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# Aromaticity of nucleic acid bases

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## Abstract

3D shape and the resulting physicochemical properties of double-helical DNA/RNA structures are determined not only by individual nucleobases, but also by their additive intermolecular interactions. Energetic contribution from aromatic π-π stacking to the stabilization of DNA/RNA is not small and sometimes even comparable to that from H-bonding. The basis of the stacking interactions lies in the π-electron structure of individual nucleobases, which can be described by various aromaticity indices. Heteroatoms and exocyclic functional groups make the electronic structure of nucleobases different from aromatic hydrocarbons. Consequently, the cyclic π-electron delocalization is not the only factor responsible for the relative stability of their tautomers. This Review puts the spotlight on interplay between aromaticity of purine and pyrimidine nucleobases and their tautomeric preferences, as well as on the effects of different noncovalent interactions (hydrogen bonding, metal ion coordination, and π-π stacking) on π-electron delocalization of five- and six-membered rings in individual nucleobases and their complexes.



**This article is categorized under:**

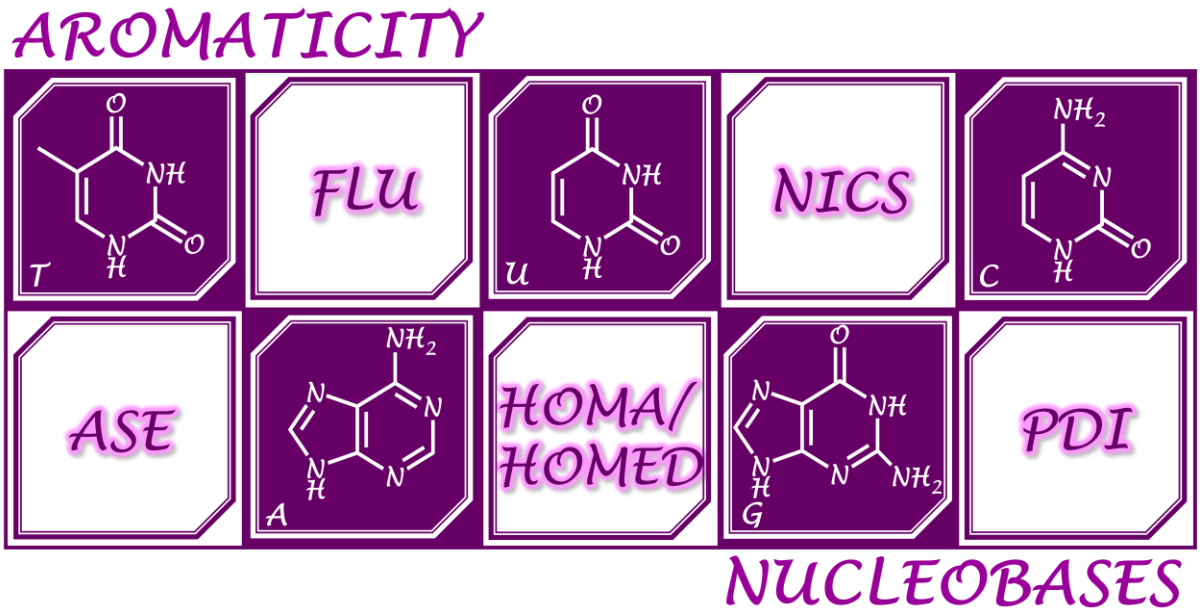
Electronic Structure Theory > Density Functional Theory

Structure and Mechanism > Molecular Structures

**KEYWORDS**

aromaticity, adenine, guanine, cytosine, thymine, uracil


## Graphical/Visual Abstract and Caption



## 1. INTRODUCTION

DNA bases are usually classified as derivatives of two parent compounds – purine and pyrimidine. Although nucleobases within each group have the same skeleton and look quite similar, exocyclic functional groups in the six-membered ring are a key feature responsible for their properties and biological individuality.[1] In biological systems, all nucleobases are usually present in canonical forms, which are more preferred to create Watson-Crick base pairs,[2] i.e. adenine-thymine (AT) and guanine-cytosine (GC). However, the presence of their rare tautomers under certain circumstances and in certain environments cannot be excluded.[3,4] Changes in π-electron delocalization in different tautomeric forms often explain particular tautomeric preferences. The description of relationships between tautomeric equilibria and π-electron delocalization for different systems, including nucleobases, has been done in the review by Raczyńska et al.[5] The main point is that for cyclic molecules without exocyclic groups a π-electron delocalization plays a primary role in the tautomeric preference, while for complex systems more internal and external factors are decisive for stability of the dominant tautomer. Moreover, in DNA/RNA helices nucleobases participate in intermolecular interactions, which may also lead to substantial changes in their electronic structure and, in consequence, to changes in their physicochemical properties. In this viewpoint, the knowledge of the changes in the electronic structure of nucleobases is of the utmost importance.

Electronic structure of molecules is usually considered in terms of σ- and π- electron structures. In the case of conjugated π-electron systems, including nucleobases, the sigma core is assumed to be less flexible, while the π-electron system is more sensitive towards various types of perturbations and therefore is very likely responsible for the resulting changes in molecular properties. Aromaticity, in its classical view, can be considered as a property of cyclic π-electron systems, and currently is well described for a large variety of molecules by energetic, [6] geometric,[7] magnetic,[8] and electronic[9] descriptors. Although there is some general trend in correlations between different aromaticity indices, it was found that aromaticity is a statistically multidimensional phenomenon, and different aromaticity criteria for a limited group of compounds may not correlate with each other.[10,11,12,13,14,15] Application of a given aromaticity index to a series of structurally similar species should lead to reliable results if the same level of computation is maintained for all systems. Some authors recommend to use a set of aromaticity descriptors based on different properties instead of a single index to get more trustworthy conclusions.[16,17] Besides, aromaticity indices are very useful in case of analysing changes in aromaticity due to impact of some intramolecular (e.g. effect of functional group) or intermolecular (e.g. H-bonding, π-stacking, etc.) interactions. In particular, consideration of the interactions with metal cations and hydrogen bonding between nucleobases seems to be the most attractive, because one of the possible mechanisms of DNA mutation[18] is associated with stabilization of rare nucleobases' tautomers by metal ions and mismatch in the base pairing.

At the beginning, the term aromaticity and its meaning appeared for cyclic π-electron hydrocarbons. In 1931, Hückel linked the number of π-electrons and unusual stability of benzene.[19,20,] Later the Hückel (4N+2) π-electrons rule (N is a positive integer, N=1 for benzene) for determining aromaticity of planar cyclic hydrocarbons was formulated.[21] Subsequently, the aromaticity concept has been applied to heterocyclic organic compounds. However, heteroatoms differ from carbon atom by electronegativity and valence. In addition, heteroatoms may include not only σ- and π-orbitals

(characteristic for CC bonds), but also lone electron pairs or electron vacancies. In conjugated carbocycles, every $sp^2$ hybridized carbon atom contributes one electron to the π-electron system. In nucleobases, along with the carbon atom there are two types of nitrogen atoms: (i) imine type (e.g. in pyridine), which contributes one $2p_z$ electron to the cyclic π-electron system, and (ii) amine type (e.g. in pyrrole) with a lone pair involved in aromatic system. Besides, exocyclic functional groups can also participate in π-electron delocalization throughout the system.

## 2. QUANTITATIVE CRITERIA OF AROMATICITY

### 2.1 Energy-based approaches

The resonance energy (RE) introduced by Pauling et al.[22,23] can be estimated as the difference between energy of benzene and energy of its olefinic analog, which could be identified with the Kekulé structure of benzene with localized double and single bonds (eq. 1).

$$\mathrm{RE} = -\Delta H_a^{\circ}(\mathrm{benzene})_g - [3E(\mathrm{C{=}C}) + 3E(\mathrm{C\text{-}C}) + 6E(\mathrm{C\text{-}H})] \quad (1)$$

where $\Delta H_a^{\circ}(\mathrm{benzene})_g$ denotes a heat of atomization of gaseous benzene, and $E$ is an energy of the particular bonds (double C=C, single C-C and C-H) estimated from the heat of atomization of ethene, ethane, and methane.

More generally, RE is a difference in energies of a given molecule with π-electron delocalization (i.e. the aromatic one) and the reference system with (completely) localized double and single bonds. In more advanced approaches, when isodesmic[24,25] or homodesmotic[26,27] reactions are used, the energy difference is called aromatic stabilization energy (ASE), for details see Cyrański.[6] When the RE or ASE concepts are applied to heterocyclic systems, a special care should be taken because complications arise in building proper reference systems.

### 2.2 Geometry-based approaches

It was observed very early that the CC bond lengths in aromatic molecules are in between the typical values for single and double bonds.[28] Julg and François were the first[29] who took advantage of this observation and defined aromaticity index $A_j$. Unfortunately, its limitation only to hydrocarbon systems was a big disadvantage. The next step was taken five years later. The mean value of all bond lengths $R_{av}$ was replaced by a conceptual parameter named as optimal bond lengths, $R_{opt}$, expected to be realized in fully aromatic compounds.[30] The new aromaticity index HOMA (Harmonic Oscillator Model of Aromaticity) reads as eq. 2:

$$\mathrm{HOMA} = 1 - \frac{\alpha}{n}\sum_{i}^{n}(R_{\mathrm{opt}} - R_i)^2 \quad (2)$$

where α is a normalization constant chosen to give HOMA = 0 for a model non-aromatic system and HOMA = 1 for a system where all bonds are equal to $R_{opt}$, $n$ is the number of bonds taken into summation, and $R_i$ are the experimental or computed bond lengths. For example, HOMA=0.998 for benzene, while HOMA=-0.220 for cyclooctatetraene.[31]

Significant progress was associated with the extension of the HOMA index to heterocyclic systems,[32] where the formula for HOMA is presented as eq. (3):

$$\mathrm{HOMA} = 1 - \frac{1}{n}\sum_{i}^{n} \alpha_j (R_{\mathrm{opt,j}} - R_{\mathrm{j,i}})^2 \qquad (3)$$

where subscript *j* denotes the type of the bond, i.e. CC, CN, CO, CP, CS, NN, NO, etc. Values of the $R_{opt,j}$ and $\alpha_j$ parameters for CC and CN bonds are $\alpha_{CC}$ = 257.7 and $\alpha_{CN}$ = 93.52, $R_{opt,CC}$ = 1.388 Å and $R_{opt,CN}$ =1.334 Å, respectively.

HOMA approach was later modified by Raczyńska et al.[33] and named as HOMED (Harmonic Oscillator Model of Electron Delocalization), then by Frizzo and Martins[34] and named as HOMHED (Harmonic Oscillator Model of Heterocycle Electron Delocalization). However, in both cases the basic idea of HOMA index was unchanged.

A very important advantage of HOMA-like approaches is the possibility of using them not only for particular ring(s) or the whole π-electron system, but also for any planar π-electron fragment of the molecule, thus providing information on its π-electron delocalization.

Values of the above-mentioned geometry-based aromaticity indices for five- and six-membered heterocycles, published since 2000, can be found in the review by Krygowski et al.[7]

**2.3 Magnetism-based aromaticity descriptors**

For over 20 years, the most commonly used magnetic criterion of aromaticity has been the nucleus independent chemical shift (NICS) introduced by Schleyer in 1996.[35] It does not require a reference compound and is easy to compute by quantum chemical programs. NICS was defined as the negative value of the absolute shielding calculated in the geometric center of the ring system. Now it is also calculated at other points[36] inside or around molecules.[16] It is well known that the magnetic response properties are tensors[37] and their trace, for a number of reasons, may be very different.[38] Schleyer recommended a component corresponding to the principal axis perpendicular to the ring plane, $NICS_{zz}$, as a preferred measure for characterizing the aromaticity of π-system.[39] Another type of this index is NICS value at 1 Å above molecular plane named NICS(1).[16] The more negative NICS value, the higher aromatic character of the ring. NICS values may be calculated at various levels of computation but the data obtained at different levels should not be compared. However, comparison is reliable when for a given series of compounds NICSs are calculated within the same computational approach.[40] Unlike magnetic susceptibility exaltation, Λ,[41,42] all NICS indices describe local aromaticity, i.e. the aromaticity of a particular ring. If NICS is used as a measure of global aromaticity in polycyclic system through summation of NICS values of the individual rings, there is some "double-counting" of the magnetic contribution from the separate rings.[43] However, excellent correlations between ΣNICS(1) and ASE values, as well as between $\Sigma NICS(1)_{zz}$ and Λ were reported for some sets of compounds.[44] Very recently, NICS-XY-Scan-based additivity scheme for aromaticity of a wide range of polycyclic aromatic compounds even with B, N, O, and S heteroatoms has been proposed.[45,46]

The state of the art of magnetic criteria for aromaticity was recently presented by Gershoni-Poranne and Stanger.[8,47]

**2.4 Electron delocalization indices**

In the last decades, popular indices of aromaticity based on electronic delocalization measures have appeared. The *para*-delocalization index (PDI)[48] proposed in 2003 represents the first index that could be classified unquestionably in the category of electronic indices. The PDI is calculated using the delocalization index (DI)[49] as defined in the framework of the Quantum Theory of Atoms in Molecules (QTAIM) of Bader.[50] The PDI is an average of all DI of *para*-related carbon atoms in a given six-membered ring. The DI value between atoms A and B, $\delta$(A,B), for monodeterminantal closed-shell wavefunctions, is obtained from Eq. 4,

$$\delta(A,B) = 4 \sum_{i,j}^{occ.MO} S_{ij}(A) S_{ij}(B) \tag{4}$$

The summation in Eq. 4 runs over all occupied molecular orbitals. $S_{ij}(A)$ is the overlap between molecular orbitals *i* and *j* within the basin of atom A. DIs in Eq. 4 reduce to Wiberg-Mayer bond orders[51] if the integrations over atomic basins are replaced by a Mulliken-like partitioning of the corresponding integrals. In this case, the PDI is called average two-center index (ATI).[52]

The aromatic fluctuation index (FLU) measures the uniformity of the electron delocalization along the molecular ring and its bonding difference with respect to some aromatic reference,[53] using the following equation:

$$FLU(\mathcal{A}) = \frac{1}{N} \sum_{i=1}^{N} \left[ \left( \frac{V(A_i)}{V(A_{i-1})} \right)^{\alpha} \left( \frac{\delta(A_i, A_{i-1}) - \delta_{ref}(A_i, A_{i-1})}{\delta_{ref}(A_i, A_{i-1})} \right) \right]^2 \tag{5}$$

where the ring considered is formed by atoms in the string {***A***} = {$A_1$, $A_2$, … $A_N$}, $A_0 \equiv A_N$ and the atomic delocalization is defined by Eq. 6,

$$V(A) = \sum_{A \neq B} \delta(A,B) \tag{6}$$

and $\alpha$ is a function that ensures that the ratio of electronic delocalizations in Eq. 5 is always greater or equal to 1,

$$\alpha = \begin{cases} 1 & V(A_i) > V(A_{i-1}) \\ -1 & V(A_i) \leq V(A_{i-1}) \end{cases} \tag{7}$$

The reference values of C-C and C-N bonds are taken from benzene and pyridine in their ground state. FLU (Eq. 5) is close to 0 in aromatic species and increases as the molecule departs from the aromatic reference.

A full account of electronic indices of aromaticity is provided in Ref. 9, along with their comparison with other aromaticity indices.[54,55,56]

## 3. AROMATICITY OF THE NUCLEIC ACID BASES

Two nucleic acid types, DNA and RNA, contain five nucleobases: adenine, cytosine, guanine, thymine, and uracil. All of them are π-electron heterocyclic compounds that exhibit partial aromatic character. First study of nucleobases aromaticity[57] showed that purine nucleobases are significantly more aromatic compared to pyrimidine ones and revealed the following sequence of decreasing aromaticity: adenine > guanine > cytosine > thymine ≈ uracil. The aforementioned sequence based on structural aromaticity indices were later confirmed by electronic and magnetic aromaticity indices.[58,59] In general, the aromaticity of the nucleobases decreases significantly with the number of exocyclic groups attached to the ring with double bonds. Some aromaticity indices for five- and six-membered rings of nucleobases are collected in Table 1.

**Table 1.** Aromaticity indices for five- and six-membered rings of nucleobases.

| | Adenine | Guanine | Cytosine | Thymine | Uracil |
|---|---|---|---|---|---|
| HOMA5[a] | 0.860 | 0.871 | - | - | - |
| HOMA6[a] | 0.976 | 0.682 | 0.672 | 0.466 | 0.503 |
| HOMED5 | 0.883[c] | - | - | - | - |
| HOMED6 | 0.995[c] | - | 0.785[d] | - | 0.60[e] |
| FLU5[b] | 0.028 | 0.025 | - | - | - |
| FLU6[b] | 0.012 | 0.045 | 0.044 | 0.079 | 0.099[f] |
| PDI[b] | 0.060 | 0.032 | 0.042 | 0.026 | 0.026[f] |
| NICS(0)5[a] | -11.9 | -12.4 | - | - | - |
| NICS(1)5[a] | -7.2 | -9.7 | - | - | - |
| NICS(0)6[a] | -9.8 | -3.1 | -1.3 | -1.5 | -1.2 |
| NICS(1)6[a] | -8.9 | -3.8 | -3.2 | -2.2 | -1.9 |

[a)] Data taken from ref. 57. [b)] Data taken from ref. 59. [c)] Data taken from ref. 60. [d)] Data taken from ref. 61. [e)] Data taken from ref. 62. [f)] Data taken from ref. 63.

Nucleobases are derivatives of well-known heteroaromatic systems, such as purine and pyrimidine, which in turn might be related to other simple nitrogen-containing heterocycles and even to benzene. The effect of such structural modifications on π-electron delocalization of benzene and pyrrole has been studied by natural resonance theory (NRT) and nucleus independent chemical shift (NICS).[64] First, the endo-substitution of benzene (NICS=-9.7 ppm) with nitrogen atom(s) decreases aromaticity of the ring by 1.5 ppm for pyridine (-8.2 ppm) and by 3.2 ppm for pyrimidine (-6.5 ppm). Second, the effect of exocyclic substituents can be seen for their amino- and keto-derivatives. The illustrative example is sequence: pyrimidine (-6.5 ppm), 2-aminopyrimidine (-4.7 ppm), and 2-amino-pyrimidone (-1.8 ppm). Very substantial decrease in aromaticity is observed if carbonyl group participates in π-electron delocalization. Nucleobases contain at least one type of functional group. In particular, adenine contains an amino group, thymine and uracil contain carbonyl groups, whereas guanine and cytosine contain both amino and carbonyl groups. Appearance of the amino group has a small effect on the aromaticity of rings, while presence of double-bonded carbonyl group induces an outflow of electron density from the ring and leads to significant decrease in aromaticity, as observed in uracil (-1.2 ppm) and thymine (-1.6 ppm). Third,

the aromaticity changes due to fusion of rings. For example, in adenine, 4-aminopyrimidine unit increases its aromaticity measured by NICS from -5.5 to -7.3 ppm, whereas imidazole becomes less aromatic changing NICS value from -14.0 to -11.9 ppm. Similar observations can be done using the HOMA aromaticity index.[65]

### 3.1 Modified nucleobases

A strategy to expand the natural nucleobases by inclusion of a benzene moiety was proposed to enlarge the genetic alphabet. Such modified bases combine the hydrogen bond properties of the natural bases with the hydrophobicity of the benzene ring. Pyrimidine nucleobases were modified by addition of benzene ring, whereas purine bases were modified by insertion of the benzene ring between two heterocyclic components of the base, leading to the so-called x-bases, as shown in Figure 1. The influence of the insertion/addition of a benzene ring to the natural nucleobases on the local aromaticity of adenine, guanine, cytosine and thymine bases was examined by HOMA, NICS, FLU, and PDI indices.[59] A good correspondence between the different indices has been documented. In particular, rings with more negative NICS values also have larger HOMA and PDI indices and lower FLU values. The obtained HOMA and FLU values revealed as a general trend that addition or insertion of the benzene ring reduces the aromatic character of the neighboring heterocyclic ring(s). Interestingly, the inserted or fused benzene ring becomes also less aromatic, but not as much as observed in naphthalene, where HOMA decreases by 0.193.[66]

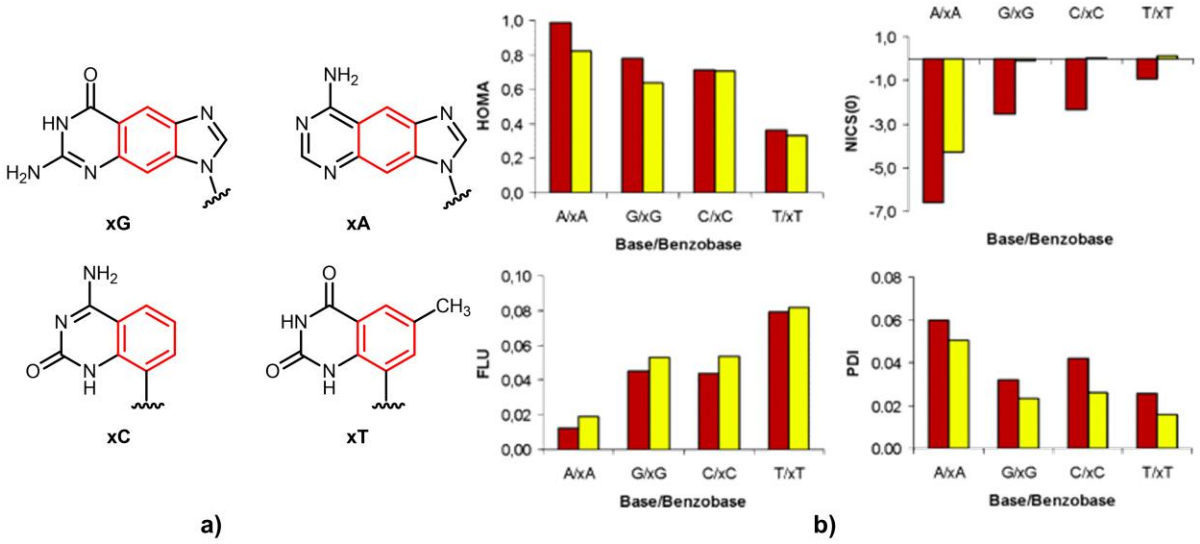



**Figure 1.** Structures of expanded nucleobases (a) and comparison of the local aromaticity descriptors for the six-membered ring of natural (red) and benzo-fused (yellow) bases (b). Adapted with permission from Ref. 59. Copyright (2006) American Chemical Society.

Very recently, aromaticity in 2-pyridone and its sulfur and selenium analogs as representative heterocyclic models of canonical nucleobases were studied.[67] It was shown that there is a significant reduction of aromaticity upon replacement of exocyclic carbonyl oxygen with S and Se; $NICS(1)_{zz}$ is -10.03, -7.16 and -5.84 ppm, respectively. This was explained by the lower electronegativity of S and Se compared to O, which makes the C=X (X = S, Se) bond less polarizable. As a result, the separation of charges between C and X significantly decreases, and the lower value of positive charge density in

the carbon center prevents the delocalization of electrons. However, in their dimers, the high polarization of the C=X bond leads to enhanced delocalization of electrons in the rings.

### 3.2 Aromaticity and tautomerism

*3.2.1 Adenine*

Adenine contains two labile protons that lead to existence of twenty-three prototropic tautomers: nine with the amino group and fourteen with the imino group. All of them are discussed in detail by Raczyńska and co-workers.[60,68,69] They considered adenine as a unit consisting of two subunits, imidazole and 4-aminopyrimidine; as well as derivative of purine. Their different aromatic character estimated by HOMED index in the gas phase and in water are presented in Figure 2. It should be noted that in this case *exo* C-N bond (with *exo* -$NH_2$ or =NH groups) was also included in the HOMED calculations. In this way, the π-electron delocalization estimated by HOMED applies to the whole π-electron system of a given molecule. CH and NH labels indicate protonation at carbon or nitrogen atoms, respectively. All CH tautomers exhibit a clearly lower aromaticity than the NH ones. Interestingly, in all cases the mean HOMED values show a slightly greater aromaticity estimated in a water solution than in the gas phase. The good correlations between HOMED values and relative energies of tautomers[69] indicate a decrease of aromatic character with stability decrease for both isolated and solvated molecules (Figure 2). The correlations are better for imidazole and purine than for 4-aminopyrimidine and adenine due to the presence of an exocyclic functional group (-$NH_2$) in the latter.

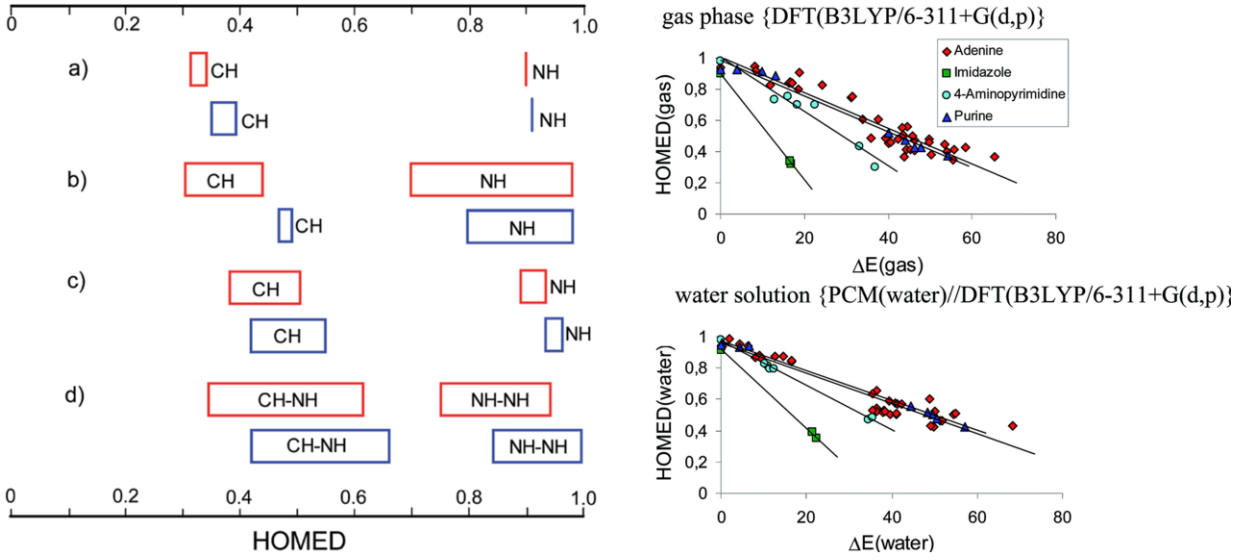



**Figure 2**. *Left*: Variability of HOMED values for tautomers of imidazole (a), 4-aminopyrimidine (b), purine (c), and adenine (d) in the gas phase (red color) and in water solution (blue color). *Right*: Correlations between HOMED indices and relative energies of tautomers. Adapted from Ref. 69 with permission from The Royal Society of Chemistry.

A detailed study of aromaticity of the four most stable tautomers of adenine (Scheme 1) was also carried out by NICS and HOMA indices.[70] In case of HOMA, both local (for five- and six-membered rings) and global aromaticity indices were calculated. Table 2 shows the obtained HOMA values for

purine[71] and adenine. In purine, the aromaticity of both rings changes with the stability of tautomers. The most stable tautomers are the most aromatic. Similar tendencies are observed in the case of adenine tautomers, however, the presence of the exocyclic amino group results in a slight deviation from tendency as compared to the purine tautomers. Two of the most stable adenine tautomers (7H and 9H), with the protonation site in the five-membered ring, exhibit higher aromaticity of the six-membered ring than the five-membered one according to HOMA (Table 2). The opposite is true for two other tautomers with protonation site in the six-membered ring. It is directly connected with location of the aromatic sextet as shown in Scheme 1. If the NH unit is located in a five-membered ring, both rings contain 6π electrons and fulfill the Hückel 4N+2 rule (tautomers 7H and 9H). If the NH unit is in a six-membered ring, five-membered ring contains 5π electrons and six-membered ring contains 7π electrons, so such rings (in 3H and 1H) do not fulfill the Hückel 4N+2 rule.[72] It should be noted that the amino group at the position 6 increases aromaticity of both five- and six-membered rings compared to purine in all four tautomers according to HOMA values. Among the two types of NICS presented in Table 2, only NICS(0) correlates well with HOMA for purines and in an acceptable way for adenine. Substituents at the C2 or C8 position of purine and adenine tautomers slightly changes the relative stability of the tautomers due to specific interactions between them and neighbouring N lone electron pair or NH unit.[73]

**Scheme 1.** Structures of most stable amino tautomers of adenine.

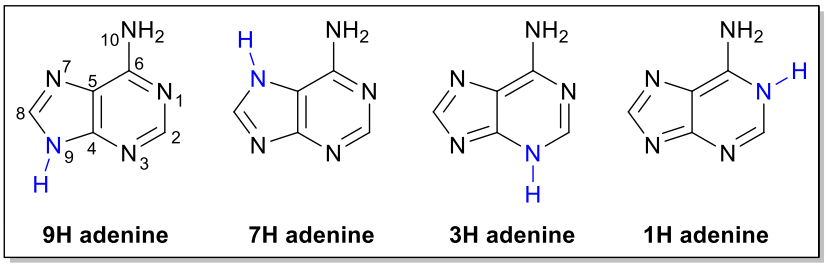



**Table 2**. Relative energies, $\Delta E_{rel}$, HOMA and NICS indices for selected tautomers of purine,[a] adenine,[b] and guanine.[c]

| | $\Delta E_{rel}$ /kcal mol$^{-1}$ | HOMA5 | HOMA6 | HOMAtot[d] | NICS(0)5 | NICS(0)6 | NICS(1)$_{zz}$5 | NICS(1)$_{zz}$6 |
|---|---|---|---|---|---|---|---|---|
| **Purine** | | | | | | | | |
| 9H | 0.00 | 0.834 | 0.976 | 0.915 | -11.7 | -8.8 | -26.2 | -29.9 |
| 7H | 3.97 | 0.832 | 0.966 | 0.914 | -11.7 | -9.4 | -26.8 | -31.1 |
| 3H | 9.88 | 0.772 | 0.819 | 0.854 | -10.5 | -8.0 | -30.7 | -24.1 |
| 1H | 13.09 | 0.667 | 0.665 | 0.778 | -9.9 | -7.5 | -31.2 | -23.7 |
| **Adenine** | | | | | | | | |
| 9H | 0.00 | 0.869 | 0.984 | 0.926 | -11.8 | -7.2 | -25.8 | -22.2 |
| 7H | 7.68 | 0.857 | 0.982 | 0.923 | -11.6 | -8.7 | -26.1 | -25.6 |
| 3H | 7.80 | 0.923 | 0.920 | 0.928 | -11.6 | -6.7 | -31.5 | -17.8 |
| 1H | 17.96 | 0.857 | 0.797 | 0.862 | -10.8 | -6.8 | -31.5 | -18.7 |
| **Guanine** | | | | | | | | |
| 9H,1H amino-oxo | 0.83 | 0.878 | 0.704 | 0.761 | -12.4 | -3.1 | -25.7 | -6.3 |

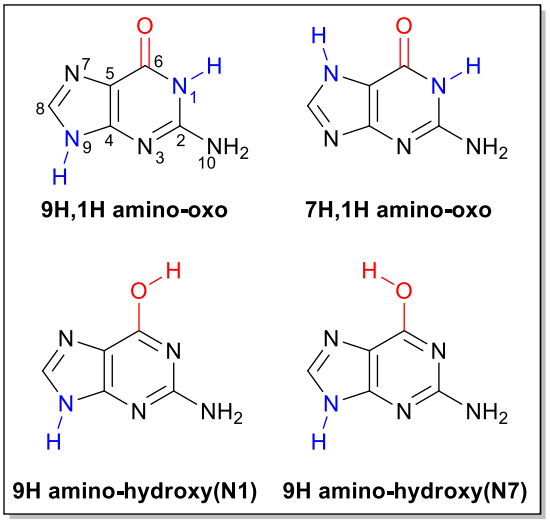
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7H,1H amino-oxo | 0.00 | 0.922 | 0.784 | 0.831 | -12.3 | -3.8 | -26.6 | -7.7 |
| 9H amino-hydroxy(N1) | 1.59 | 0.846 | 0.981 | 0.916 | -11.1 | -6.9 | -24.0 | -17.8 |
| 9H amino-hydroxy(N7) | 2.02 | 0.856 | 0.981 | 0.918 | -11.3 | -6.8 | -24.0 | -17.8 |

[a] Data taken from ref. 71. [b] Data taken from ref. 70. [c] Data taken from ref. 74. d) all bonds in purine system are taken into account.

*3.2.2 Guanine*

Among all nucleobases, guanine has the largest number of tautomers, namely 36 including rotamers. The thermodynamics and kinetics of intramolecular proton transfer were computationally studied at the MP2 and CCSD(T) levels.[75] The obtained results revealed that the four lowest tautomeric structures are located within *ca.* 1 kcal/mol (Scheme 2). All of them were observed experimentally.[76,77]

**Scheme 2.** The most stable tautomers of guanine.




Guanine consists of fused pyrimidine and imidazole rings with two functional groups (oxo/hydroxy and amino/imino). Study of aromaticity in its most stable tautomers showed that changes in aromaticity of imidazole ring are negligible, whereas aromaticity of pyrimidine ring can change noticeably.[74] The difference originates from the effects caused by functional groups attached to the pyrimidine ring. The carbonyl group has the largest effect on aromaticity (Table 2). It induces an outflow of the electron density from the ring and causes a dramatic decrease in aromaticity (ΔHOMA6 = 0.3). A number of specific intramolecular interactions between the functional groups and ring heteroatoms leads to a lack of correlation between the relative stability of guanine tautomers and their aromaticity in comparison with adenine, where the stability of its tautomers is consistent with the aromaticity characteristics.

*3.2.3 Uracil*

Uracil can exist in 13 NH tautomers and 5 rare CH tautomers.[62] It is the least aromatic nucleobase with NICS = -1.2 ppm and HOMA6=0.5. Important to note that its most stable tautomer with two carbonyl groups is energetically more stable than all other tautomers by >10 kcal/mol, as shown in

Table 3.[78] The most favourable tautomers of uracil are presented in Scheme 3. For **u1**, the lone electron pair of each nitrogen atom is n–π cross-conjugated with π-electrons of the ring and *exo*-C=O group. The additional n–π cross conjugation with the lone electron pair of the oxygen atom in exo-OH group takes place for the NH-OH tautomers (**u2**, **u3**). For di-OH tautomers (**u4**, **u5**), six π-electrons are conjugated in the ring and additionally with the lone electron pair of each oxygen atom in exo-OH groups. Thus, π-electrons are strongly delocalized in the ring and throughout the tautomeric system, including the exo-OH groups. The CH tautomers are the least delocalized structures because the C-ring atom has $sp^3$ hybridization and $CH_2$ group is only σ–π cross-hyperconjugated with π-electrons of the ring and *exo*-C=O group. The additional n–π cross conjugation is possible between lone electron pair of the oxygen atom in *exo*-OH group and π-electrons of the ring. As in the case of guanine tautomers, there is no direct relationship between stability of uracil tautomers and their π-electron delocalization (Figure 3). Most likely intramolecular interactions as shown in Scheme 3 stabilize one tautomer and destabilize the others.

**Scheme 3.** The most stable tautomers of uracil and intramolecular interactions in them.

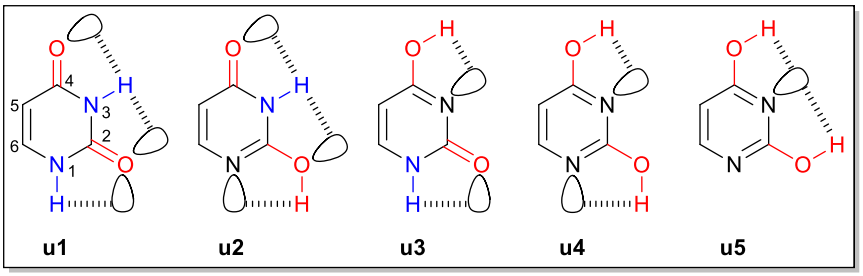



**Table 3**. Relative energy, $E_{rel}$, HOMA and NICS aromaticity indices for selected tautomers of uracil,[a] thymine[b] and cytosine.[c]

| Tautomer | $E_{rel}$/kcal/mol[d] | HOMA6 | NICS(0) | NICS(1) | NICS(1)zz |
|---|---|---|---|---|---|
| **Uracil** | | | | | |
| u1 | 0.00 | 0.505 | - | - | - |
| u2 | 11.10 | 0.637 | - | - | - |
| u3 | 12.00 | 0.714 | - | - | - |
| u4 | 12.80 | 0.990 | - | - | - |
| u5 | 14.00 | 0.994 | - | - | - |
| **Thymine** | | | | | |
| t1 | 0.00 | 0.490 | -1.5 | -2.2 | -2.1 |
| t2 | 10.83 | 0.629 | -2.8 | -4.3 | -8.2 |
| t3 | 12.38 | 0.718 | -2.7 | -4.2 | -7.5 |
| t4 | 13.02 | 0.984 | -7.2 | -8.8 | -20.9 |
| t5 | 14.17 | 0.987 | -7.3 | -8.9 | -21.1 |
| **Cytosine** | | | | | |
| c1 | 0.00 | 0.699 | -1.3 | -3.3 | -5.7 |
| c2 | 0.54 | 0.976 | -5.3 | -7.5 | -18.1 |
| c3 | 1.29 | 0.981 | -5.4 | -7.6 | -18.3 |
| c4 | 1.81 | 0.534 | -0.6 | -1.2 | 0.5 |

| | | | | | |
|---|---|---|---|---|---|
| c5 | 3.56 | 0.512 | -0.6 | -1.2 | 0.4 |
| c6 | 6.97 | 0.800 | -1.8 | -3.6 | -6.7 |

[a] Data taken from ref. 79. [b] Data taken from ref. 80. [c] Data taken from ref. 81. [d] Energy data for uracil taken from ref. 78.

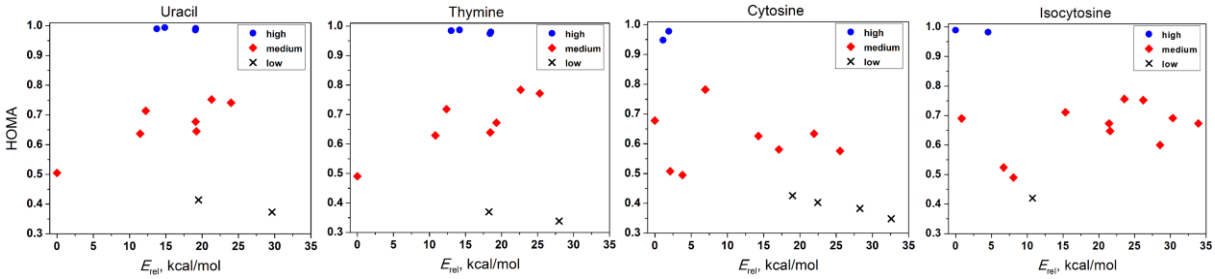


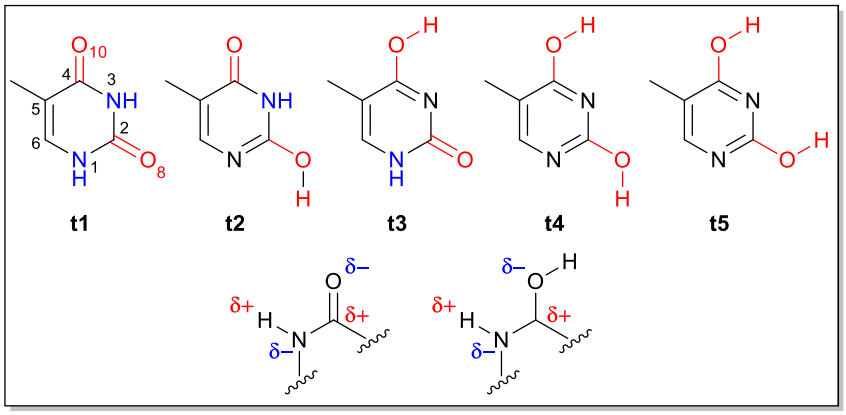

**Figure 3.** Relationship between aromaticity index HOMA6 and relative energy of uracil, thymine, cytosine, and isocytosine tautomers. Blue circles, red diamonds and black crosses denote tautomers with high, medium and low aromaticity. Data taken from refs 79 and 80, respectively.

Interesting correlations between HOMA values and stability of anionic forms of different 5,6-substituted uracil derivatives have been recently reported.[82,83] In the gas phase, the N1 anions are more stable and aromatic than the N3 ones. In aqueous solution, the solvent more efficiently stabilizes the more polar N3 anions and aromaticity of N3 anions increases considerably, making both anions almost equally stable.

*3.2.4 Thymine*

Thymine is also known as 5-methyluracil and can also exist in 13 NH tautomeric forms.[80] Thymine differs from uracil only by the presence of the methyl group, but similarly contains two carbonyl groups, which are the reason for the low aromaticity of the six-membered ring. Similar to uracil, the four most aromatic tautomers with HOMA6 > 0.9 are not the most stable. They have two *exo*-OH groups leading to a typical aromatic π-sextet in the ring. Contrary to that, the most stable tautomer in the gas phase, as well as in solution and the solid state,[84,85] is the 1H,3H-diketo form (or di-NH form, **t1**), which contains two carbonyl groups. These groups participate in π-electron delocalization. When considering the five most stable tautomers (Scheme 4) in terms of aromaticity and stability, it appears that the six-membered ring of the most stable tautomer (**t1**) contains formally 8 π-electrons, next two ones (**t2**, **t3**) – 7 π-electrons, and the least stable tautomers (**t4**, **t5**) – 6 π-electrons.

**Scheme 4.** Structures of the most stable thymine tautomers and possible stabilizing bond dipole interactions.

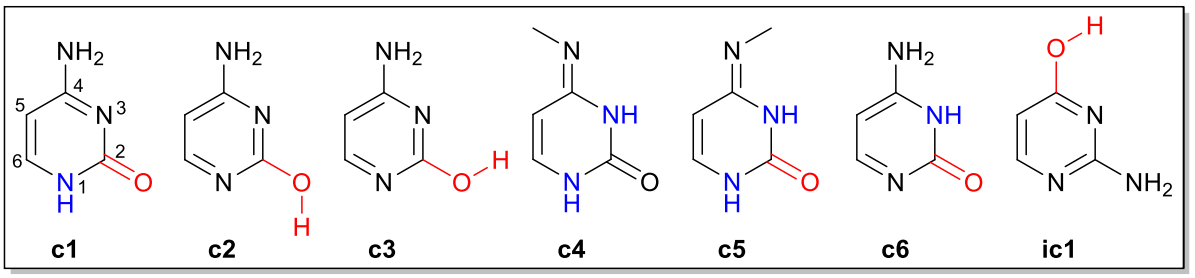



According to NBO analysis, the number of π-electrons in the ring are between 6.98 (**t1**) and 6.24 (**t4**).[80] Consequently, the ring of the most stable tautomer contains more than 6 π electrons and does not follow the (4N+2) Hückel rule. HOMA and NICS values in Table 3 confirm its low aromatic character. In general, the stability of thymine tautomers does not directly correlate with their aromatic character (Figure 3). The key issue is presence of functional groups and their electronic character. In particular, changes of the imine-type of N atom, which contributes with only one $2p_z$ electron to the π-electron system, to the amine-type, with a pair of π-electrons, significantly increase the aromaticity of the ring. Consequently, transfer of protons towards C=O groups changes their electronic character and makes the ring more aromatic.

Why tautomer with 8 π-electrons and low aromaticity is the most stable? It was shown that stabilization of the 1H,3H-diketo form is mostly achieved by the extra intramolecular interactions between $N^{\delta-}H^{\delta+}$ and $C^{\delta+}O^{\delta-}$ bond dipoles (Scheme 4).[80]

*3.2.5 Cytosine*

Cytosine is a heterocyclic system that contains two nitrogen atoms in the ring. Additionally, cytosine contains one exocyclic amino/imino group and one exocyclic oxo/hydroxy group. Cytosine has two labile protons and can form 9 prototropic tautomers, but 21 when all rotamers are included.[61] Rare CH tautomers with $E_{rel}$>20 kcal/mol will not be considered here.




**Scheme 5**. Six most stable cytosine tautomers and the most stable isocytosine tautomer.

The most stable cytosine tautomers are shown in Scheme 5. They have been studied using HOMA and NICS aromaticity indices (Table 3).[81] Two tautomers, **c2** and **c3**, are highly aromatic, since their ring is of (4N+2) π-electron type and hence well fulfils the Hückel rule. In tautomers with the oxo group, i.e. **c1** and **c6**, the aromaticity of the ring is lower since double-bonded groups attached

to the ring decrease its π-electron delocalization. Tautomers **c4** and **c5** have the lowest ring aromaticity since two double-bonded groups (oxo and imino) are attached to the ring and it contains 4N π-electrons. Therefore, it can be concluded that the functional groups involved in prototropic equilibrium play a very important role in ring aromaticity in cytosine tautomers. The protonation of these groups (as in **c2** and **c3**) substantially increases aromaticity of the six-membered ring.

The relative stability of cytosine tautomers depends on various internal and external factors. Similar to other pyrimidine nucleobases, a lack of simple correlation between the relative energy of all tautomers and their aromaticity indices was observed (Figure 3).[79] Most likely intramolecular interactions between exocyclic groups and endocyclic NH units, similar to uracil and thymine, affect the conformational preferences in cytosine tautomers.

*3.2.6 Isocytosine*

Isocytosine is one of the building blocks of the guanine together with imidazole ring. This is functionalized pyrimidine ring with two exocyclic substituents, amino and oxo groups. Presence of labile protons leads to existing of nine prototropic tautomers, which are similar to tautomers of other pyrimidine nucleobases.[79,86] When rotational isomerism of exo-OH group and geometrical isomerism of exo =N-H group are included, twenty-one tautomers are possible.

Relationship between stability of tautomers and their aromaticity was studied in the gas phase and aqueous solution by HOMA and HOMED indices.[79] In the gas phase, the most stable hydroxy-amino tautomer **ic1** (Scheme 5) is the most aromatic, HOMED6 is close to 1. This tautomer contains six π-electrons fully delocalized in the ring. The oxo-amino, hydroxy-imino, and oxo-imino tautomers are less delocalized than **ic1**, with 0.6 < HOMED6 < 0.8. For them, π-electrons and lone pairs of NH unit are π-n conjugated in the ring. Thus, such six-membered rings formally contain 7 or 8 π-electrons, similar to thymine tautomers. All CH tautomers are non-aromatic and possess the lowest HOMED6, which is close to zero.

For isocytosine tautomers, the lack of linear correlation between aromaticity index HOMA and energetic parameters was observed (Figure 3). Thus, electron delocalization is not the only factor that influences the relative stabilities of tautomers for all pyrimidine nucleobases. For example, in aqueous solution, stability of functional groups and their mutual interactions are more important. As a result, in solution the most aromatic **ic1** becomes less stable by 5.5 kcal/mol than the less delocalized oxo-amino form.

**3.3 Aromaticity and interactions**

*3.3.1 Hydrogen bonding*

Nucleobases in DNA and RNA helices interact between themselves in two basic modes: by means of intermolecular H-bonding and *via* stacking interactions. Hydrogen bonding may lead to substantial changes in aromaticity of nucleobases.[70,74,80,81] In a series of papers we analyzed two types of complexes: (i) neutral with HF, and (ii) anionic with $F^-$. All possible active centers (basic and acidic) of particular tautomers were taken into account.

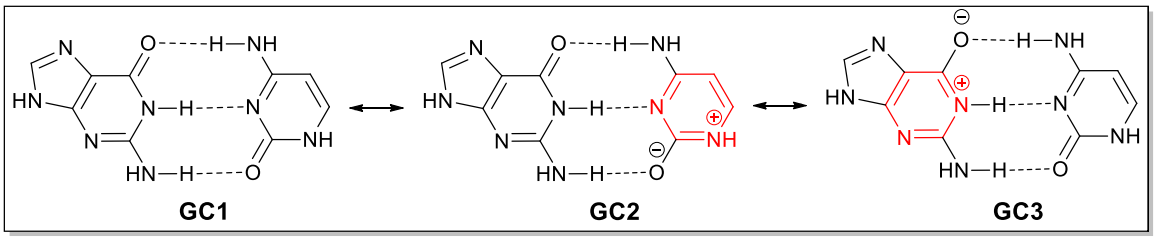
Comparison of aromaticity indices calculated for isolated tautomers and their complexes revealed that the aromaticity of the most aromatic tautomers of all nucleobases is resistant to any kind of intermolecular interactions, whereas the less aromatic tautomers can change their aromaticity up to a quarter of the HOMA index scale. The magnitude of the H-bond effect strongly depends on the site and type of intermolecular interaction. Generally, the strongest interactions cause the largest aromaticity changes. Thus, formation of the neutral H-bonds does not much influence aromaticity of nucleobases. In turn, strong charge-assisted H-bonds can induce perceptible aromaticity changes both in particular rings and in the entire heterocyclic systems. It was found that large changes in aromaticity are caused by H-bond interactions involving functional groups such as amino/imino and oxo/hydroxy, mainly due to modification of their electronic character.

The analysis of the aromaticity of the nucleobases involved in the Watson-Crick pairs showed that aromatic character of the six-membered rings increases as a consequence of H-bond formation (Table 4) in comparison with the free nucleobases (Table 1). For example, an important increase in the aromatic character of the six-membered rings of guanine and cytosine can be understood taking into account the resonance structures of the guanine-cytosine (GC) base pair depicted in Scheme 6. Hydrogen bond interactions in the GC pair stabilize the resonance structures **GC2** and **GC3** with charge separation as compared to **GC1**, and, consequently, the aromaticity of the six-membered ring of guanine increases. Similar resonance structures can be drawn to justify an aromaticity increase in the six-membered ring of thymine. The change in the environment from the gas phase to aqueous solution causes an increase in the aromaticity of the six-membered rings in AT and GC pairs,[87,88] because polar solvent stabilizes the resonance structures with charge separation (Scheme 6).

**Table 4.** Aromaticity indices for Watson-Crick paired nucleobases.[a,b]

| pair | base | HOMA5 | HOMA6 | NICS(1)5 | NICS(1)6 | FLU5 | FLU6 | PDI |
|---|---|---|---|---|---|---|---|---|
| AT | adenine | 0.867 | 0.966 | -9.7 | -8.2 | 0.027 | 0.013 | 0.056 |
| | thymine | - | 0.533 | - | -2.3 | - | 0.076 | 0.017 |
| AU | adenine | 0.867 | 0.965 | -9.7 | -8.2 | - | - | - |
| | uracil | - | 0.566 | - | -2.0 | - | - | - |
| GC | guanine | 0.853 | 0.810 | -9.2 | -4.1 | 0.028 | 0.038 | 0.034 |
| | cytosine | - | 0.715 | - | -3.1 | - | 0.043 | 0.039 |

[a] HOMA and NICS(1) data taken from ref. 57. [b] FLU and PDI data taken from ref. 59.




**Scheme 6**. Possible resonance forms of the guanine-cytosine base pair.

The specific interactions between guanine (G) and 3,4-ethylenedioxythiophene (EDOT) was examined by Teixeira-Dias et al.[89,90] They found seven possible minima. The most stable conformer

corresponds to the isomer with $N_1$–H···$O_{EDOT}$ hydrogen bond. Formation of this hydrogen bond promotes the importance of the resonance structure of guanine similar to that observed in **GC3** (Scheme 6) with increased aromaticity of the six-membered ring. Indeed, the HOMA6 value changes from 0.679 in free guanine to 0.734 in G:EDOT complex. This hydrogen bond can be classified as a resonance-assisted hydrogen bond.

The relationship between changes in the aromatic character of heterocycles and their hydrogen bonding capabilities was recently described as the phenomenon of aromaticity-modulated hydrogen bonding (AMHB).[91] A general idea is that H-bonding interactions increase cyclic (4N+2) π-electron delocalization in heterocycles. H-bonds are strengthened because of enhanced aromatic character in the resulting hydrogen-bonded complex, whereas those H-bonds that disrupt aromaticity are weakened due to reduced aromatic character in the hydrogen-bonded complex. Based on the Hückel definition of π-aromaticity for closed-shell planar rings, not all of the six-membered rings of nucleobases are formally "aromatic" due to lack of a cyclic delocalization of (4N+2) π-electrons. However, during formation of hydrogen-bonded pairs, their π-electrons are polarized that results in increased cyclic (4N+2) π-electron delocalization in the six-membered rings. For example, such "extra" aromaticity gain stabilizes the GC pair (Figure 4, resonance structures in red) providing a possible explanation for their stronger association strengths compared to analogous H-bonded systems with the same numbers, types, and patterns of hydrogen bonding interactions. Thus, nucleobase pairing interactions can polarize the π-electrons of interacting bases to increase (or decrease) cyclic (4N+2) π-electron delocalization resulting in aromaticity gain (or loss) in the paired bases. This effect promotes strengthening or weakening of the hydrogen bonds.[92]

Analysis of 57 natural and unnatural nucleobase pairs revealed an excellent linear correlation (Figure 4, $R$=0.949) between the gas-phase association energies of nucleobase pairs and the propensity of the interacting bases to gain or lose aromatic character ($\Delta DE_\pi$, where $DE_\pi$ is π-electron delocalization energy).[92] It is worth mentioning that the computed $\Delta DE_\pi$ values for all 57 base pairs are positive, indicating increased π-conjugation for all paired bases upon formation of H-bonded complexes. Higher $\Delta DE_\pi$ values indicate more aromaticity gain upon base pairing, whereas lower $\Delta DE_\pi$ values indicate little to no aromaticity gain or aromaticity loss. For example, the computed $\Delta DE_\pi$ for GC pair is high (28.4 kcal/mol) since base pairing increases aromaticity in six-membered rings of both G and C.

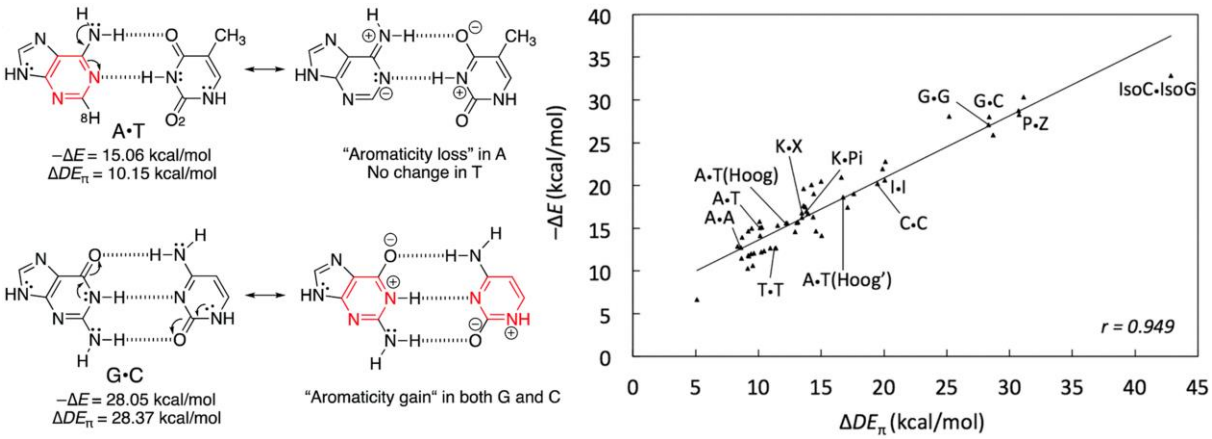


**Figure 4.** *Left*: Schematic illustration of aromaticity-modulated hydrogen bonding in Watson–Crick AT and GC pairs. Resonance structures with (4N + 2) π-electrons are in red. *Right*: Plot of interaction energy (-Δ*E*, in kcal/mol) vs. π-conjugation gain (Δ$DE_\pi$) in the gas-phase for 57 nucleobase pairs. Adapted from Ref. 92 with permission from The Royal Society of Chemistry.

According to the AMHB relationship, heterocycles with the same numbers, types, and patterns of hydrogen bond donor/acceptor moieties can exhibit different hydrogen bond strengths depending on their π-conjugation patterns.[93] However, according to the study by Guillaumes et al.,[94] the strength of the hydrogen bonds in AT pair and its smaller analogs (not even cyclic) deviates less than 1.6 kcal/mol from each other. This result can be considered as an evidence that the atoms participating in the hydrogen bonds do not need to be connected to an aromatic ring to achieve the similar strength of hydrogen bonding.

*3.3.2 Metal complexation*

The effects caused by interactions with alkali metal cations are similar to those observed in H-bonded systems and always lead to monotonic changes in aromaticity in line with the increase of metal cationic radius.[70,74] Interactions with $Li^+$ usually produce greater changes than interaction with $K^+$. Interaction energies for complexes between $Na^+$ and the most stable tautomers of adenine, guanine, thymine, and cytosine are presented in Table 5 together with their HOMA indices (see Schemes 1 to 5 for atom numbering). Many of the mentioned interactions are bifurcated and denoted as $(N_X,N_Y)\cdots Na^+$, where X and Y are the sequence number of the N atom. Despite the very strong intermolecular interactions, their effect on aromaticity of nucleobases is comparable with that observed in the case of charged H-bonds. The 9H tautomer of adenine has the most resistant π-electron system to interactions with metal cations; local and global aromaticity changes are within 0.03 HOMA units.[70] In guanine, these interactions have larger effect on aromaticity of the rings.[74] The greatest aromaticity changes are always caused by interactions with functional groups (amino/imino and oxo/hydroxy). Less aromatic tautomers (9H,1H- and 7H,1H- amino-oxo, Scheme 2) show the greatest changes in the six-membered rings, and they are decisive for total changes in aromatic character of tautomers. Similar to guanine, the π-electron system of pyrimidine nucleobases (thymine and cytosine) are more responsive than that of adenine because two exocyclic functional groups are involved in the interactions.[80,81]

**Table 5**. Total interaction energies, $E_{tot}$, and HOMA values for the complexes of most stable tautomers of nucleobases with $Na^+$.

| | $E_{tot}$/ kcal/mol | HOMA5 | HOMA6 | HOMAtot[a] |
|---|---|---|---|---|
| **Adenine[b]** | | **0.869** | **0.984** | **0.926** |
| $(N1,N10)\cdots Na^+$ | -32.18 | 0.871 | 0.974 | 0.930 |
| $N3\cdots Na^+$ | -30.26 | 0.871 | 0.962 | 0.913 |
| $(N7,N10)\cdots Na^+$ | -32.94 | 0.878 | 0.988 | 0.934 |
| **Guanine[c]** | | **0.878** | **0.704** | **0.761** |
| $(N3,N10)\cdots Na^+$ | -23.16 | 0.901 | 0.601 | 0.711 |
| $(N7,O)\cdots Na^+$ | -56.04 | 0.885 | 0.863 | 0.861 |

| | | | | |
|---|---|---|---|---|
| **Thymine**[d] | | | **0.490** | |
| O8···$Na^+$ | -33.60 | - | 0.465 | - |
| O10···$Na^+$ | -35.53 | - | 0.688 | - |
| **Cytosine**[e] | | | **0.699** | |
| (N3,O)···$Na^+$ | -51.84 | - | 0.803 | - |

[b] all bonds in purine system are taken into account [b] Data taken from ref. 70. [c] Data taken from ref. 74. [d] Data taken from ref. 80. [e] Data taken from ref. 81.

In a work by Poater et al.,[95] the changes in aromaticity due to interaction of GC pair with $Cu^+$, $Ca^{2+}$, and $Cu^{2+}$ cations were investigated. The analysis was carried out with the NICS, PDI, and HOMA indicators of aromaticity (Table 6). All indices point out an increase in the aromatic character of the six-membered rings when $Cu^+$ and $Ca^{2+}$ interact with the GC pair through $N_7$ and $O_6$ atoms of guanine. This increase in aromaticity is consistent with the fact that metal cations increase the weight of the resonance structure **GC3** in Scheme 6. As a result, the six-membered ring of the guanine in the GC pair is more aromatic when GC interacts with a metal cation. In contrast, there is a strong decrease of aromaticity of this ring for the $Cu^{2+}$-GC structure. Interaction of GC with $Cu^{2+}$ causes the removal of a π-electron from the guanine, thus reducing the aromaticity of its six-membered ring.[96]

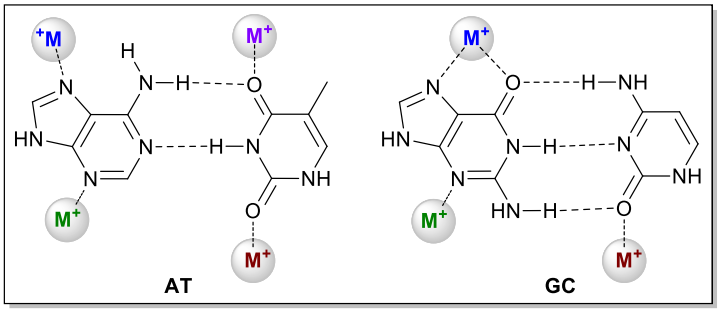
**Table 6.** NICS, PDI, and HOMA aromaticity measures of the five- and six-membered rings of guanine (G), and the six-membered ring of cytosine (C). NICS in ppm and PDI in electrons.[a]

| System | NICS | | | PDI | | HOMA | | |
|---|---|---|---|---|---|---|---|---|
| | G-5 | G-6 | C-6 | G-6 | C-6 | G-5 | G-6 | C-6 |
| **GC** | **-11.94** | **-4.10** | **-1.86** | **0.036** | **0.040** | **0.848** | **0.795** | **0.703** |
| $Ca^{2+}$-GC | -10.67 | -4.76 | -2.53 | 0.044 | 0.045 | 0.843 | 0.886 | 0.797 |
| $Cu^+$-GC | -10.64 | -4.59 | -2.25 | 0.040 | 0.043 | 0.869 | 0.898 | 0.761 |
| $Cu^{2+}$-GC | -7.37 | -2.00 | -3.07 | 0.022 | 0.040 | 0.915 | 0.760 | 0.822 |

[a] Data taken from ref. 95.

Moreover, for AT and GC pairs, π-electron delocalization in the rings of adenine and guanine may decrease or increase depending on the cation location (Scheme 7), while aromaticity of thymine and cytosine rings increases regardless to the site of interaction.[88] Considering local aromaticity of particular rings in purine nucleobases, it was observed that electronic structure of the six-membered rings is more flexible to changes caused by interactions with alkali metal cations ($Li^+$, $Na^+$ and $K^+$) than that of the five-membered ones. This can be explained by the presence of functional groups which interact with cations and cause the greatest geometric and aromaticity changes, increasing the weight of the resonance structures as **GC3** (Scheme 6). The six-membered ring of adenine is the most aromatic ring among all nucleobases and its π-electron system hardly changes due to metal binding. In aqueous solution, the effects of alkali metal cations on aromaticity of the rings are very similar to those observed in the gas phase, but are less pronounced.

**Scheme 7.** Possible coordination sites of alkali metal cations ($M^+$=$Li^+$, $Na^+$, $K^+$) for AT and GC pairs.




*3.3.3 π-π stacking interactions*

The effect of π-π stacking interactions on aromaticity of five- and six-membered rings was studied for 15 complexes between nucleic acid bases.[63] The analysis of aromaticity led to a general conclusion that the variation in the aromaticity of the rings due to π-π stacking is small. Moreover, aromaticity change in the five-membered rings is less pronounced than that in the six-membered ones. FLU and ATI indices show a decrease in the six-membered ring aromatic character, while PDI and HOMA indices are less varied and, to some extent, they remain unchanged during stacking interaction. The FLU index is more sensitive to describe the aromaticity variation in the stacked base pairs. Among 15 studied base pairs, maximum reduction in the aromaticity was observed for cytosine in its dimer complex.

A detailed study on NICS profiles along the axis through the center of five- and six-membered rings of adenine from -2 Å to +2 Å for its complex with acenaphthylene showed complex changes in $NICS_{zz}$ values, as presented in Figure 5.[97] The interface region (-2 to 0 Å) shows significantly larger negative NICS values for both five- and six-membered rings of adenine than in the isolated molecule. However, it is not associated with increase in aromaticity of adenine but rather with magnetic couplings of both molecules leading to unrealistic description of aromatic behaviour in that region, as was previously observed by Solà and co-workers.[98,99] In the external region, there is no direct overlap between electron densities of both molecules, thus changes in the $NICS_{zz}$ values in this region can be considered as changes in the magnetic field of the corresponding molecule, and the curves in the outer region (0–2 Å) are quite similar for isolated and bonded adenine. Thus, aromatic character of the monomers is preserved or only slightly increase upon π-π complexation.

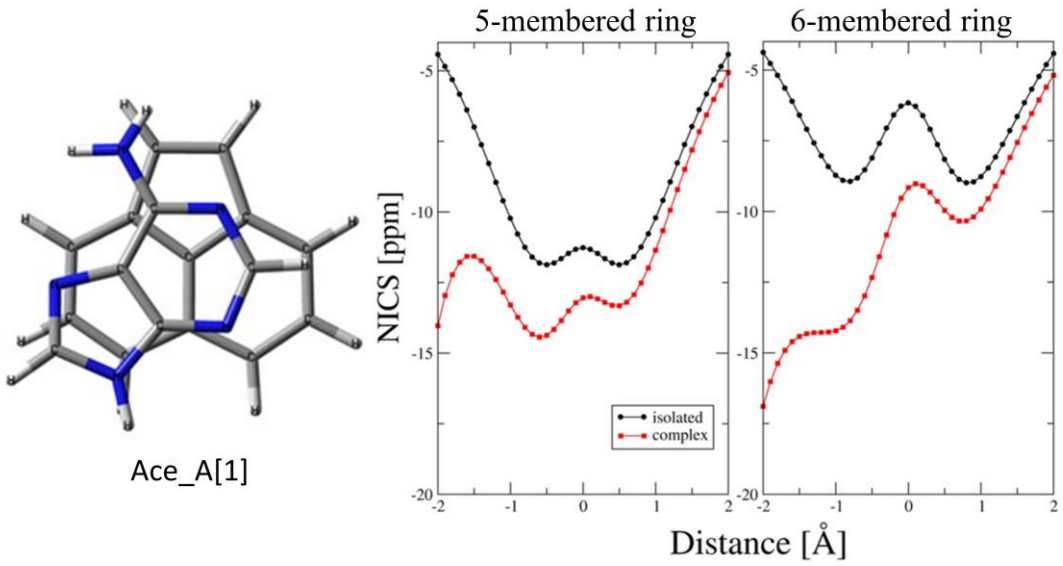



**Figure 5**. NICS profiles along the axis through the center of adenine rings from -2 to +2 Å with a step of 0.1 Å for acenaphthylene-adenine (Ace_A[1]) complex. Adapted with permission from Ref. 97. Copyright (2016) Wiley.

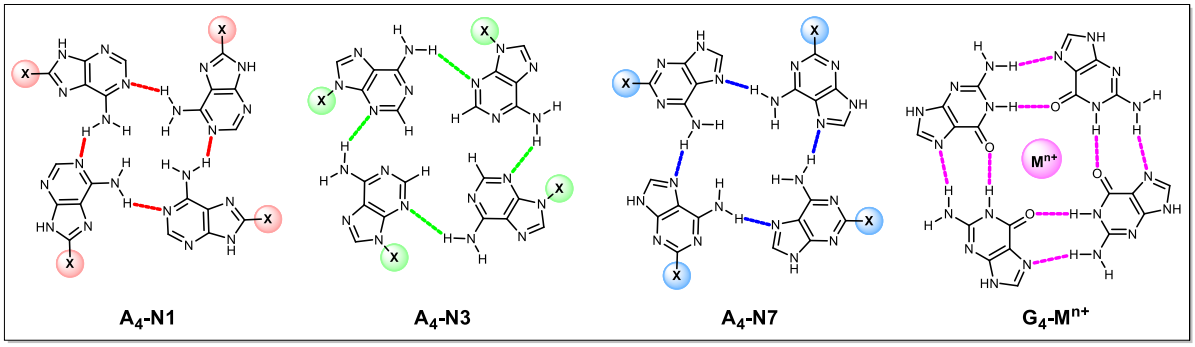
Similar results were obtained for nucleobases-graphene and nucleobases-phosphorene interactions.[100,101] The role of nucleobase aromaticity in their physisorption strength has been investigated with geometric, electronic, and magnetic aromaticity descriptors. In all cases, NICS values suggest an increase in aromaticity of nucleobases upon adsorption on graphene compared to the free nucleobases.[100,102] Except the NICS indices, all other aromaticity descriptors indicate that almost no changes in the aromatic character of the five- and six-membered rings of nucleobases are observed after binding to graphene surface. The failure of NICS indices arises from the fact that both graphene and nucleobases are able to show diamagnetic ring currents because both have an aromatic character. The total induced magnetic field at the point where the shielding is measured in the supermolecule will be larger than that of free graphene and free nucleobases due to the coupling effects, but this effect is not related to the increase or decrease of aromaticity.

*3.3.4 Quartets*

The hydrogen bonding, metal ion coordination, and aromatic π–π stacking represent three major noncovalent forces that determine a spatial structure of large biomolecules, for example, quadruplexes. Study of aromaticity of nucleobases forming the tetramer structures is very attractive because changes in π-electron delocalization are responsible for tuning π-π stacking interactions in their supramolecular assembly.

Comparison of HOMA5 and HOMA6 values for the substituted adenine monomers (with $NO_2$, Cl, F, H, Me, $NH_2$) with their values for adenine tetramers ($A_4$) clearly shows that π-electron system of the five-membered rings is almost not affected during the tetramer formation, in contrast to π-electron system of the six-membered rings.[103] Among different types of adenine quartet, aromaticity of both adenine rings in $A_4$-N3 tetramer (Scheme 8) is more sensitive to electronic nature of the substituents than in other tetramers. In general, to increase aromaticity of the five-membered rings the electron-accepting substituents should be introduced at the C2 or C8 positions,

or the electron-donating substituent at the N9 position. In turn, six-membered rings are highly aromatic (HOMA > 0.9) and slightly responsive only to the substituents at the C8 position, whereas C2- and N9-substitution does not affect them.




**Scheme 8.** Tetramers of adenine with C8, N9 and C2 positions of substitution (X=$NO_2$, Cl, F, H, Me, $NH_2$) and structure of guanine quartet ($G_4$) with metal cations ($M^{n+}$).

The aromaticity indices, including HOMA, FLU, and PDI, were calculated for five- and six-membered rings of guanine in guanine quartet ($G_4$) and its complexes with metal cations (Scheme 8).[104] According to the values of aromaticity indices (Table 7), in $G_4$ the five-membered ring is more aromatic than the six-membered one. However, its aromaticity decreases upon complexation with metal cation, whereas that of the six-membered rings follows the reverse tendency. These changes in aromaticity are connected with modification of the electron density distribution around C=O bonds after complexation with $M^{n+}$ ion. The observation in $G_4$-$M^{n+}$ complexes is very similar to the case when GC pair interacts with a metal cation and six-membered ring of the guanine becomes more aromatic.[95]

**Table 7.** Aromaticity indices for five- and six-membered rings of guanine in the guanine quartet and its metal complexes.[a]

| $M^{n+}$ | HOMA5 | FLU5 | HOMA6 | FLU6 | PDI |
|---|---|---|---|---|---|
| **$G_4$** | **0.865** | **0.0269** | **0.803** | **0.0327** | **0.0420** |
| $Al^{3+}$ | 0.815 | 0.0325 | 0.871 | 0.0277 | 0.0517 |
| $Mg^{2+}$ | 0.850 | 0.0301 | 0.891 | 0.0282 | 0.0486 |
| $Ca^{2+}$ | 0.853 | 0.0298 | 0.879 | 0.0293 | 0.0474 |
| $Na^{+}$ | 0.866 | 0.0281 | 0.855 | 0.0306 | 0.0447 |
| $Ag^{+}$ | 0.864 | 0.0283 | 0.862 | 0.0307 | 0.0446 |
| $K^{+}$ | 0.867 | 0.0279 | 0.841 | 0.0315 | 0.0440 |

[a] Data taken from ref. 104.

**Sidebar title:**

[Please include sidebars in the body of the text where appropriate]

## Conclusion

Nucleobases are extremely important heterocyclic compounds, which form our genetic code. A spatial structure of large biomolecules is determined mostly by noncovalent interactions, including hydrogen bonding and aromatic π–π stacking. Therefore, a proper description of π-electron delocalization in nucleobases is essential. However, unlike aromatic hydrocarbons, cyclic π-electron delocalization and aromaticity of nucleobases do not always correlate with their stability. This difference arises from the presence of the nitrogen atoms of amine or imine type in the cycle and exocyclic oxo/hydroxy and amino/imino groups. Intramolecular interactions among exocyclic groups are the major factor contributing to the stabilization of various tautomers of nucleobases. Stabilization by these interactions is more important than stabilization due to aromaticity. Thus, the Hückel (4N+2) rule indicating greater stability for π-electron hydrocarbons is not applicable to determine the stability of nucleobases.

Geometric, electronic, and magnetic aromaticity indices demonstrate that cyclic π-electron system of purine nucleobases are more aromatic compared to pyrimidine ones. Aromaticity of their most stable tautomers decreases in the sequence: adenine > guanine > cytosine > thymine ≈ uracil. In general, aromaticity of the nucleobases depends on the number of exocyclic functional groups attached to the ring and their electronic character. Exocyclic oxo and imino groups significantly decrease π-electron delocalization in the six-membered rings of nucleobases.

Intermolecular interactions, such as H-bonds and complexation with metal cations, induce similar changes in aromaticity of purine and pyrimidine bases. In most cases, the strongest interactions of both types cause the largest aromaticity changes. Greater changes occur when exocyclic groups participate directly in the intermolecular interactions. Such changes are more pronounced in the six-membered rings than in the five-membered ones. In the case of purine nucleobases, changes in the pyrimidine ring overweight changes in the imidazole one and play a decisive role in the total aromaticity changes.

Aromatic character of nucleobases is preserved or only slightly enhanced upon π-π stacking interactions. Electronic FLU index appears to be more sensitive in describing such subtle aromaticity changes. Importantly, the increase in NICS index measured at the interface region of two stacked aromatic molecules cannot be associated with an increase in aromaticity of monomer but rather with magnetic couplings of both molecules.

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## Research Resources

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## Conflict of Interest

**The authors have declared no conflicts of interest for this article.**

## Further Reading

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