Why do A•T and G•C Self-Sort? Hückel Aromaticity as a Driving Force for Electronic Complementarity in Base Pairing

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Density functional theory computations and block-localized wavefunction analyses for 57 hydrogen-bonded base pairs document excellent linear correlation between the gas-phase association energies and the degree of aromaticity gain of paired bases (r = 0.949), challenging prevailing views of factors that underlie the proposed electronic complementarity of A•T(U) and G•C base pairs. Base pairing interactions can polarize the π-electrons of interacting bases to increase (or decrease) cyclic 4n+2π electron delocalization, resulting in aromaticity gain (or loss) in the paired bases, and become strengthened (or weakened). The potential implications for improving nucleic acid force-fields and for designing robust unnatural base pairs are discussed.

Introduction

More than sixty years have passed since the proposal of the double helix structure of DNA,[1] yet fundamental aspects of the recognition properties of nucleobase pairs remain puzzling. How does Nature choose the optimal hydrogen bonding complement for a specific nucleobase (and can we mimic this selectivity)? Given a mixture of adenine (A), thymine (T)/uracil (U), guanine (G), and cytosine (C) in the primordial soup, why does A pair with T (or U) and G with C instead of to themselves? In this work, we report computational evidence suggesting that aromaticity gain (or loss) in paired bases can strengthen (or weaken) base pairing interactions, having direct relevance for rationalizing the electronic complementarity of A•T(U) and G•C pairs in DNA and RNA and for designing unnatural hydrogen-bonded base pairs.

In their seminal work, Kyogoku, Lord, and Rich first evoked the attractive idea that the A•T(U) and G•C pairs might exhibit special electronic features, i.e., "electronic complementarity," favoring their specific associations.[2,3] Measurements of the association constants (K_{assoc}) of these nucleobases and their derivatives in chloroform revealed noticeably higher K_{assoc} values for the A•U (100 M^{-1}) pair, compared to A•A (~3 M^{-1}) and U•U (~6 M^{-1}), and the G•C (10^4-10^5 M^{-1}) pair, compared to G•G (10^3-10^4 M^{-1}) and C•C (~28 M^{-1}).[2,3] The recognition of A•U caught special attention since the self-associated A•A and U•U also formed two hydrogen bonds. It was proposed that the A•U pair might exhibit additional attractive C–H…O interactions between the H8 of A and the O2 of U (Figure 1a).[4,5] Others pointed out, however, that in both the Watson-Crick and Hoogsteen configurations of A•U, the C–H…O interactions were distal, nonlinear, and thus at most weak interactions.[6-9]

Here, we show that the aromatic characters of nucleobases (i.e., their "π-conjugation patterns") influence their association strengths to complementary bases through a reciprocal aromaticity-modulated hydrogen bonding (AMHB) relationship.[10,11] Base pairing interactions that increase aromaticity (i.e., enhance cyclic 4n+2 π-electron delocalizations) of the interacting bases exhibit stronger than expected hydrogen bonds, while those that decrease aromaticity (i.e., disrupt cyclic 4n+2 π-electron delocalizations) of the interacting bases display weaker associations. In a related work, Cyranski et al. showed indeed that hydrogen bonding at the C=O positions of A, T, G, and C base pairs increased the aromatic characters of the respective rings.[12] Fliegl et al. reported that the interaction strengths of several hydrogen-bonded dimers, including the Watson-Crick, A•T and G•C pairs, correlated to their computed diamagnetic susceptibilities.[13] Energy decomposition analyses for A•T and G•C quantified the effects of resonance-assistance.[7,14] Demonstrative examples of AMHB, in squaramide complexes[15,16] and polymers,[17] in dimers of five and six membered arrays,[10,11] and in multipoint hydrogen bonded arrays[18] also have been reported.

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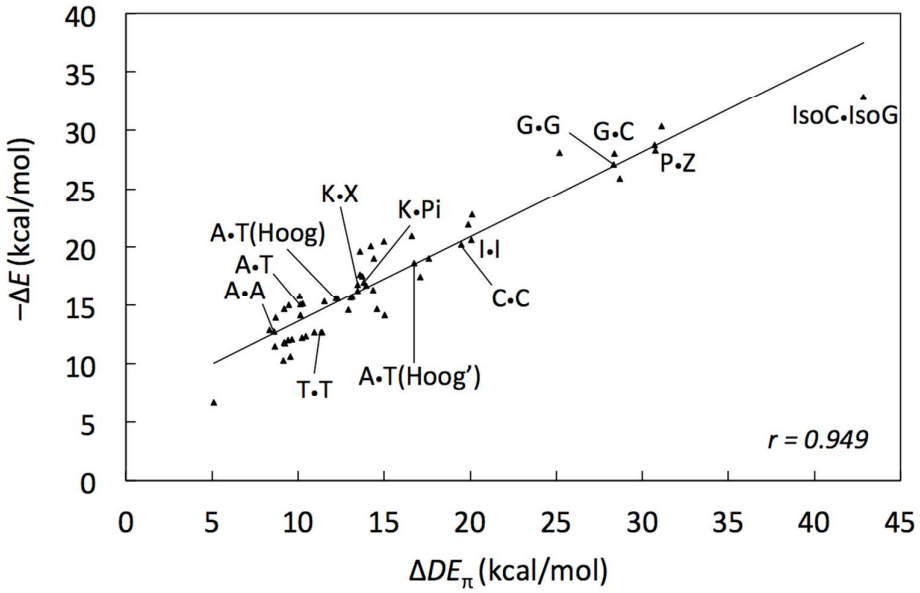

Figure 2. Plot of base pairing interaction energy (–Δ*E*, in kcal/mol) vs. π-conjugation gain (ΔDE_π) in the gas-phase for all 57 base pairs. Plot of –Δ*E* vs. ΔDE_π for selected base pairs in chloroform is provided in Figure S8 of the SI.

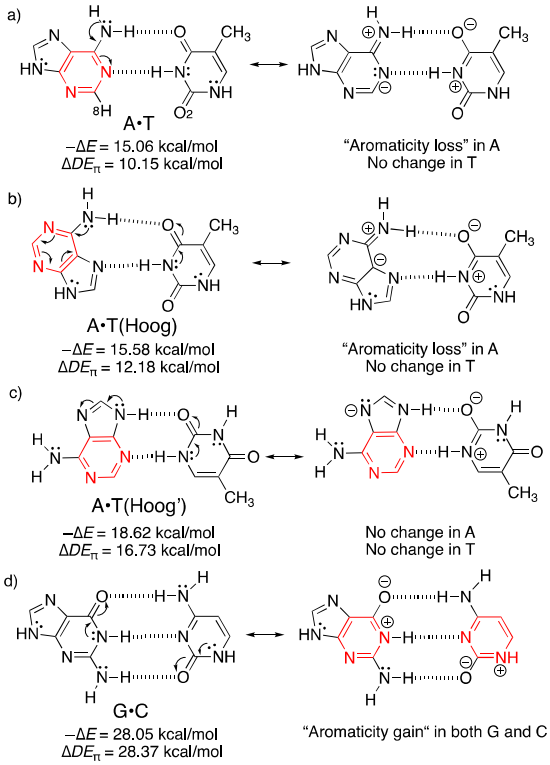

Figure 1. Aromaticity-modulated hydrogen bonding (AMHB) in the a) Watson-Crick A•T, b) natural Hoogsteen A•T, c) most stable Hoogsteen A•T, and d) Watson-Crick G•C pairs. Resonance structures with formal cyclic 4*n*+2π electron delocalizations are in red. Computed interaction energies (–Δ*E*) and the estimated π-conjugation gain ($-\Delta DE_\pi$) effects also are shown.

Schematic illustrations of aromaticity-modulated hydrogen bonding in the A•T and G•C base pairs are shown Figure 1. In both the Watson-Crick and natural Hoogsteen configurations of A•T (Figures 1a and 1b), hydrogen bonding interactions polarize the ring π-electrons of the bases modestly, leading to decreased aromatic character in A, while T remains non-aromatic. In the most stable A•T configuration, A•T(Hoog') (Figure 1c), hydrogen bonding interactions polarize the ring π-electrons, but result in no gain or loss of aromatic character in either base. In the Watson-Crick G•C pair (Figure 1d), hydrogen bonding interactions polarize the ring π-electrons of both G and C, leading to increased aromatic character in both bases (note resonance form in red), and the resulting "aromaticity gain" stabilizes the G•C complex in addition to the three hydrogen bonds present. We show that in this way, base pairs with the same numbers and types of hydrogen bonds can exhibit notably different pairing strengths depending on the π-conjugation pattern of the base.

Results and Discussion

Based on a survey of 57 natural and unnatural base pairs, excellent linear correlation (r = 0.949, Figure 2) was found between the gas-phase association energies of each base pair (a•b) ($\Delta E = E_{a\bullet b} - E_a - E_b$) and the propensity of the interacting bases to gain or lose aromatic character (ΔDE_π, see below). Geometries for all structures were optimized with a constrained C_s symmetry at ωB97X-D/6-311+G(d,p) employing Gaussian09[19] (see details in the Supplementary Information, SI). Base pairs subject to obvious steric effects were excluded from the study.

Since aromaticity is related to the degree of π-electron delocalization in molecules, the effects of aromaticity gain or loss

can be quantified by the amount of increase in π-electron delocalization upon base pairing, and is evaluated here by the block-localized wavefunction (BLW) analysis.[20-22] BLW quantified the π-electron delocalization energy (DE_π) of the base pairs and bases by comparing the fully delocalized wavefunction (ψ_{deloc}) of the system considered to that of a hypothetical localized wavefunction (ψ_{loc}), in which all π-electrons were mathematically constrained to resemble a strict π-electron-localized Lewis structure; $DE_\pi = \psi_{loc} - \psi_{deloc}$. The increase in π-electron delocalization energy (ΔDE_π) (as a result of base pairing) is evaluated by the computed DE_π value for the base pair considered (a•b) minus that of the interacting bases (a and b); $\Delta DE_\pi = DE_{a•b} - (DE_a + DE_b)$ (see details in the SI). All BLW computations were performed at B3LYP/6-31G(d) employing the GAMESS-2013-R1 program.[23]

Following this procedure, the computed ΔDE_π values for all 57 base pairs were positive, indicating increased π-conjugation for all paired bases upon hydrogen bonding. The amount of π-conjugation gain differs depending on whether there is an increase or decrease in aromatic character in the paired bases. Higher ΔDE_π values indicate more aromaticity gain upon base pairing; lower ΔDE_π values indicate little to no aromaticity gain or aromaticity loss. For example, the computed ΔDE_π values for the Watson-Crick and natural Hoogsteen A•T pairs (10.2 and 12.2 kcal/mol, aromaticity loss in A, no change in T, Figures 1a and 1b) are lower compared to that of the most stable A•T configuration, A•T(Hoog'), (16.7 kcal/mol, no change in aromaticity for A or T, Figure 1c). The computed ΔDE_π for G•C (28.4 kcal/mol) is even higher since base pairing increases aromaticity in both G and C (Figure 1a).

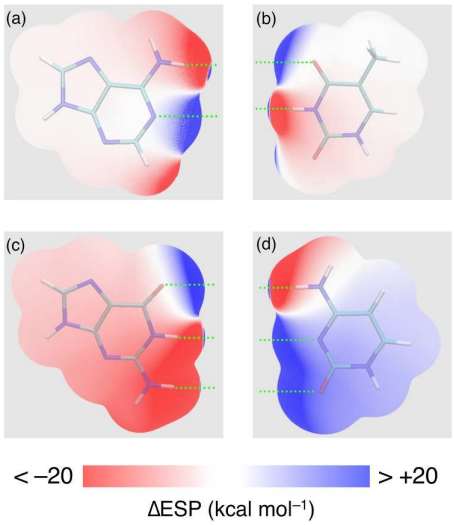

Figure 3. Computed electrostatic potential difference maps, ΔESP, for a) adenine, b) thymine c) guanine, and d) cytosine, upon base pairing to A•T and G•C.

Accordingly, the computed electrostatic potential (ΔESP) difference maps for the Watson-Crick, A•T and G•C, pairs show stark differences, indicating very different polarizabilities for A, T, G, and C (Figure 3). The ΔESP plots of A and T (upon pairing to form A•T) showed relatively little electron polarization, while those of G and C (in G•C) showed notable polarization. Positive ΔESP values (blue) indicate a more repulsive surface, and negative ΔESP values (red) a more attractive surface upon base pairing. Each plot was generated by comparing the computed ESP values of the paired bases minus that of the isolated bases at a 0.001 a.u. isosurface (generated by the Multiwfn program,[24,25] see details in the SI). We note that previous benchmarking studies of the performance of various force-fields[26] against quantum mechanical methods documented better agreement for the computed interaction energies of base pairs such as A•T, A•A, and T•T (aromaticity loss or no change), relative to base pairs such as G•C and G•G (aromaticity gain). It is tempting to make the connection that such variations, i.e., differences in the polarizability of nucleobases because of their π-conjugation patterns, may explain why fixed-charged approaches adopted by popular force-fields,[27,28] might understabilize certain interactions but overstabilize others.

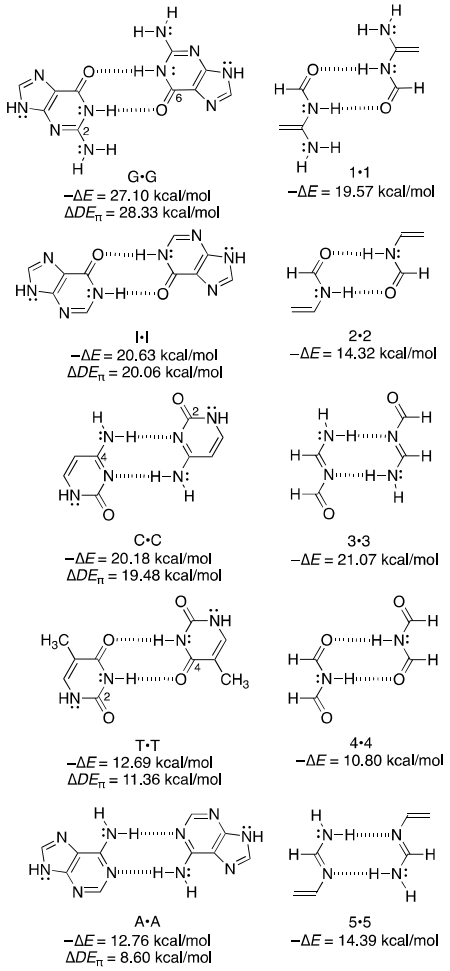

Figure 4. Computed $-\Delta E$ and ΔDE_π values for the self-associated G•G, I•I, C•C, U•U, A•A pairs, and $-\Delta E$ values for their acyclic references, 1•1, 2•2, 3•3, 4•4, and 5•5. See also Figure S2 in the SI.

Considering the potential for aromaticity gain or loss in base pairs could help explain variations in their association strengths. For example, it has been suggested that, among the doubly hydrogen-bonded, self-associated, G•G, C•C, T•T, A•A pairs, G•G and C•C displayed especially high association strengths due to additional

attractive secondary electrostatic interactions (SEI);[29] in G•G, between the amino groups on C2 and the carbonyl groups on C6, and in C•C, between the amino groups on C4 and the carbonyl groups on C2. In T•T, there are additional repulsive SEI's between the C2 and C4 C=O groups. These attractive interactions are absent in A•A. More recent studies suggested the important effects of steric repulsion on base pairing in G•G vs. C•C.[30] We show here that, in addition to the SEI and possible steric effects, the strong association of G•G (as well as its closely related inosine analog, I•I) may be attributed to prospects for significant aromaticity gain in the paired G (and I) bases; note the aza-2-pyridone moieties of G•G and I•I (Figure 4). In C•C and T•T, base pairing has little to no effect on the aromatic character of either monomer. In A•A, base pairing reduces the aromatic character of the paired A units; note the 2-hydroxypyridine moiety of A•A (Figure 4). Relevant resonance forms are shown in Figure S2 of the SI.

Direct comparisons of the computed $-\Delta E$ values for G•G, I•I, C•C, T•T, A•A, to those of their hydrogen-bonded acyclic dimer references (1•1, 2•2, 3•3, 4•4, 5•5) document the energetic effects of AMHB (Figure 4). Notably, the computed $-\Delta E$ values for G•G (27.1 kcal/mol) and I•I (20.6 kcal/mol) are 6 to 8 kcal/mol higher compared to those of their acyclic references, 1•1 (19.6 kcal/mol) and 2•2 (14.3 kcal/mol), which display the same primary and secondary electrostatic interactions but are preclude of aromaticity gain. In contrast, the computed $-\Delta E$ values for C•C (20.2 kcal/mol) and T•T (12.7 kcal/mol) closely follow those of their acyclic references, 3•3 (21.1 kcal/mol) and 4•4 (10.8 kcal/mol), suggesting that key factors relevant to the hydrogen bond strengths of C•C and T•T are adequately captured by their acyclic references. The computed $-\Delta E$ for A•A (12.8 kcal/mol) is modestly lower than 5•5 (14.4 kcal/mol), as expected by aromaticity loss of A upon base pairing.

Recognizing the effect of AMHB also has important implications for synthetic efforts in "expanding the genetic alphabet." Several research groups have demonstrated elegant examples of artificial replication processes mimicking DNA, by using "unnatural" base pairs.[31-34] Although the designs of unnatural base pairs have focused primarily on optimizing geometric complementarity (in which hydrogen bonds may or may not be present), the correlation shown in Figure 2 suggests, that for hydrogen-bonded pairs, aromaticity gain (and loss) may serve as an effective strategy for modulating the robustness of unnatural base pairs, such as the isoC•isoG, P•Z, K•Pi, K•X pairs discussed below.

As shown in Figure 5, the computed $-\Delta E$ values for both isoC•isoG (32.9 kcal/mol) and P•Z (28.3 kcal/mol) are 5 to 10 kcal/mol higher than their acyclic reference 3•1 (22.9 kcal/mol), due to increased aromaticity in the isoC, isoG, P, Z moieties upon base pairing. In sharp contrast, the computed $-\Delta E$ values for both K•Pi (17.0 kcal/mol) and K•X (16.8 kcal/mol) are close to that of their acyclic reference 6•4 (15.8 kcal/mol), indicating little non-additivity beyond the primary and secondary electrostatic effects present (base pairing decreases the aromatic character of K, and has little to no effect on the aromatic character of Pi and X). Relevant resonance forms are shown in Figure S3 of the SI. A plot showing linear correlation, between $-\Delta E$ vs. ΔDE_π, for 1•1, 2•2, 3•3, 4•4, 5•5, 3•1, 6•4 is provided in Figure S9 of the SI.

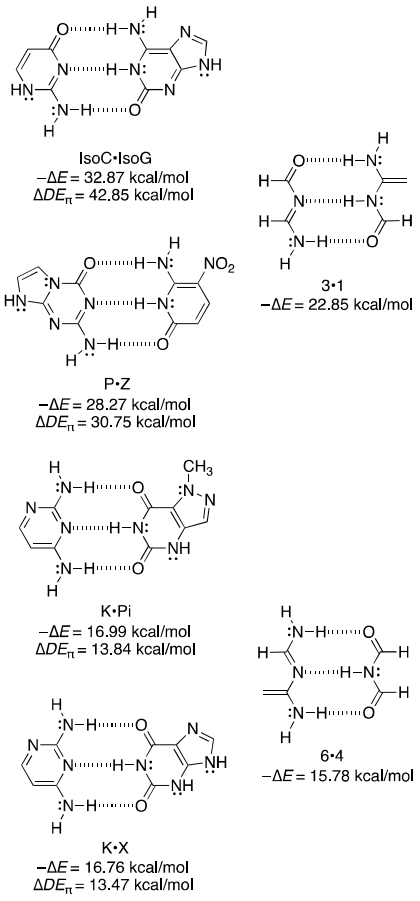

Figure 5. Computed $-\Delta E$ and ΔDE_π values for isoC•isoG, P•Z, K•Pi, K•X, and $-\Delta E$ values of their acyclic references. See also Figure S3 in the SI.

Overall, our findings suggest that while primary and secondary electrostatic interactions[29] have clear energetic consequences for base pairing (e.g., $-\Delta\Delta E$ = 8.8 kcal/mol for 1•1 vs. 4•4, and 7.1 kcal/mol for 3•1 vs. 6•4), the effects of AMHB are comparable in magnitude (e.g., $-\Delta\Delta E$ = 7.5 kcal/mol for 1•1 vs. G•G, and 10.0 kcal/mol for 3•1 vs. isoC•isoG), and therefore should be considered when evaluating base pairing strengths.

Conclusions

It is perhaps curious that adenine is the only fully "aromatic" nucleobase in the genetic code according to the Hückel $4n+2\pi$ electron rule for aromaticity. None of the other bases in DNA or RNA, i.e., thymine, uracil, cytosine, guanine, inosine, are $4n+2\pi$ electron "aromatic," despite having a closed-shell, cyclic, π-conjugated structure. What emerges from our finding is the suggested possibility that the π-conjugation patterns "encoded" to nucleobases have real chemical significance for modulating, understanding, and perhaps simulating base pairing interactions in DNA and RNA.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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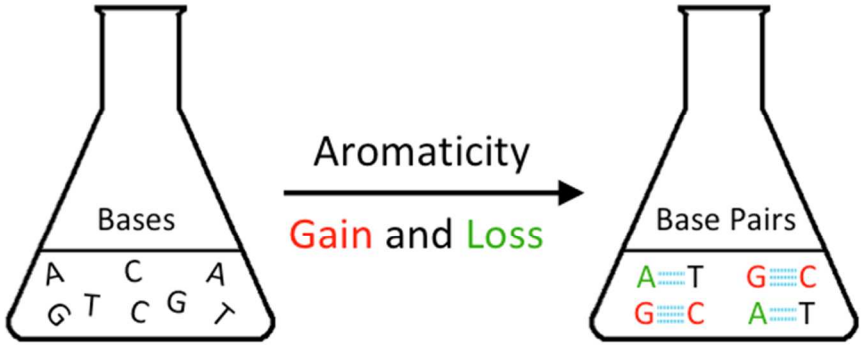
Notes and references

1 J. D. Watson, F. H. C. Crick, *Nature*, 1953, **171**, 737-738.
2 Y. Kyogoku, R. C. Lord, A. Rich, *Biochim. Biophys. Acta*, 1969, **179**, 10-17.
3 Y. Kyogoku, R. C. Lord, A. Rich, *Proc. Natl. Acad. Sci. U.S.A.*, 1967, **57**, 250-257.
4 K. S. Jeong, T. Tjivikua, A. Muehldorf, G. Deslongchamps, M. Famulok, J. Rebek Jr., *J. Am. Chem. Soc.*, 1991, **113**, 201-209.
5 G. A. Leonard, K. McAuley-Hecht, T. Brown, W. N. Hunter, *Acta Crystallogr., Sect. D*, 1995, **51**, 136-139.
6 J. R. Quinn, S. C. Zimmerman, J. E. Del Bene, I. Shavitt, *J. Am. Chem. Soc.*, 2007, **129**, 934-941.
7 C. Fonseca Guerra, F. M. Bickelhaupt, J. G. Snijders, E. J. Baerends, *Chem. Eur. J.*, 1999, **5**, 3581-3594.
8 A. Asensio, N. Kobko, J. J. Dannenberg, *J. Phys. Chem. A*, 2003, **107**, 6441-6443.
9 P. Hobza, J. Sponer, E. Cubero, M. Orozco, F. J. Luque, *J. Phys. Chem. B*, 2000, **104**, 6286-6292.
10 J. I. Wu, J. E. Jackson, P. v. R. Schleyer, *J. Am. Chem. Soc.*, 2014, **136**, 13526-13529.
11 T. Kakeshpour, J. I. Wu, J. E. Jackson, *J. Am. Chem. Soc.*, 2016, **138**, 3427-3432.
12 M. K. Cyrański, M. Gilski, M. Jaskólski, T. M. Krygowski, *J. Org. Chem.*, 2003, **68**, 8607-8613.
13 J. F. Beck, Y. Mo, *J. Comput. Chem.*, 2006, **28**, 455-466.
14 H. Fliegl, O. Lehtonen, D. Sundholm, V. R. I. Kaila, *Phys. Chem. Chem. Phys.*, 2011, **13**, 434-437.
15 D. Quiñonero, A. Frontera, P. Ballester, P. M. Deyà, *Tetrahedron Lett.*, 2000, **41**, 2001-2005.
16 D. Quiñonero, R. Prohens, C. Garau, A. Frontera, P. Ballester, A. Costa, P. M. Deyà, *Chem. Phys. Lett.*, 2002, **351**, 115-120.
17 V. S. Talens, P. Englebienne, T. T. Trinh, W. E. M. Noteborn, I. K. Voets, R. E. Kieltyka, *Angew. Chem.*, 2015, **127**, 10648-10652.
18 C.-H. Wu, Y. Zhang, K. v. Rickley, J. I. Wu, *Chem. Commun.*, 2018, **54**, 3512-3515.
19 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O¨. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian 09, revision D.01, Gaussian, Inc., Wallingford, CT, 2013.
20 Y. Mo, J. Gao, S. D. Peyerimhoff, *J. Chem. Phys.*, 2000, **112**, 5530-5538.
21 Y. Mo, L. Song, Y. Lin, *J. Phys. Chem. A*, 2007, **111**, 8291-9301.
22 Y. Mo, in *the Chemical Bond: Fundamental Aspects of Chemical Bonding*, ed. G. Frenking, and S. Shaik, Wiley, Weinheim, Germany, 2014, pp 199.
23 M. W. Schmidt, K. K. Baldridge, J. A. Boatz, S. T. Elbert, M. S. Gordon, J. H. Jensen, S. Koseki, N. Matsunaga, K. A. Nguyen, S. Su, T. L. Windus, M. Dupuis, J. A. Montgomery, *J. Comput. Chem.*, 1993, **14**, 1347-1363.
24 T. Lu, F. Chen, *J. Comput. Chem.*, 2012, **33**, 580-592.
25 T. Lu, F. Chen, *J. Mol. Graph. Model.*, 2012, **38**, 314-323.
26 P. Hobza, M. Kabelac, J. Sponer, P. Mejzlik, J. Vondrasek, *J. Comput. Chem.*, 1996, **18**, 1136-1150.
27 W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.*, 1995, **117**, 5179-5197.
28 D. A. Case, T. E. Cheatham III, T. Darden, H. Gohlke, R. Luo, K. M. Merz Jr., A. Onufriev, C. Simmerling, B. Wang, R. J. Woods, *J. Comput. Chem.*, 2005, **26**, 1668-1688.
29 W. L. Jorgensen, J. Pranata, *J. Am. Chem. Soc.*, 1990, **112**, 2008-2010.
30 S. C. C. van der Lubbe, C. Fonseca Guerra, *Chem. Eur. J.*, 2017, **23**, 10249-10253.
31 E. T. Kool, *Acc. Chem. Res.*, 2002, **35**, 936-943.
32 J. A. Piccirilli, T. Krauch, S. E. Moroney, S. A. Benner, *Nature*, 1990, **343**, 33-37.
33 M. Ishikawa, I. Hirao, S. Yokoyama, *Tetrahedron Lett.*, 2000, **41**, 3931-3934.
34 T. Ohtsuki, M. Kimoto, M. Ishikawa, T. Mitsui, I. Hirao, S. Yokoyama, *Proc. Natl. Acad. Sci. U.S.A.*, 2001, **98**, 4922-4925.


Bases
A C A
G T C G T
Aromaticity
Gain and Loss
Base Pairs
A T G C
G C A T