

Differential stabilization of adenine quartets by anions and cations

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Abstract We have investigated the structures and stabilities of four different adenine quartets with alkali and halide ions in the gas phase and in water, using dispersion-corrected density functional theory at the BLYP-D/TZ2P level. First, we examine the empty quartets and how they interact with alkali cations and halide anions with formation of adenine quartet–ion complexes. Second, we examine the interaction in a stack, in which a planar adenine quartet interacts with a cation or anion in the periphery as

well as in the center of the quartet. Interestingly, for the latter situation, we find that both cations and anions can stabilize a planar adenine quartet in a stack.

Keywords Adenine quartets · Anion binding · Cation binding · Density functional calculations · Solvent effects

Introduction

The biological significance of tetrastranded nucleic acid structures is becoming increasingly recognized [1], and numerous applications of quadruplexes are emerging [2–4]. DNA and RNA quadruplexes are composed of nucleobase quartets, in which the bases are essentially coplanar and interact through hydrogen bonds. There is the possibility to accommodate different strand directions in quadruplexes. By far the most stable and at the same time longest known nucleobase quartet is that of guanine (G_4). It displays a cyclic arrangement of four pairs of hydrogen bonds between $N(2)H_2$ and $N7$ sites on one hand, and between $N(1)H$ and $O6$ sites on the other [5]. Nucleobase quartets of all other individual DNA/RNA bases as well as combinations of different nucleobases are now known [6]. As a consequence of their—compared with G_4 —lower stabilities, they come in a larger structural diversity, and frequently use G_4 as a platform. For example, the uracil quartet (U_4) may be planar [7–9] or saddle-shaped [10], it may involve different hydrogen-bonding patterns [6, 11], or it may even contain a water molecule in the hydrogen-bonding scheme [12]. In heteronucleobase quartets, e.g., GCGC (C is cytosine) [6] or GAGA (A is adenine) [13], individual nucleobase pairs can interact differently through hydrogen bonds.

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For adenine quartets (A_4), at least three variants have been experimentally shown to exist (Scheme 1). In all cases these quartets display four cyclic hydrogen bonds only, which extend from the N(6)H₂ groups of adenine, which function as the donors, yet use different acceptor sites, namely, N1 (A_4 -N1), N3 (A_4 -N3), and N7 (A_4 -N7). A_4 -N7 [14] and A_4 -N3 [15] have been characterized by X-ray crystallography in tetrastranded and octastranded RNA molecules, respectively, whereas type A_4 -N1 has been detected by NMR spectroscopy [16, 17]. All three quartets have also been the subject of computational studies [11, 18, 19]. Quartet structures of adenines can also be part of polymeric ribbons of adenine pairs [20, 21], but the latter will not be considered further here.

Major stabilizing factors of G_4 as well as of thymine quartets (T_4) and U_4 are metal cations (Na^+ , K^+ , NH_4^+ , Ca^{2+} , Sr^{2+} , etc.), which are located in the center of the quartet, or are centrally sandwiched between adjacent, stacked quartets [22]. The interactions relieve the mutual repulsion between the four exocyclic oxygen atoms of the four nucleobases, which point toward the center of the quartet.

With A_4 , there are different options to be considered (see above): whereas A_4 -N3 enables favorable interactions between a cation and the lone electron pairs of the N1 positions [15], in A_4 -N1 and A_4 -N7 the exocyclic amino groups point toward the center, which appears to be favorable for having a stabilizing interaction with an anion through hydrogen-bonding. Metal binding to A_4 -N3 has been observed experimentally [15] and has been rationalized by a computational study [19]. In work reported by Pan et al. [14], A_4 -N7 was found to interact with a Na^+ ion, which prompted us to look into the possibility of the interaction of adenine quartets with anions. Anion binding to nucleobases, a feature quite common in positively charged model nucleobases (charge brought about by metal coordination or protonation), is even observed in real DNA and RNA structures [23]. There are also scattered reports on association patterns between supramolecular metal-nucleobase assemblies and anions, with anions interacting with the periphery however [24, 25]. Recent calculations

on the stability of a NaCl ion pair bonded to a stack composed of G_4 (with its known affinity for Na^+) and A_4 of A_4 -N7 further corroborate such a possibility [26].

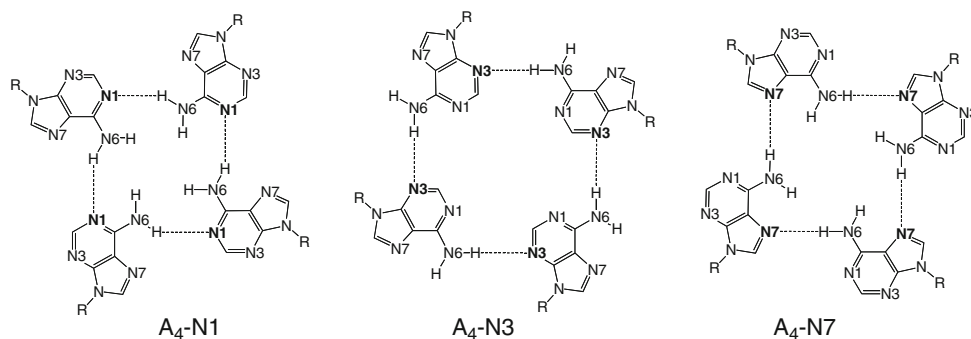
In the work reported here, we computationally investigated the interaction of the adenine and 9-methyladenine quartets (A_4 -N1, A_4 -N3, and A_4 -N7) with various monovalent cations (Li^+ , Na^+ , and K^+) as well as monovalent anions (F^- , Cl^- , and Br^-) using dispersion-corrected density functional theory (DFT-D). We also included an additional quartet, A_4^* , in the calculations, which contains the rare imino tautomers of adenine (Scheme 2). Although there is no experimental evidence for the existence of such A_4^* structures at present, on the mono(nucleobase) level, the relevance of rare nucleobase tautomers is probably underestimated [27]. Finally, binding of cations and anions to the outside of adenine quartets has been investigated. Groove binding of metal ions is undoubtedly relevant for double-stranded DNA [24, 28] and has recently also been observed for DNA quadruplexes [29].

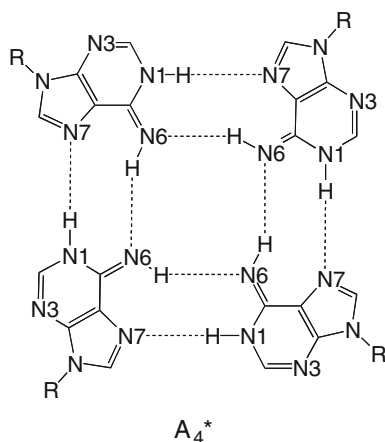
First, we discuss the adenine quartets without ions in three different symmetries (i.e., planar C_{4h} , bowl-shaped C_4 , and saddle- or box-shaped S_4) in the gas phase and in water. Then, we include the cations and anions to investigate the influence of the ions on the structures and energies. Finally, we report results where we simulated the situation in a stack of quartets by keeping A_4 planar in C_{4h} symmetry (with the exception of the amino groups, which were allowed to pyramidalize) and allowing the respective ion to move along the C_4 axis.

Computational methods

All calculations were performed using the Amsterdam Density Functional (ADF) program developed by Baerends et al. [30–41], and the Quantum Regions Interconnected by Local Descriptions (QUILD) program developed by Swart and Bickelhaupt [42, 43]. The QUILD program is a wrapper around ADF (and other programs) and is used for its superior geometry optimizer, which is based on adapted delocalized coordinates [42]. The numerical integration

Scheme 1 Three types of adenine quartets





Scheme 2 A_4^* of the rare imino tautomers of adenine

was performed using the procedure developed by Boerrigter et al. [37] and te Velde and Baerends [38].

The molecular orbitals were expanded in a large uncontracted set of Slater-type orbitals containing diffuse functions: TZ2P (no Gaussian functions are involved) [39]. The basis set was of triple- ζ quality for all atoms and was augmented with two sets of polarization functions, i.e., 2p and 3d on H, 3d and 4f on Li, C, N, O, F, Na, Cl, and K, and 4d and 4f on Br. The 1s core shells of Li, C, N, O, F, and Na, the 1s2s2p core shells of Cl and K, and the 1s2s2p3s3p core shell of Br were treated by the frozen-core approximation [33]. An auxiliary set of *s*, *p*, *d*, *f*, and *g* Slater-type orbitals was used to fit the molecular density and to represent the Coulomb and exchange potentials accurately in each self-consistent-field cycle [40].

The calculations were done with DFT using the BLYP functional [44, 45] with dispersion corrections as developed by Grimme [46, 47] (BLYP-D). In this approach, the BLYP density functional is augmented with an empirical correction for long-range dispersion effects, described by a sum of damped interatomic potentials of the form C_6R^{-6} added to the usual DFT energy [46, 47]. Equilibrium structures were optimized using analytical gradient techniques [41].

Geometries were optimized for species in the gas phase and in aqueous solution (vide infra). All stationary points in the symmetries C_{4h} , C_4 , and S_4 in Tables 1, 2, and 3 (for R is H) were checked to be minima through vibrational analysis. In most cases, no imaginary frequencies were found. In a few cases, small imaginary frequencies occurred, which were, however, shown to be spurious using an explicit potential-energy scan. For the dispersion-corrected functional, the basis set superposition error on the bond energy was not calculated because the dispersion correction [46] was developed such that the small basis set superposition error effects are absorbed into the empirical potential.

Table 1 Bond energies, ΔE_{Bond} (kcal/mol), for the adenine (R is H) and 9-methyladenine (R is CH_3) quartets in the gas phase and in water

	R is H			R is CH_3		
	C_{4h}	C_4	S_4	C_{4h}	C_4	S_4
Gas phase						
$A_4\text{-N1}$	-25.5	-30.2	-46.0	-25.5	-30.1	-49.0
$A_4\text{-N3}$	-33.1	-32.7	-32.8	-33.3	-32.8	-39.9
$A_4\text{-N7}$	-31.9	-33.1	-45.5	-32.5	-33.9	-52.3
A_4^*	-0.6	-1.7		-0.2	-1.5	
Water						
$A_4\text{-N1}$	-10.9	-17.0	-33.2	-10.3	-16.7	-36.0
$A_4\text{-N3}$	-16.8	-16.3	-17.0	-17.1	-17.1	-19.3
$A_4\text{-N7}$	-16.2	-16.3	-27.2	-16.0	-15.6	-31.2
A_4^{*a}	8.6	6.6		9.6	7.3	

Computed at the BLYP-D/TZ2P level. See Eq. 2. Quartets with R is H were verified to be equilibrium structures through vibrational analysis

^a Destabilized complex, separated by a transition state from dissociation within C_{4h} symmetry into individual bases. This barrier occurs as it is unfavorable to break eight hydrogen bonds at the same time

Solvent effects in water were estimated using the conductor-like screening model [48, 49], as implemented in the ADF program [50]. For the settings, see [51, 52] (the radii of Li^+ , Na^+ , K^+ , F^- , Cl^- , and Br^- were determined such that they reproduce the experimental solvation energy of the ions). According to the work of Riley et al. [53], the dispersion correction does not need to be modified for solvated systems.

The bond energy ΔE_{Bond} of the quartet is defined as

$$\Delta E_{\text{Bond}} = \Delta E_{\text{Quartet}} - 4\Delta E_{\text{Adenine}}, \quad (1)$$

where $\Delta E_{\text{Quartet}}$ is the energy of the quartet, optimized in C_{4h} , C_4 , or S_4 symmetry, and $\Delta E_{\text{Adenine}}$ is the energy of the adenine or 9-methyladenine, optimized in C_1 symmetry. In the case where an ion is added to the quartet, the bond energy is defined in a similar way:

$$\Delta E_{\text{Bond}} = \Delta E_{\text{Complex}} - 4\Delta E_{\text{Adenine}} - \Delta E_{\text{Ion}}, \quad (2)$$

where $\Delta E_{\text{Complex}}$ is the energy of the complex, optimized in C_{4h} , C_4 , or S_4 symmetry. Note that also in the case of the rare imino-tautomer quartet A_4^* , ΔE_{Bond} in Eqs. 1 and 2 still has the regular adenine tautomers as a reference and, therefore, comprises both the tautomerization energy of the four nucleobases plus the complexation energy between them in A_4^* .

For the calculations where the situation in the stack was simulated by keeping the adenine quartet flat, we also defined the energy of stabilization of the quartet by the ion:

$$\Delta E_{\text{stab}} = \Delta E_{\text{Complex}, C_4} - \Delta E_{\text{Quartet}, C_{4h}} - \Delta E_{\text{Ion}}. \quad (3)$$

The energy of the complex, $\Delta E_{\text{Complex}, C_4}$, was obtained from a constrained optimization, in which the quartet was

Table 2 Bond energies, ΔE_{Bond} (kcal/mol), in the gas phase between four adenine bases (R is H) or four 9-methyladenine bases (R is CH₃) and an ion for equilibrium structures in C_{4h}, C₄, and S₄ symmetry

Quartet	Ion	R is H			R is CH ₃		
		C _{4h}	C ₄	S ₄	C _{4h}	C ₄	S ₄
A ₄ -N1	Li ⁺		-125.5	-151.0		-129.3	-158.1
	Na ⁺		-106.7	-119.7		-113.3	-126.3
	K ⁺		-90.2			-95.1	
	F ⁻	-111.7	-114.4		-109.1	-112.0	
	Cl ⁻		-77.8			-75.4	
	Br ⁻		-70.7			-68.4	
A ₄ -N3	Li ⁺			-139.4			-143.4
	Na ⁺			-116.8			-121.0
	K ⁺	-91.5	-92.0	-91.9	-94.2	-94.8	-97.7
	F ⁻	-53.3	-54.0		-52.7	-54.1	
	Cl ⁻		-35.6			-35.5	
	Br ⁻		-32.9			-32.7	
A ₄ -N7	Li ⁺		-129.3	-145.3		-134.8	-159.0
	Na ⁺		-117.1	-116.3		-122.4	-129.2
	K ⁺		-94.6			-101.0	
	F ⁻	-113.2			-110.7		
	Cl ⁻		-80.8			-78.7	
	Br ⁻		-74.2			-72.2	
A ₄ *	Li ⁺	-121.3	-123.6		-125.2	-128.0	
	Na ⁺		-95.4			-99.7	
	K ⁺		-65.5			-69.0	

Computed at the BLYP-D/TZ2P level. See Eq. 2. Quartets with R is H were verified to be equilibrium structures through vibrational analysis. *Empty cells* indicate that the structures of the respective symmetry are labile with respect to deformation toward one of the other point-group symmetries

kept C_{4h}-symmetric, except for the amino groups, which were allowed to pyramidalize, and the ion was allowed to move along the C₄ symmetry axis. The energy of the quartet, $\Delta E_{\text{Quartet, C4h}}$, was obtained from a C_{4h} optimization.

Results and discussion

Adenine quartets

First, we consider the formation of the various types of adenine quartets (i.e., A₄-N1, A₄-N3, A₄-N7, and A₄*), both in the gas phase and in aqueous solution, but in the absence of any ions. Here, we focus on the relative stability of the aforementioned geometrical shapes of these quartets, namely, planar, bowl-shaped, and saddle-shaped, which correspond to stationary points of C_{4h}, C₄, and S₄ point-group symmetry, respectively. The corresponding bond energies, i.e., energies of formation of the

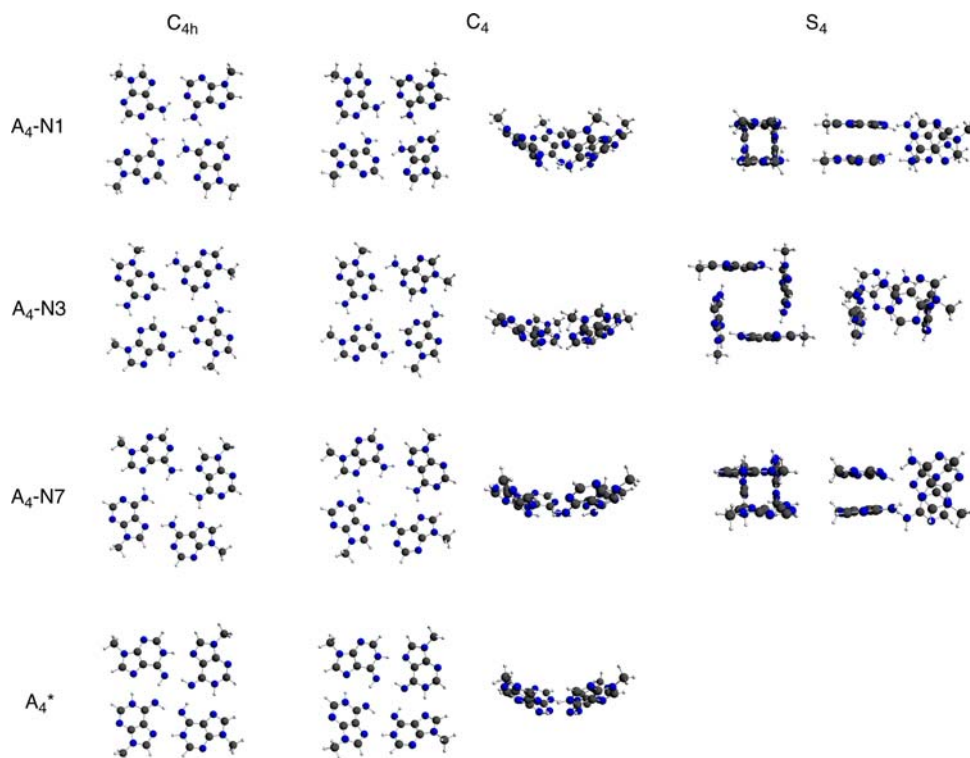
Table 3 Bond energies, ΔE_{Bond} (kcal/mol), in water between four adenine bases (R is H) or four 9-methyladenine bases (R is CH₃) and an ion for equilibrium structures in C_{4h}, C₄, and S₄ symmetry

Quartet	Ion	R is H			R is CH ₃		
		C _{4h}	C ₄	S ₄	C _{4h}	C ₄	S ₄
A ₄ -N1	Li ⁺		-25.6	-49.8		-26.6	-50.9
	Na ⁺		-37.5	-46.8		-38.4	-41.9
	K ⁺		-34.3	-32.3		-35.0	-34.5
	F ⁻	-27.1	-31.3		-26.8	-31.0	
	Cl ⁻		-23.9			-23.7	
	Br ⁻		-23.7			-23.6	
A ₄ -N3	Li ⁺			-33.6			-33.6
	Na ⁺			-37.7			-38.1
	K ⁺	-31.0	-29.5		-30.2	-29.5	
	F ⁻		-6.6			-8.2	
	Cl ⁻		-10.2			-11.6	
	Br ⁻		-12.6			-13.9	
A ₄ -N7	Li ⁺		-29.2	-47.5		-29.8	-51.2
	Na ⁺		-34.7	-43.5		-36.3	-48.1
	K ⁺		-32.1	-32.6		-33.3	-36.0
	F ⁻	-26.7	-26.8		-26.5	-26.2	
	Cl ⁻		-21.7			-21.1	
	Br ⁻		-22.0			-21.6	
A ₄ *	Li ⁺	-13.2	-17.7		-12.3	-16.5	
	Na ⁺		-16.0			-15.3	
	K ⁺		-6.5			-5.2	

Computed at the conductor-like screening model (COSMO)-BLYP-D/TZ2P level. See Eq. 2. Quartets with R is H were verified to be equilibrium structures through vibrational analysis. *Empty cells* indicate that the structures of the respective symmetry are labile with respect to deformation toward one of the other point-group symmetries

quartets from four adenine or four 9-methyladenine bases, are collected in Table 1 (see also Eq. 1). The structures of the quartets in water are shown in Fig. 1. The three regular quartets, i.e., A₄-N1, A₄-N3, and A₄-N7, have an S₄-symmetric structure as the global minimum, both in the gas phase and in water. For A₄-N1 and A₄-N7 these global minima are saddle-shaped, characterized by two stacks of two adenine bases which are rotated with respect to each other by 90° and mutually bind through N-H...N hydrogen-bonding. For A₄-N3 the global minimum is box-shaped with the four bases positioned as the sides of a box. The corresponding bond energies ΔE_{Bond} relative to four separate bases (see Eq. 1) are some -30 to -52 kcal/mol in the gas phase and -17 to -36 kcal/mol in water. Note that this result is essentially not changed by substitution of the hydrogen atom (R is H) at N9 by a methyl group (R is CH₃), although the bond energies are a few kilocalories per mole greater in the latter case (R is CH₃).

Fig. 1 C_{4h} -, C_4 -, and S_4 -symmetric equilibrium structures of empty 9-methyladenine quartets in water (for C_4 and S_4 , the top and side views are shown)



In stacks, the DNA-base quartets adopt planar structures; therefore, an important quantity in connection with forming such stacks is the planarization energy, i.e., the energy needed to go from the global minimum of the quartet to the planar, C_{4h} -symmetric structure. The planarization energy is in all cases endothermic. In the case of the unsubstituted adenine bases (i.e., for R is H), the planarization of A_4 -N1 and A_4 -N7 is associated with an endothermicity of 22.3 and 11 kcal/mol, respectively. On the other hand, the planarization energy of A_4 -N3 (0.2 kcal/mol only) is very slightly endothermic, actually close to thermoneutral.

Furthermore, we find that A_4^* , consisting of the rare imino tautomers, is not stable. In water, it is destabilized by some 7 kcal/mol with respect to four regular adenine bases, with either R is H or R is CH_3 . Also in the gas phase, it is hardly stabilized with respect to four regular adenine bases, only by -1.7 kcal/mol. The reason is that the bond energy for the formation of A_4^* relative to four rare tautomers is not sufficiently stabilizing to compensate the endothermic tautomerization energy. For example, in water, the tautomerization of four 9-methyladenine bases toward the four corresponding imino tautomers amounts to 32.0 kcal/mol (not shown in Table 1). This can not be fully compensated by the bond energy of -22.4 kcal/mol in water between these four tautomers in the C_{4h} -symmetric A_4^* (not shown in Table 1).

Adenine quartets binding cations and anions

Next, we address the question of whether the various adenine quartets can bind alkali cations and halide anions and, if so, how strongly they bind them. In particular, we explore the differential stabilization of the various quartets by the formation of these quartet–ion complexes. The corresponding bond energies, i.e., energies of formation of the quartet–ion complexes from four adenine (R is H) or four 9-methyladenine (R is CH_3) bases in the gas phase and in aqueous solution are collected in Tables 2 and 3, respectively (see also Eq. 2). Selected structures of quartet–ion complexes in water are shown in Figs. 2 and 3.

The planar, C_{4h} -symmetric structure, which could correspond to a situation in the stack, is in most cases not a stationary point, neither in the gas phase nor in water. The lability of the planar quartet–ion structures is related to the size of the ion, which is either too big or too small to properly fit into the center cavity of the quartet. In the gas phase, F^- is the only halide anion that is small enough to fit into the center of the planar A_4 -N1, A_4 -N3, and A_4 -N7 structures, with bond energies ΔE_{Bond} of -111.7 , -53.3 , and -113.2 kcal/mol, respectively (see Table 2). Furthermore, Li^+ is small enough to fit into the center of planar A_4^* , yielding a bond energy of -121.3 kcal/mol. On the other hand, K^+ is big enough to fit into the center of planar A_4 -N3, resulting in a bond energy of -91.5 kcal/mol (see

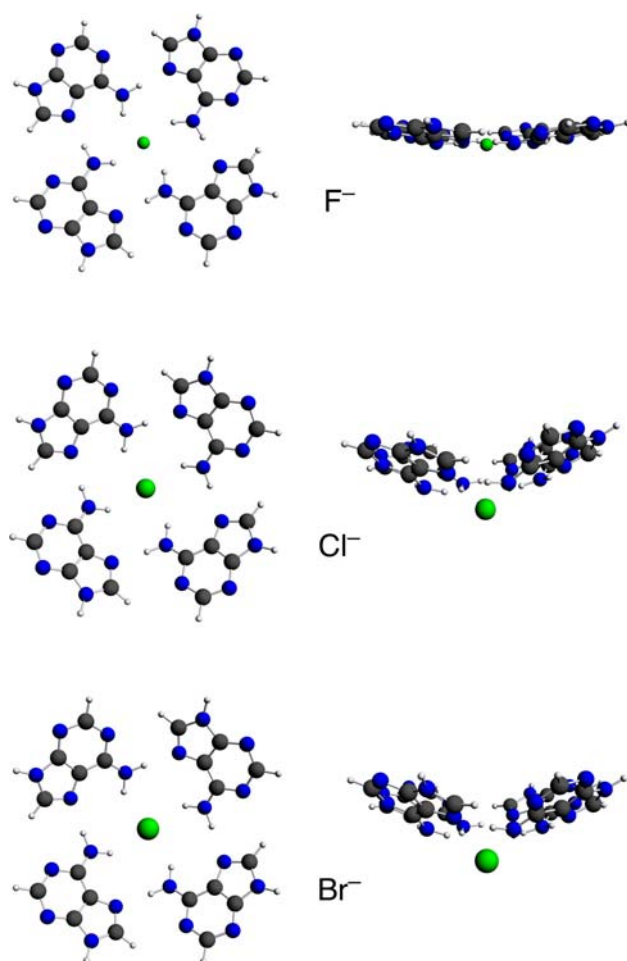


Fig. 2 Top and side views of the C_4 -symmetric equilibrium structure of A_4 -N7 binding halide anions (F^- , Cl^- , and Br^-) in water

Table 2). The Pauling radius [54] is very similar for F^- (1.36 Å) and K^+ (1.33 Å) and this seems to be the optimal size to fit into the center of the quartet.

In aqueous solution, the planar structures are even less stable as all bond energies are reduced owing to solvent effects. Thus, the only stable planar, C_{4h} -symmetric adenine quartet–ion complexes are those of fluoride with A_4 -N1 and A_4 -N7, which both have a bond energy ΔE_{Bond} of some 27 kcal/mol (see Table 3). The only other planar quartet–ion complex among our model systems is that of the rare imino tautomers with the lithium cation, with a bond energy of -13.2 kcal/mol (see Table 3).

The global energy minima for quartet–ion complexes are therefore mostly nonplanar, similar to the situation for the empty quartets. However, at variance with the latter, which are saddle- or box-shaped (S_4), it depends on the type of ion if we deal with a bowl-shaped (C_4) or a saddle- or box-shaped (S_4) complex. Focusing now on the condensed phase, the halide anions stabilize preferentially the bowl-shaped (C_4 -symmetric) structures of A_4 -N1, A_4 -N3,

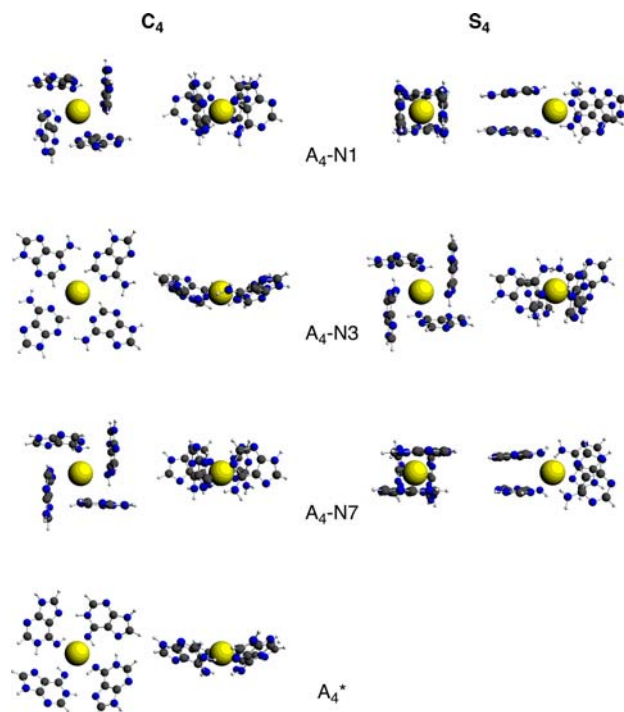


Fig. 3 Top and side views of C_4 - and S_4 -symmetric equilibrium structures of adenine quartets binding a potassium cation in water

and A_4 -N7 (see Table 3). The bond energies ΔE_{Bond} in water of the halide anions with, for example, A_4 -N7 amount to -26.8 , -21.7 , and -22.0 kcal/mol, respectively, for F^- , Cl^- , and Br^- . The corresponding structures are visualized in Fig. 2. There, one can see that F^- fits into the center of the planar quartet, whereas the quartet becomes increasingly bowl-shaped as the size of the anion becomes larger as we move from F^- to Cl^- to Br^- . The reason for this deformation is threefold: (1) as the halide becomes effectively larger, it no longer fits into the central cavity of a planar quartet structure and, thus, moves out of this cavity; (2) to retain the $N(6)\text{--}H\text{--}X^-$ hydrogen bonds and to accomplish an optimal interaction with the anion, the quartet deforms so as to align the $N(6)\text{--}H$ bonds toward the halide anion; (3) this happens such that overlap between the anion and the π -electron system of the adenine bases is avoided (see Fig. 2).

On the other hand, the alkali cations stabilize preferentially the saddle- or box-shaped (S_4) structures of the regular adenine quartets (see Table 3). Note, however, that the preference for saddle- or box-shaped (S_4) over bowl-shaped (C_4) quartet–alkali cation complexes becomes smaller as we move from Li^+ to Na^+ to K^+ . In the case of the K^+ complexes of A_4 -N1 and A_4 -N3 (but not for that of A_4 -N7), there is even a switch in preferential stabilization from saddle- or box-shaped to bowl-shaped (see Table 3). Figure 3 shows the bowl-shaped C_4 and saddle- or box-shaped S_4 structures of the adenine quartets with K^+ in

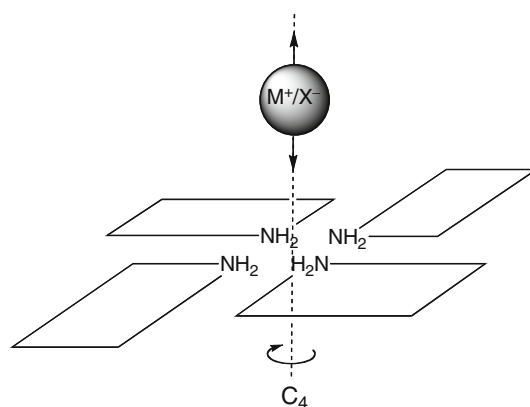
water. The structures of these K^+ complexes are similar to those of the empty quartets, except for the C_4 structures of A_4-N1 and A_4-N7 . What happens is that the quartets align the lone pairs on the nitrogen atoms at the central cavity toward the cation. In the idealized, planar representation in Scheme 1, this is the amino N6 atom in A_4-N1 and A_4-N7 and the aromatic N1 in A_4-N3 . Indeed, these atoms are involved in the bonding with the alkali cation. However, as can be seen in Fig. 3, A_4-N1 and A_4-N7 rearrange somewhat and fold around the cation. The main reason is that, in this way, these quartets can engage additional nitrogen atoms from the aromatic ring (namely, N7 in the case of A_4-N1 and N1 in the case of A_4-N7) in the bonding with the alkali cation.

The addition of either halide anions or alkali cations leads to the preferential stabilization of A_4-N1 , which is also the most stable adenine quartet in the absence of these ions (compare the data in Tables 1, 3). All trends are more or less identical for adenine (R is H) and 9-methyladenine (R is CH_3) quartets. Interestingly, although the tautomeric form A_4^* does not bind to halide anions, it does bind to alkali cations, which stabilize this rare tautomer quartet with respect to four regular adenine bases (see Table 3).

Models of adenine quartets in stacks binding cations and anions

Finally, we explore the quartet–ion interactions under circumstances as they occur in stacks of adenine quartets in which the latter preserve a planar geometry. As pointed out earlier, planar quartet geometries in most cases do not correspond to the intrinsic global minimum. The planarization energy, which ranges from only a few tenths of a kilocalorie per mole for A_4-N3 up to 22 kcal/mol for A_4-N1 (vide supra), must be compensated in the stacks by the gain in π – π stacking interaction. Here, we focus on the interaction of the ion with one quartet in a geometry corresponding to the situation in the stack. To simulate this situation, we optimized the quartet–ion complexes under the constraint that the adenine bases were kept in one plane except for the nitrogen and hydrogen atoms of the N(6) H_2 amino groups of A_4-N1 and A_4-N7 . The ion was allowed to move along the C_4 symmetry axis and for A_4-N1 and A_4-N7 the amino groups were allowed to pyramidalize (see Scheme 3). Note, however, that these models are not equilibrium structures on their own!

The planar quartets that simulate the situation in quartet stacks appear to have substantial bonding interactions with ions, both in the gas phase and in water, as can be seen in Tables 4 and 5, respectively. As found for the equilibrium structures discussed earlier, the stabilization due to ion binding is weakened if we go from the gas phase to aqueous solution. In the following, we focus on the situation in water (see Table 5).



Scheme 3 The C_4 -symmetric systems in Tables 4 and 5 were optimized under the constraint that all atoms of the bases were kept in one plane, except for the nitrogen and hydrogen atoms of the N(6) H_2 amino groups of A_4-N1 and A_4-N7 , which were allowed to pyramidalize; the cation or anion was allowed to move along the C_4 symmetry axes

In the case of coordination in the central cavity or along the C_4 axis of the quartet, we find bond energies for alkali cations of up to -29 kcal/mol (see Table 5). This corresponds to stabilization energies ΔE_{stab} between alkali cations and a C_{4h} quartet of regular adenine bases (i.e., A_4-N1 , A_4-N3 , and A_4-N7) of up to -6 kcal/mol for A_4-N1 and up to -12 kcal/mol for A_4-N3 . Note that A_4-N7 does not form stable complexes with the ion in the quartet center: at a distance of approximately 0.1 \AA above the quartet plane, there is a dip in the potential energy surface at elevated energies of 4 – 21 kcal/mol. Note, however, that this quartet, A_4-N7 , forms stable complexes in which the ion binds at the periphery of the quartet, with stabilization energies of up to -10 kcal/mol in the case of Na^+ (see the C_s -symmetric complexes in Table 5). In the peripheral coordination mode, the alkali cations bind to an adenine N3 atom of A_4-N7 . Likewise, peripheral coordination is also preferred in the case of A_4-N1 , in which case the alkali cations also bind to an adenine N3 atom. Nevertheless, A_4-N1 can also form coordination complexes with the ion in the central cavity, in which case the N(6) H_2 amino groups pyramidalize such that the N–H bonds point away and the N lone-pair-type orbital (i.e., the fourth-highest occupied molecular orbital) points toward the alkali cation. This is illustrated in on the left of Fig. 4 for $A_4-N1 \cdots Na^+$, which for the purpose of bonding analyses is taken here in the gas phase. The fourth-highest occupied molecular orbital of the quartet interacts with and donates 0.1 electrons into the sodium $3s$ atomic orbital.

In the case of the rare imino-tautomer quartet A_4^* , the stabilization energy even reaches values of -22.3 kcal/mol for coordination of Na^+ in the central cavity. These are significantly more stabilizing ΔE_{stab} values than in the case of the regular adenine quartet–cation complexes. In this

Table 4 Bond energies, ΔE_{Bond} (kcal/mol), stabilization energies, ΔE_{stab} (kcal/mol), hydrogen-bond distances, r_{HB}^{a} (Å), and vertical separation, z (Å), in the gas phase for the complex of four 9-methyladenine bases and an ion

Quartet	Ion	C_{4h}			C_4				C_s	
		ΔE_{Bond}	ΔE_{stab}	r_{HB}^{a}	ΔE_{Bond}	ΔE_{stab}	r_{HB}^{a}	z^{b}	ΔE_{Bond}	ΔE_{stab}
A ₄ -N1	None	-25.5		2.91	-25.5		2.91		-25.5	
	Li ⁺	-32.4	-6.9	3.12	-105.5	-80.0	2.85	0.73	-83.2	-57.7
	Na ⁺	-48.8 ^c	-23.3 ^c	3.28 ^c	-88.3	-62.8	2.88	1.30	-66.4	-40.9
	K ⁺	-47.1 ^c	-21.6 ^c	3.30 ^c	-70.2	-44.7	2.90	1.89	-54.5	-29.0
	F ⁻	-109.1	-83.6	3.08	-109.1	-83.6	3.08	0.00		
	Cl ⁻	-60.3	-34.8	3.49	-64.0	-38.5	3.04	1.93		
	Br ⁻	-49.4	-23.9	3.75	-56.1	-30.6	3.03	2.24		
A ₄ -N3	None	-33.3		3.00	-33.3		3.00		-33.3	
	Li ⁺	-101.4	-68.1	2.88	-101.4	-68.1	2.88	0.00	-86.5	-53.2
	Na ⁺	-103.9	-70.6	2.89	-103.9	-70.6	2.89	0.00	-70.4	-37.1
	K ⁺	-94.2	-60.9	2.99	-94.2	-60.9	2.99	0.00	-58.3	-25.0
	F ⁻	-52.7	-19.4	3.06	-52.7	-19.4	3.06	0.00	- ^d	
	Cl ⁻	-22.1	11.2	3.32	- ^e	- ^e	- ^e	- ^e	-54.8	-21.5
	Br ⁻	-14.0	19.3	3.47	- ^e	- ^e	- ^e	- ^e	-51.1	-17.8
A ₄ -N7	None	-32.5		2.88	-32.5		2.88		-32.5	
	Li ⁺	-50.4	-17.9	2.77	-91.1	-58.6	2.82	0.61	-90.2	-57.7
	Na ⁺	-44.3	-11.8	2.86	-82.5	-50.0	2.83	1.00	-73.4	-40.9
	K ⁺	-33.1	-0.6	2.97	-69.2	-36.7	2.85	1.53	-60.4	-27.9
	F ⁻	-110.7	-78.2	2.86	-110.7	-78.2	2.86	0.00		
	Cl ⁻	-76.5	-44.0	3.00	-76.7	-44.2	2.98	0.57		
	Br ⁻	-66.7	-34.2	3.07	-68.8	-36.3	2.97	1.28		
A ₄ [*]	None	-0.2		2.87 (3.07)	-0.2		2.87 (3.07)		-0.2	
	Li ⁺	-125.2	-125.0	2.92 (3.19)	-125.2	-125.0	2.92 (3.19)	0.00	-58.4	-58.2
	Na ⁺	-92.5	-92.3	3.24 (3.53)	-93.0	-92.8	2.97 (3.21)	1.22	-40.5	-40.3
	K ⁺	-54.0	-53.8	3.76 (4.11)	-68.7	-68.5	2.92 (3.13)	1.92	-26.8	-26.6

Computed at the BLYP-D/TZ2P level. See Eqs. 1, 2, and 3

^a Hydrogen-bond distances defined for each quartet as the distance between the proton donor and the proton acceptor. N7–N1 hydrogen-bond distance in A₄^{*} in parentheses

^b Vertical separation between ion and plane of quartet

^c A₄-N1 switches connectivity from regular N(6)–H6⋯N(1) to the other N(6)–H6'⋯N(1) hydrogen bond

^d F⁻ abstracts a proton from the amino group

^e Ion does not bind

way, the rare imino tautomers can be stabilized relative to separate regular adenine bases through coordination of A₄^{*} with alkali cations (see the ΔE_{Bond} values in Table 5). However, the corresponding Na⁺ and K⁺ complexes of the regular A₄-N1 and A₄-N3 remain more stable than those of A₄^{*} (see the ΔE_{bond} values in Table 5). Furthermore, we note that the alkali cations take up positions that are in general above the planar quartet structure, i.e., by 0.1 (A₄-N3⋯K⁺) to 2.9 Å (A₄-N1⋯K⁺). Only in the case of A₄^{*}⋯Li⁺ does the cation go into the center of the planar quartet structure.

Interestingly, regular A₄-N1 and A₄-N7 (see Scheme 1) can also firmly bind halide anions in their central cavity with bond energies of up to -27 kcal/mol and stabilization

energies that reach approximately -17 kcal/mol for A₄-N1⋯F⁻ (see Table 5). Note that peripheral coordination is not possible for these adenine quartets as there are no proper hydrogen-bond-donor groups in the periphery (see Scheme 1). Thus, A₄-N7, for example, binds F⁻, Cl⁻, and Br⁻ with stabilization energies of -10.5, -5.0, and -5.7 kcal/mol, respectively (see Table 5). In the latter two complexes, the N(6)H₂ amino groups of the quartet (which is otherwise kept planar to mimic the stacking situation) pyramidalize such that the N–H bonds point toward the anion. This is illustrated on the right in Fig. 4 for A₄-N1⋯Cl⁻, which for the purpose of bonding analyses is taken here in the gas phase. The lowest unoccupied molecular orbital of the quartet interacts with and accepts

Table 5 Bond energies, ΔE_{Bond} (kcal/mol), stabilization energies, ΔE_{stab} (kcal/mol), hydrogen-bond distances, r_{HB} (Å), and vertical separation, z (Å), in water for the complex of four 9-methyladenine bases and an ion

Quartet	Ion	C_4				C_s	
		ΔE_{Bond}	ΔE_{stab}	r_{HB}^a	z^b	ΔE_{Bond}	ΔE_{stab}
A ₄ -N1	None	-10.3		2.95			
	Li ⁺	- ^c	- ^c	- ^c	- ^c	-17.8	-7.5
	Na ⁺	-16.3	-6.0	2.92	1.47	-20.5	-10.2
	K ⁺	-13.5	-3.2	2.94	2.05	-17.8	-7.5
	F ⁻	-26.8	-16.5	3.11	0.00		
	Cl ⁻	-15.8	-5.5	3.02	2.19		
A ₄ -N3	None	-17.1		3.03			
	Li ⁺	- ^d	- ^d	- ^d	- ^d	-25.6	-8.5
	Na ⁺	-28.9	-11.8	2.93	0.1	-25.8	-8.7
	K ⁺	-28.9	-11.8	3.02	0.1	-23.7	-6.6
	F ⁻	- ^d	- ^d	- ^d	- ^d	-20.2	-3.1
	Cl ⁻	- ^d	- ^d	- ^d	- ^d	-17.8	-0.7
A ₄ -N7	None	-16.0		2.91			
	Li ⁺	5.4	21.4 ^e	2.87	0.87	-22.2	-6.2
	Na ⁺	-9.0	7.0 ^e	2.87	1.19	-26.1	-10.1
	K ⁺	-11.7	4.3 ^e	2.89	1.75	-23.3	-7.3
	F ⁻	-26.5	-10.5	2.89	0.00		
	Cl ⁻	-21.0	-5.0	2.88	1.55		
A ₄ *	None	9.6		2.91 (3.07)			
	Li ⁺	-12.3	-21.9	2.95 (3.20)	0.00	3.8	-5.8
	Na ⁺	-12.7	-22.3	2.91 (3.11)	1.50	-0.1	-9.7
	K ⁺	-5.5	-15.1	2.91 (3.09)	2.09	2.6	-7.0

Computed at the COSMO-BLYP-D/TZ2P level. See Eqs. 1, 2, and 3

^a Hydrogen-bond distances defined for each quartet as the distance between the proton donor and the proton acceptor. N7–N1 hydrogen-bond distance in A₄* in parentheses

^b Vertical separation between ion and plane of quartet

^c Ion does not bind

^d Ion binds very weakly at about 5.0 Å, which is not relevant for the situation in a stack of quartets

^e Destabilized complex, separated by a transition state from dissociation

0.09 electrons from the chloride 3p atomic orbitals. The F⁻ anion is small enough to be accommodated within the A₄-N7 complex, but the larger Cl⁻ and Br⁻ adopt positions 1.55 and 1.91 Å above the plane of the quartet (see Table 5). These trends for the artificial A₄-N7...X⁻ models (which, as we recall, mimic the situation in a larger stack) reflect similar but more pronounced trends in the corresponding but more tightly bound, bowl-shaped (C₄) equilibrium structures (see Table 3).

In the case of A₄-N3, the situation is in a sense inverted compared with that for A₄-N1 and A₄-N7. In A₄-N3, there are no hydrogen-bond-donor groups in the inner cavity and consequently no stable central-cavity coordination complexes A₄-N3...X⁻ are found. However, in A₄-N3, we have good hydrogen-bond donors at the periphery, namely, N(6)-H bonds pointing outward. This leads to the formation of peripheral-coordination complexes A₄-N3...X⁻ that are weakly bound by up to -3.1 kcal/mol for A₄-N3...F⁻ (see Table 5).

Conclusions

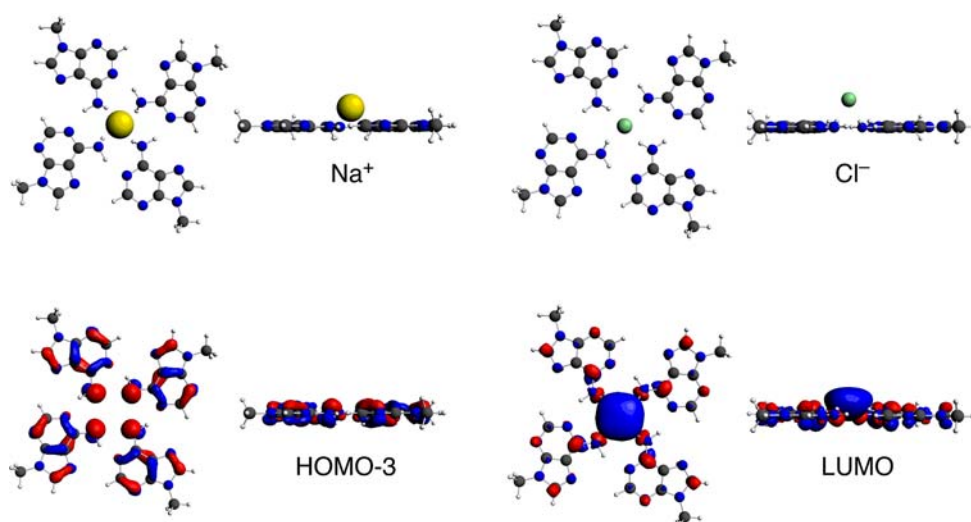
We have shown, using dispersion-corrected DFT, that adenine quartets can bind both cations and anions in or above their central cavity in the gas phase as well as in aqueous solution. Our study comprised three regular adenine quartets (A₄-N1, A₄-N3, and A₄-N7) as well as the rare imino-tautomer quartet (A₄*). The global minima of these quartets are in general bowl-, saddle-, or box-shaped. In the case of the fluoride complexes of A₄-N1 and A₄-N7 and the lithium complex of A₄* there also exist planar equilibrium structures. The isolated adenine quartets prefer a nonplanar geometry in the gas phase and in water. This preference does not change upon adding a cation or an anion.

The ion-quartet interaction in a stacking environment was modeled by keeping the quartet artificially planar and only allowing nonplanarity of the inner N(6)H₂ amino groups of A₄-N1 and A₄-N7 (pyramidalization) and the ion (can adopt its optimal position in or above the central cavity). This leads again to substantial stabilization for cations and anions. Interestingly, the anions bind to A₄-N1 and A₄-N7 preferentially via coordination in (F⁻) or above (Cl⁻, Br⁻) the central cavity. In these cases, the inner N(6)H₂ amino groups pyramidalize such that the N-H bonds point toward the anions. This geometry optimizes electrostatic and donor-acceptor orbital interactions. Coordination of anions to the quartet's peripheral N(6)-H bonds is the preferred mode in the case of A₄-N3.

The cations prefer peripheral coordination to a nitrogen atom in the case of A₄-N1 and A₄-N7. But coordination at the central cavity is also possible for A₄-N1, with stabilization energies of -3.2 kcal/mol for K⁺ and -6.0 kcal/mol for Na⁺ in water. In the case of A₄-N3, coordination at the central cavity of cations is even the preferred ion-binding mode, with stabilization energies of approximately -12 kcal/mol for both Na⁺ and K⁺.

Our findings are relevant in connection with the role of adenine quartets in tetrastranded nucleic acids (as derived from negatively charged oligonucleotides) or in artificial analogues (as derived from uncharged adenine

Fig. 4 *Top:* Top and side views of planar A_4-N1 interacting in the gas phase with a sodium cation (*left*) or a chlorine ion (*right*). *Bottom:* Corresponding A_4-N1 fourth-highest occupied molecular orbital (*HOMO-3*), which donates charge into sodium $3s$ atomic orbitals (*left*), and A_4-N1 lowest unoccupied molecular orbital (*LUMO*), which accepts charge from the chloride $3p$ atomic orbital (*right*)



moieties). Thus, whenever the quartets are forced into planarity by stacking interactions and having the amino groups in the interior, halide anions and in particular the smallest one, fluoride, are good choices for stabilizing such an arrangement.

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