electronic Excitation Energies ( $\Delta E$  in eV) of Six Singlet-Excited States of the Three Most Stable 5-HT conformers (**GmGp**, **GmGm** and **GmAt**) Using The TDDFT Method at the B3LYP/6-311+G(2d,2p) Level of Theory, Together with Their Corresponding Ground State HOMO-n/LUMO+n Gap Energies (HLG, in eV).

	Excited	Major orbital cont	tribution	) (nm)	f	$\Delta E$	HLG
	state	Character	Coefficients	_ ~ (mn)	1	(eV)	(eV)
GmGp	$1^{1}A$	HOMO→LUMO	0.69821	336.77	0.0002	3.681 6	0.1590
	$2^{1}A$	HOMO-1→LUMO	0.69706	309.89	0.0025	4.000 9	0.1701
	3 <sup>1</sup> A HOMO	HOMO→LUMO+1	0.63991	287.66	0.0725	4.310 1	0.1781
		HOMO-1→LUMO+1	0.57370	265.91	0.0711	4.662 6	0.1892
	5 <sup>1</sup> A	HOMO→LUMO+2	0.65512	250.20	0.0230	4.955 3	0.2050
	6 <sup>1</sup> A	HOMO→LUMO+3	0.64966	241.13	0.0410	5.141 8	0.2099
GmGm	$1^{1}A$	HOMO→LUMO	0.70296	357.58	0.0015	3.467 4	0.1485
	$2^{1}A$	HOMO-1→LUMO	0.70081	318.36	0.0179	3.894 5	0.1658
	3 <sup>1</sup> A	HOMO→LUMO+1	0.64599	290.68	0.0874	4.265 4	0.1759
	$4^{1}A$	HOMO-1→LUMO+1	0.47024	258.91	0.0452	4.788 7	0.1932
	5 <sup>1</sup> A	HOMO→LUMO+2	0.58807	256.43	0.0284	4.835 1	0.1998
	6 <sup>1</sup> A	HOMO→LUMO+3	0.60372	253.90	0.0143	4.883 3	0.1988
GmAt	$1^{1}A$	HOMO→LUMO	0.70189	454.78	0.0004	2.726 2	0.1183

$2^{1}A$	HOMO-1→LUMO	0.70167	402.00	0.0006	3.084 2	0.1321
3 <sup>1</sup> A	HOMO→LUMO+1	0.66497	292.91	0.0777	4.232 8	0.1746
$4^{1}A$	$HOMO \rightarrow LUMO+3$	0.47448	281.13	0.0025	4.410 2	0.1831
5 <sup>1</sup> A	HOMO→LUMO+2	0.50302	280.26	0.0037	4.423 8	0.1811
6 <sup>1</sup> A	HOMO→LUMO+4	0.65231	274.22	0.0138	4.521 3	0.1881

**Table S10.** Energy Components (in kcal/mol) of The Three  $NH_4^+/5$ -hydroxyindole ComplexesCalculated Using The Kitaura-Morokuma (KM) and The Reduced Variational Space (RVS) EnergyDecomposition Analyses at The HF/6-31G(d) and The HF/6-311+G(d,p) Levels of Theory.

		HF/6-31G(d)		HF/6-311+G(d,p)			
Energetic components <sup><i>a</i></sup>	<b>Gp</b> -complex	Gm- complex	At- complex	<b>Gp</b> -complex	Gm- complex	At- complex	
КМ							
$\Delta E_{\mathrm{ES}}$	-11.34	-10.86	-1.52	-11.69	-12.13	-1.39	
$\Delta E_{\mathrm{EX}}$	10.37	11.99	0.98	12.20	14.71	1.19	
$\Delta E_{ m PL}$	-9.0	-8.32	-4.18	-43.30 <sup>b</sup>	nd <sup>c</sup>	-6.27	
$\Delta E_{ m CT}$	-3.79	-3.50	-1.41	-3.52	-3.51	-1.16	
$\Delta E_{ m MIX}$	1.49	1.68	0.55	35.03 <sup>b</sup>	nd	2.41	
BSSE	0.76	0.80	0.30	0.41	0.38	0.17	
$\Delta E_{\mathrm{TOT}}$	-11.51	-8.21	-5.28	-10.87	nd	-5.05	
RVS							
$\Delta E_{\mathrm{CEX}}$	-0.97	1.13	-0.54	0.51	2.58	-0.20	
$\Delta E_{ m PL}$	-8.04	-6.72	-3.91	-9.30	-7.88	-4.34	
$\Delta E_{ m CT}$	-2.69	-2.88	-0.90	-1.98	-2.23	-0.55	
BSSE	-0.29	-0.25	-0.17	-0.23	-0.24	-0.10	
$\Delta E_{ m TOT}$	-11.99	-8.72	-5.52	-11.0	-7.77	-5.19	

<sup>*a*</sup> ES is the electrostatic term, PL is the polarization term, EX is the exchange repulsion, and CT is the charge transfer term. MIX term accounts for the coupling term, which is calculated by the difference between the SCF energy and the summation of the ES, PL, EX and CT contributions. BSSE is the basis set superposition error. For RVS, the ES and EX are included in the same term CEX.

<sup>b</sup> Overestimation of the energy. See text for explanations.

<sup>c</sup> no determination due to the eigen values that did not converged for calculation of this energetic term.