ELSEVIER

Finding and visualizing nucleic acid base stacking

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Base stacking is one of the primary factors stabilizing nucleic acid structure. Yet, methods for locating stacking interactions in DNA and RNA are rare and methods for displaying stacking are rarer still. We present here simple, automated procedures to search nucleic acid molecules for base–base and base–oxygen stacking and to display these interactions graphically in a manner that readily conveys both the location and the quality of the interaction. The method makes no a priori assumptions about relative base positions when searching for stacking, nor does it rely on empirical energy functions. This is a distinct advantage for two reasons. First, the relative contributions of the forces stabilizing stacked bases are unknown. Second, the electrostatic and hydrophobic components of base stacking are both poorly defined by existing potential energy functions.

INTRODUCTION

In 1953 Watson and Crick[1] proposed a structure for DNA based on fiber diffraction photographs and molecular modeling. Their model consisted of a sugar–phosphate double helix winding smoothly around a common axis with hydrogen-bonded bases stacked in the interior. More precise crystallographic studies later proved this basic structure correct[2] but also showed that DNA is highly flexible and capable of significant deviations from "canonical" structure.[3] Nucleic acids have also been shown to adopt conformations not at all similar to the original Watson and Crick model (as reviewed in Ref. 4).

In a regular double helix (e.g., the A, B, and Z conformational families) the hydrogen bonds between paired bases serve mainly to hold the strands together. In general, hydrogen-bonding patterns remain constant in regular nucleic acid helices and obey standard Watson–Crick base pairing. Likewise, small changes in the six dihedral angles of the local sugar–phosphate backbone (i.e., α, β, γ, δ, ϵ, and ζ) can easily absorb changes in base pair geometry.[5] The fact that base pairs alone in solution have a natural tendency to form helices is another indication that the sugar–phosphate plays a rather passive role in nucleic acid structure. The interactions between the planar, hydrophobic bases stacked neatly between the strands impart to nucleic acids their distinctive helical structure.[6] Several structural phenomena are known to be sequence dependent (e.g., DNA polymorphism and curvature). This implies that base-stacking interactions are responsible to a large degree for helix variability.

In spite of the importance of stacking interactions, automated methods for finding stacked bases in nucleic acids and representing them graphically are not readily available. By contrast, finding a hydrogen bond is a comparatively simple task. One need only locate a hydrogen bound to an electronegative atom and search for suitable acceptors within the sphere of influence of that hydrogen. At that point a line connecting the hydrogen and its heavy atom acceptor is sufficient to illustrate the hydrogen bond, and varying the thickness or color of this line is sufficient to illustrate its quality. For stacking interactions, space-filling or van der Waals models give some information but since such models are not transparent they block the view to atoms beyond the solvent-accessible surface of the molecule. It therefore becomes necessary to manipulate the model in space in order to examine every pair of adjacent bases. This leads to the second problem—van der Waals models are cumbersome and difficult to manipulate manually, or as a computer graphics image, for all but small nucleic acids. Real-time manipulation of a transfer RNA space-filling model, for example, requires considerable computer power. Also, space-filling models do not call attention directly to stacked bases, nor do they easily convey information regarding the quality of the stacking interaction.

Two general approaches can be used to search for stacking. It is possible to assess the quality of a stacking arrangement using an energy function or by examining spatial ori-

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entation between two bases. We exclude the former case for the following reasons. Stacking is composed of hydrophobic and electrostatic interactions. Electrostatics plays an important role in long-range sequence effects[7,8] but its role in individual base–base stacking interactions can be effectively ignored.[9] Hydrophobicity is a considerably more important factor in base stacking. However, electrostatics and hydrophobicity are poorly described by existing potential energy force fields, especially when considering stacking.[10]

The second approach is to search for stacking based on spatial orientation. There are two competing schools of thought regarding optimal stacking geometry. The first is embodied in the classic work of Bugg et al,[11] which suggests that large base overlap is the exception rather than the rule in stacking. They contend that induction, the interaction between polar exocyclic substituents and the delocalized electrons of the adjacent aromatic ring system, confers stability. More recent work, however, challenges this view of stacking. Extensive *ab initio* calculations show no significant stabilization from induction[12] and suggest that packing forces[10,12] may have contributed to the conclusions of Bugg et al.[11] Furthermore, their results show that the degree of ring overlap determines the strength of a stacking interaction.[12]

Although neither the induction nor the maximal ring overlap view of stacking geometry is entirely correct by itself, we favor the more recent data. In this article, stacking is defined as the parallel, or nearly parallel, interaction of two planar hydrophobic bases in which some degree of ring overlap is present. This definition is more stringent than that used in most studies.

We present here a method for finding and displaying base–base and base–oxygen stacking that suffers from none of the shortcomings associated with potential energy functions (i.e., finding) or space-filling models (i.e., displaying). Our algorithms use a set of geometric criteria to find stacking. These criteria are both intuitive and easy to implement and can be set to whatever stringency is desired for the analysis. Also, the search algorithms are sufficiently general that they can be used on nucleic acids not possessing regular helical structure, because helical coordinates are not taken into account.

METHODS

Finding base–base stacking

A sequence of criteria must be satisfied before a pair of bases is declared stacked (Figure 1). First, a distance vector d_{ij} connecting the geometric centers of two potentially stacked bases is constructed. For purines, the geometric centers of both the pyrimidine and imidazole rings are used. If this distance is below the user-defined cutoff, the unit normal vectors extending from the α faces (as defined by Rose et al.[13]) of the mean planes of both rings are calculated using the Cremer and Pople method.[14] Briefly, the mean plane of a ring is defined by two vectors

$$r_1 = \sum_{j=1}^{N} r_j \cos[2\pi(j-1)/N]$$

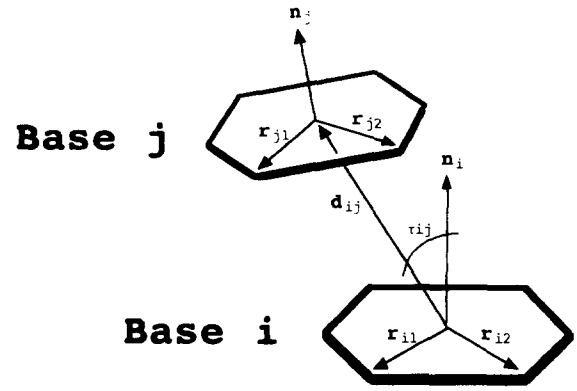

Figure 1. Search algorithm for finding stacked bases.

$$r_2 = \sum_{j=1}^{N} r_j \sin[2\pi(j-1)/N]$$

where r_j is the positional vector of each ring atom with respect to the ring geometric center and N is the number of ring atoms (i.e., five for imidazoles and six for pyrimidines). The normal vectors for bases i and j are calculated as

$$n_i = \frac{r_{i1} \times r_{i2}}{|r_{i1} \times r_{i2}|}$$

$$n_j = \frac{r_{j1} \times r_{j2}}{|r_{j1} \times r_{j2}|}$$

If the mean plane vectors are calculated from atom positions clockwise around the ring, then the positive direction of the normal vector extends from the α face of the base. If the angle between these vectors (or its supplement)

$$\theta_{ij} = \cos^{-1}(n_i \cdot n_j)$$

is less than the user-defined value, the overlap of the bases is determined using the angle between the unit distance vector connecting the ring geometric centers and one of the base normal vectors:

$$\tau_{ij} = \cos^{-1}\left[n_i \cdot \frac{d_{ij}}{|d_{ij}|}\right]$$

If τ_{ij} (or its supplement) is less than the user-defined value then the bases are considered stacked. If any of these criteria are not satisfied, the bases are assumed to be unstacked.

Finding base–oxygen stacking

Base–oxygen stacking is neither common nor widely accepted as a true stacking interaction. Its energetics are poorly understood compared to base–base stacking. With the notable exception of Z-DNA, it is rarely, if ever, seen in regular nucleic acid helices. However, this type of stacking occurs in RNA hairpin loops,[15] cytosine and various other crystals,[11,16] and in the recently defined crystal structure of guanylyl-2′,5′-cytidine dihydrate.[17] In fact, base–ribose stacking is common in nucleotides linked by 2′,5′-phosphodiester bonds.[17–19] Internucleotide 2′,5′-phosphodiester linkages have been detected in interferon-treated

cells and in simulated prebiotic conditions.[20–22] It has also been suggested that in the anticodon loop of transfer RNA, one base interacts with a phosphate oxygen.[23,24] Base–oxygen stacking may also occur in some of the unusual nucleic acid structures (e.g., pseudoknots, ribozymes, RNase P, and parallel helices) that are presently emerging.[4]

To search for this type of stacking a cylinder extending 3.0 Å normal to both base faces is created (Figure 2a). The radius of this cylinder is equal to the distance from the ring geometric center to the midpoint of the bond connecting C-4 and C-5, thus creating a circle approximately inscribing the base rings (Figure 2b). This bond is chosen because it is present in both purines and pyrimidines. If a phosphate or furanose oxygen lies within this cylinder, it is considered stacked on the base (Figure 2c). The height z of the oxygen above the base and its lateral distance r from the ring geometric center are determined:

$$\varphi = \cos^{-1}\left(\frac{V \cdot S}{|V||S|}\right)$$

$$z = |V| \cos \varphi$$

$$r = |V| \sin \varphi$$

where V is the positional vector of the oxygen atom with respect to the ring geometric center and S is 3.0 Å times the base unit normal vector described in Figure 1. If z is less than or equal to 3.0 Å and r is less than or equal to the radius of the ring, then the oxygen is considered stacked on the base.

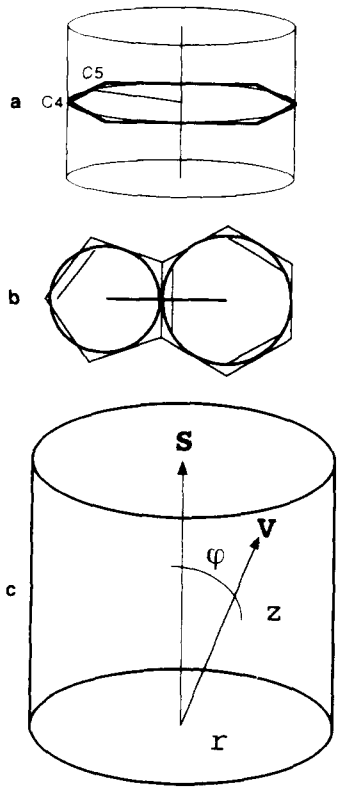

Figure 2. Search algorithm for finding base–oxygen stacking. (a) A cylinder is extended normal to both base faces. (b) The radius of the cylinder is equal to the ring geometric center to the midpoint of the bond connecting C-4 and C-5, giving circles approximately inscribing the imidazole and pyrimidine rings. (c) The position of the oxygen is calculated relative to the cylinder.

Selecting the search parameters

Choosing reasonable stacking criteria is complicated by the high degree of fluctuation in the helical parameters most important to stacking (i.e., twist, roll, and slide as defined by the Cambridge Convention[25,26]). Available crystallographic and theoretical surveys of stacked bases were examined to obtain starting rough values for the d_{ij}, θ_{ij}, and τ_{ij} parameters.[8,26] Two regular nucleic acid helices, the B-form Dickerson dodecamer[27] and an A-form RNA duplex,[28] were analyzed with these parameters under the assumption that every base is stacked to some degree with its nearest intrastrand neighbors. The starting estimates were adjusted to give the most stringent conditions that still preserved the latter criterion. These parameters were further tuned against a molecule with less regular structure. Namely, the nuclear magnetic resonance (NMR) structure of an RNA hairpin loop[15] was analyzed. The following set of conditions successfully located all of the stacked bases defined by Cheong et al.[15] with no false positives: d_{ij} less than 4.5 Å, θ_{ij} less than 23°, and τ_{ij} less than 40°.

The two parameters used to find base–oxygen stacking interactions (Figure 2) were arbitrarily chosen. If the oxygen lies within the cylinder extending 3.0 Å from either the imidazole or pyrimidine ring perpendicular to the base plane, the oxygen is considered stacked on the ring. The radius of the cylinder is equal to the radius of the circle approximately circumscribing the given ring. This criterion was tested against the crystal structures of guanylyl-2′,5′-cytidine dihydrate[17] and Z-DNA.[29] In G2′p5′C the guanine is in the *syn* configuration and its pyrimidine ring is stacked on the neighboring cytidine sugar. This structure bears a striking resemblance to a d(GpC) dinucleotide in Z conformation. In every d(GpC) step of Z-DNA, the guanines (also in *syn* configuration) stack on the neighboring cytidine sugar. The cylinder criterion successfully finds all such stacking arrangements with no false positives.

Graphical output

Base–base and base–oxygen stacking are represented differently so that they may be easily distinguished. When two stacked bases are found as described above, a cylinder containing a random scattering of points is extended 3.4 Å (i.e., the van der Waals distance) normal to both bases (Color Plate 1a). In this case, the radius of the cylinder is equal to the distance between the ring geometric center (pyrimidine or imidazole) to the C-4 atom. The C-4 atom is chosen because it is present in all commonly occurring bases. Thus, the cylinder approximately circumscribes the base ring. In the final graphical representation of base–base stacking, only the points in the overlapping volume of the two cylinders are shown (Color Plate 1b, cytosine–cytosine interstrand stacking). In this manner, attention is immediately focused not only on the stacking interaction but also on the size of the overlapping region. This gives some indication of

the quality of stacking, or, in this case, the degree of ring overlap. If this is too stringent for the desired analysis the exocyclic substituents of the bases can be checked for stacking by the criteria described in Figure 2, except in this case the cutoff value of z is 3.4 Å instead of 3.0 Å. If an exocyclic heavy atom is stacked on a neighboring base (i.e., satisfies the z and r criteria of Figure 2) the radius of the display cylinders is increased. For example, the radii of pyrimidine display cylinders can be increased to cover the distance between the ring geometric center to the O-2 oxygen in order to show stacking of keto oxygens over base rings. However, the overlapping region of the two cylinders will be considerably larger. Rather than use all points in the overlapping region, only those points within the original cylinders circumscribing the base rings are shown (Figure 3).

In the case of base–oxygen stacking, cones are used rather than cylinders (Color Plate 1b, guanine pyrimidine rings stacked on adjacent intrastrand 2′-deoxyriboses). The position of the furanose oxygen with respect to the geometric center of the base ring is conveyed by the slant of the cone. For example, an oxygen directly above the geometric center of the base ring and aligned with the base normal vector will exhibit a perfect cone. Notice that the base–ribose stacking cones in Color Plate 1b are slightly slanted.

Note that the display method builds on the search algorithm so that calculations are not duplicated unnecessarily. For example, the base normal vectors calculated to search for base–base stacking are also used by the display method to position the cylinders. Likewise, for base–oxygen stacking, the vector describing the position of the oxygen relative to the base geometric center is used to position the display cone.

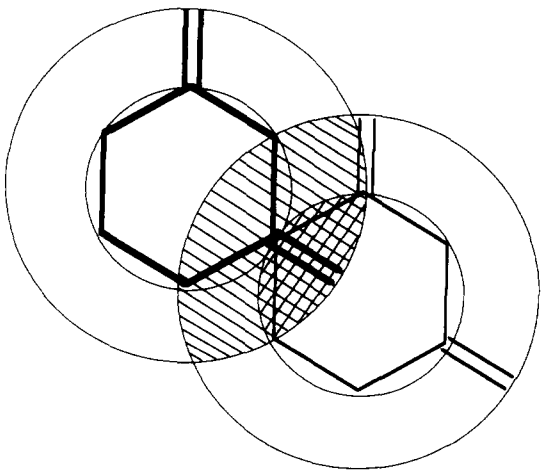

Figure 3. Optional base–base stacking display method for cases where ring overlap is not present but the bases could be considered stacked due to exocyclic substituents situated over neighboring rings (i.e., induction). Notice that one of the keto groups of the upper pyrimidine (thick lines) is stacked over the lower pyrimidine ring (thin lines). Rather than use the entire overlapping region (hatched area), only the area of the upper cylinder lying within the lower base ring is displayed (cross-hatched area).

Program description

The program housing the search and display algorithms is called ANUCLYZE and is written in Fortran77 (approximately 1 200 lines of source code). The program is entirely command-line driven. Because of this the Unix Fortran77 library is required at compile time. It accepts any file in the Brookhaven Protein Data Bank (PDB) format[30] whose atom names follow IUPAC nomenclature for nucleic acids. After analysis, ANUCLYZE outputs a single text file containing the search results and either one or two graphics files in PDB format showing base–base and/or base–oxygen stacking. The program XmMol[31] was used for all graphics displays. Color Plates 1–4 were produced by the program Scene Viewer (Silicon Graphics, Inc.) using ANUCLYZE output files.

We have considered the possibility that the algorithms presented here could be used to examine intercalating agents and even rare cases when amino acid side chains are involved in stacking arrangements. The method can easily be adapted to interactions between any planar compounds. As such, it is possible for the user to add new ring systems to the program library.

In addition to stacking analysis, ANUCLYZE also finds hydrogen bonds in nucleic acids. In the case of hydrogen bonds involving keto oxygens, the program takes the angle of the lone pair electrons into account.

Since the program was originally designed as a tool to analyze large numbers of structures (e.g., from a molecular dynamics or Monte Carlo simulation) it will also process a series of structures and output data in terms of the percentage of structures containing the hydrogen bonds and stacking arrangements found. To analyze 1 000 DNA decamers, for example, takes approximately 3 min on a Hewlett-Packard 700 workstation.

RESULTS

The method has been tested on several different structures, including nucleic acids without regular helical geometry. For reasons of space only the well-known and well-characterized A-, B-, and Z-helical geometries are presented here. Nonetheless, they effectively illustrate the level of performance of the method.

The first example is the crystal structure of a B-DNA dodecamer d(CGCGAATTCGCG)$_2$.[27] In a regular helix such as this it is easy to assume that all adjacent bases are properly stacked. Notice that the presence and quality of stacking (or, in this case, the degree of ring overlap) are clearly demonstrated (Color Plate 2). Although it is not readily apparent from traditional representations, CURVES analysis[32] reveals a dislocation in the helix axis (Figure 4). This can potentially unstack bases near the dislocation, as illustrated for the dCpG step in Color Plate 2. It is surprising that stacking is disrupted at a dCpG step since calorimetric studies show that this particular nearest-neighbor pair is the most thermodynamically stable.[33] It is important to note that the bases shown as unstacked are often considered stacked in spite of the fact that there is very little overlap of any base atoms.[3] If the radius of the display cylinders is increased as

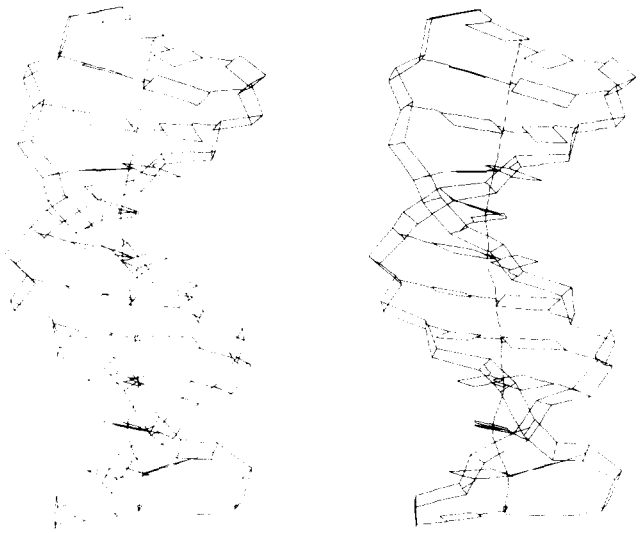

Figure 4. CURVES analysis [32] showing the dislocation in the helix axis of the Dickerson dodecamer [27].

described above (Figure 3), a small degree of stacking is illustrated.

The crystal structure of A-RNA $[U(UA)_6A]_2$[28] serves as a good positive control for the search and display algorithms. Specifically, interstrand adjacent purines are known to overlap in A-form helices.[34] Color Plate 3 shows that our method successfully locates cross-strand stacking. Notice that the pyrimidine rings of the purine bases are involved in interstrand stacking while the imidazole rings are involved in intrastrand stacking.

The last example, a half-turn of Z-DNA $d[CG)_3]_2$,[29] demonstrates how our method identifies both base–base and base–ribose stacking (Color Plate 4). Color Plate 4a shows the correct pattern of base–ribose stacking in alternating cytosine–guanine dinucleotides. Color Plate 4b shows only the base–base stacking. Color Plate 4c shows for the first time the elaborate network of base–base and base–ribose stacking interactions that helps stabilize Z-DNA. Notice in particular the central axis of stacking that extends the length of the helix. This axis consists of base–ribose stacking as well as intra- and interstrand base–base stacking.

CONCLUSIONS AND FUTURE DIRECTIONS

We have presented a rapid, accurate, and flexible method for finding and displaying stacking interactions in nucleic acids. Our search algorithm makes no *a priori* assumptions about the relative positions of bases within a sequence or about the helical character, nor does it use potential energy functions to determine whether bases are stacked. Stacking is decided on the basis of geometry alone. This is advantageous because stacking energetics are poorly understood.

Three examples demonstrating the capability of our search and display method were shown. For reasons of space we chose to show only the A-, B-, and Z-conformational families since they are well known and well characterized. The method successfully located all stacked bases, including known interstrand stacking interactions in A-RNA and Z-DNA. In the latter case, the display method immediately reveals the elaborate pattern of intra- and interstrand base–base stacking and base–ribose stacking.

Although we obtained good results, the cutoff values for the d_{ij}, θ_{ij}, and τ_{ij} parameters defining base–base stacking can be set by the user to whatever stringency is desired for a given analysis. In the future, we hope to perform a rigorous parametrization of d_{ij}, θ_{ij}, and τ_{ij} using all of the structures available in the Rutgers Nucleic Acid Database.[35] This should lead to a more general set of parameter values as well as a set of sequence-specific values.

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