Lecture 18. The structure and conformation of A, B and Z DNA, DNA bending

For a compilation of nucleic acid structure tutorials in 3D see:

https://sites.google.com/view/nucleic-acid-chemistry/jmol
Watson Crick base pair is the basic unit of RNA and DNA duplexes. It has a pseudo C2-axis of symmetry that will result in an antiparallel duplex.
Helical parameters

**Helix axis.** Line at the center of the helix parallel to the direction of the helix.

**Pitch** - The distance traveled along the helix axis for a complete turn.

**Helical repeat** - The number of monomer units per complete turn.

**Helical rise per residue, \( h \)** - Distance along the helix axis between the residues.

**Twist angle** - Angle at which the pseudo dyad axis rotates on going from one base pair to the following base pair.

**Diameter** - Twice the largest radius in the helix.
Arnott A RNA Structure (arn0035.pdb)

Helical repeat 11 bp/turn
Pitch 28 A
Diameter 26 A, 2.6 nm
Fat right handed helix

Dickerson Dodecamer (bdl001.pdb)

Helical repeat 10.5 bp/turn
Pitch 34 A, 3.4 nm
Diameter 20 A, 2.0 nm
Skinny right handed helix
The pi stacking distance in A RNA and B DNA is the same (3.4 Å), but because the bases in RNA are tilted relative to the helical axis, the rise per residue is less.

**A RNA structure**

**B DNA structure**
RNA duplex has a hole down the center because bases pushed out.

B DNA has a solid core because the base pairs are stacked at the helix axis.
As a consequence of the displacement of the base pairs relative to the helix axis, and the sugar phosphate backbone, grooves are formed. To see the grooves, you have to tilt the helix so you can look down on it.

- **A RNA**
  - Minor groove too shallow for drug binding
  - Major groove entrance too narrow to allow binding

- **B DNA**
  - Minor groove perfect for small molecule binding
  - Major groove perfect for protein alpha helix binding
The grooves of DNA are receptors for proteins and small molecules.

major groove can accommodate a protein alpha helix

minor groove can accommodate molecules like distamycin or Hoescht

cro repressor alpha helices
To understand the conformation of DNA and RNA duplexes we need to understand something about the conformational properties of the sugar phosphate backbone.

- Conformations of 5-membered rings
- Conformations of glycosidic linkages
- Conformations of phosphodiester bonds
- Conformations of single bonds
Torsion Potentials

The side chain has many rotatable bonds and degrees of freedom, but only certain conformations are favored, mainly gauche and anti for sp3-sp3 bonds. 180 for sp2-sp2

**Staggered preferred**

\[ S_1 = +1 \]
\[ n_1 = 3 \]

**Eclipsed preferred**

\[ S_1 = -1 \]
\[ n_1 = 2 \]
Bond angle definitions and major conformations in B/A DNA

this one looks to be in a higher energy conformation

+g

A/B

+p/a

+g/a

lone pair anti to O3’

lone pair anti to O5’

A/B

eclipsed with C1’-H1’

purines

pyrimidines

lone pair anti to O5’

lone pair anti to O3’

-3

-3

-3
The torsion angles found in A and B conformations all fit into roughly gauche and anti conformations

**The major difference between A and B forms is in the delta angle**

<table>
<thead>
<tr>
<th></th>
<th>A-DNA</th>
<th>B-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>-g</td>
<td>-50</td>
</tr>
<tr>
<td>β</td>
<td>a</td>
<td>188</td>
</tr>
<tr>
<td>γ</td>
<td>+g</td>
<td>41</td>
</tr>
<tr>
<td>δ</td>
<td>+g</td>
<td>79</td>
</tr>
<tr>
<td>ε</td>
<td>a</td>
<td>-146</td>
</tr>
<tr>
<td>ζ</td>
<td>-g</td>
<td>-78</td>
</tr>
<tr>
<td>χ</td>
<td>-120</td>
<td></td>
</tr>
<tr>
<td>Rise</td>
<td>2.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Twist</td>
<td>31.1</td>
<td>36.1</td>
</tr>
<tr>
<td>Inclination</td>
<td>12.0</td>
<td>2.4</td>
</tr>
<tr>
<td>x-displacement</td>
<td>4.10</td>
<td>0.8</td>
</tr>
</tbody>
</table>

DOI: [10.1093/nar/gki538](http://10.1093/nar/gki538)
**Phosphodiester bond conformations**

Because of the anomeric effect whereby a lone pair donates into the $\sigma^*$ orbital of the P-O bond, the O-P-O-R torsion angle is at minimum at +/- 60° (+/- gauche), not 180° (anti).
Glycosyl bond conformations
two general conformations, syn and anti

purines favor anti, but can adopt syn
pyrimidines favor anti much more than syn and are rarely found in the syn conformation because of large steric clash
Definition of the bond angles in deoxyribose ring system

The torsion bonds are defined by the sugar ring atoms. Example, $t_1$ is defined by O4'-C1'-C2'-C3'.

The torsion angles are not independent of each other, and instead of 5 degrees of freedom there are only two degrees of freedom described.

A flat 5-membered ring would have ring bond angles (C1’-C2’-C3’) of 108° which is almost identical to the angle for sp³ hybridized carbon. In such a case, however, all the torsion angles (C1’-C2’-C3’-C4’) would be eclipsed, and in their highest energy conformation.
5-membered rings pucker to relieve eclipsing interactions to form envelope and twist conformations

Envelope Form

- 4 atoms of ring in the plane
- 1 atom out of plane
- 1 eclipsed bond (1-2-3-4) (angle =0)
- best to have oxygen in the eclipsed bond because it has lone pair orbitals
Twist Form

- 3 atoms in plane
- 1 atom below plane (#4)
- 1 atom above plane (#5)
- No eclipsed bonds
- transition intermediate between envelope conformations
Pseudorotation pathway smoothly interconnects twist with endo forms

O4'-endo-C1'-exo

C1'-endo

C2'-endo

C2'-endo-C1'-exo

O4' down
C1' down

C1' up
C2' up

C1' up
C2' up
Pseudorotation Cycle for Ribose Ring

The different allowed conformations of a five-membered ring can be described by the pseudorotation equation:

\[ t_i = t_{\text{max}} \cos(P+144(i+3)) \]

where \( P \) is the phase angle
\( t_{\text{max}} \) is the maximum degree of pucker

The equation will give you the values of each of the five angle \( t_0, t_1, t_2, t_3, \) and \( t_4 \), for a given \( P \) and \( t_{\text{max}} \). To figure out the angle \( t_2 \) simply plug in \( P, t_{\text{max}}, \) and \( i=2 \). Do the same for the other angles.
Potential Energy Surface for Ribose Ring System

countour plot: each line represents a specific energy (like an altitude on a topographic map)

Circle describes a path with a constant $t_{\text{max}}$

minima can be seen at 18 degrees (C3'-endo) and at 164 degrees (C2'-endo) with a low barrier between the two

There is a maximum at 270 degrees
Origin of the high energy of the O4'-exo conformation of deoxyribonucleosides

Bad VDW interaction due to eclipsed bond
Relationship between sugar pucker and helix type

Because the base pairs have a twist angle of about 35° and are centered over the helix axis, the DNA backbone in B DNA has farther to go (7 Å) than in A RNA (5.9 Å) which has the base pairs pushed out away from the axis.

The torsion angle that is directly related to the interphosphate linkage is delta (τ3), because the C4’-C3’ bond is parallel to the helix axis.

The C2’-endo conformation of B DNA makes the delta bond 180° whereas in RNA, with a shorter interphosphate distance can use the 3’-endo conformation with a small bond angle.

δ = anti (180°)  
δ = + gauche (+80°)
RNA/DNA hybrids as found in Okazaki fragments are in the A form.
RNA duplexes more stable than DNA duplexes

-ΔG/bp vs AT content

RNA/DNA with <30% pyrimidines in the DNA are less stable than DNA/DNA. Hybrids with >70% pyrimidines in the DNA were more stable than DNA/DNA.

Biochemistry 1995, 34, 10807—10815
Peptide Nucleic Acids

Peptide backbone in place of sugar phosphate backbone reduces electrostatic repulsion

DNA

PNA

Conformation of PNA dictated by planar amides (PNA duplex, 1pup.pdb)

Thick bonds are non-rotatable, boxes enclose atoms confined to a plane

Figure: pna_dup2.ppt
PNA obeys Watson Crick Base Pairing Rules and forms more stable duplexes

Order of increasing stability:
DNA/RNA = DNA/DNA < PNA/DNA < PNA/RNA

<table>
<thead>
<tr>
<th>TABLE 1 Melting temperatures $T_m$ (°C) for PNA–DNA, PNA–RNA, DNA–DNA and DNA–RNA complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>First strand sequence*</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PNA:DNA (parallel) TGTACGTGACAACTAT† DTAGATCACAT‡ AGTGATGCTAC‡</td>
</tr>
<tr>
<td>56.1 38.0 38.0</td>
</tr>
<tr>
<td>PNA:DNA (antiparallel) 69.5 51.0 49.0</td>
</tr>
<tr>
<td>PNA:RNA (parallel) 51.2 ND ND</td>
</tr>
<tr>
<td>PNA:RNA (antiparallel) 72.3 ND ND</td>
</tr>
<tr>
<td>DNA:DNA 53.3 33.5 33.5</td>
</tr>
<tr>
<td>DNA:RNA 50.6 ND ND</td>
</tr>
</tbody>
</table>

Absorbance versus temperature curves were measured at 260 nm in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0, as described in ref. 11. $T_m$, the temperature at which half of the molecules are hybridized, was obtained by fitting triplicate melting curves at 4 μM of each strand to a modified two-state model with linear sloping baseline.

Written 5'-3' for oligonucleotides and N to C terminal for PNA.
† The PNA terminates in a carbamoyl amide.
‡ The PNA terminates in a lysine amide.

<table>
<thead>
<tr>
<th>TABLE 2 Thermodynamic parameters for the formation of PNA–DNA, PNA–RNA, DNA–RNA and DNA–DNA duplexes with the sequence TGTACGTGACAACTA present in the PNA strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA:RNA  PNA:RNA  DNA:DNA  PNA:DNA</td>
</tr>
<tr>
<td>$\Delta H^\circ$ (kcal mol$^{-1}$)* -128.9 -128.5 -106.3 -106.6</td>
</tr>
<tr>
<td>$\Delta S^\circ$ (EU)* -372.8 -345.9 -298.2 -285.8</td>
</tr>
<tr>
<td>$\Delta G^\circ$ (kcal mol$^{-1}$)* -13.3 -21.2 -13.4 -18.0</td>
</tr>
<tr>
<td>$T_m$ (°C, 8 μM)* 50.1 72.2 53.5 68.8</td>
</tr>
</tbody>
</table>

Measured in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0.
* Obtained from linear plots of $1/T_m$ versus log(concentration)$^{20}$. 
Single base mismatches greatly effect the thermodynamic stability of DNA duplexes

Order of decreasing stability of base pairs in DNA

\[
\text{G-C} > \text{A-T} > \text{G-G} > \text{G-T} \geq \text{G-A} > \\
\text{T-T} \geq \text{A-A} > \text{T-C} \geq \text{A-C} \geq \text{C-C}.
\]

Sequence-specific variations in base pair parameters in B DNA

Roll angle - Deviation of the angle about C6-C8 for a base pair that is assigned zero for that in which the base pair plane is perpendicular to the helix axis.

Tilt - Deviation of the pseudodyad axis from 0 for that in which the base pair plane is perpendicular to the helix axis.

Propellar twist - Angle between the planes of each individual base of the base pair.

Displacement D - The distance between the helix axis and the center of the base pair.

Center of the base pair - The point where the C6-C8 bond crosses the pseudodyad axis.
The relative orientation between bases and base pairs is sequence dependent.
Propeller twisting causes sequence dependent steric clashes between base pairs result in reorganization of the base pair conformations.

Calladine developed an back of the envelop method of analyzing the clashes and prediction of the responses.

- Minor groove clash at 5'-Py-Pu-3'
- Major groove clash at 5'-Pu-Py-3'
Clashes minimized by shifting BP over so G’s don’t interact in Major or minor groove

Clash due to large twist angle

Clash minimized by decreasing twist angle

Minor groove clash at 5’-Py-Pu-3’ minimized by rolling bp away from each other.
Discovery of left-handed DNA

CD of poly (dG-dC) at pH 7 25 °C

Upon raising the salt concentration, the circular dichroism spectrum (CD) flipped from a right-handed split CD to a left-handed split CD according to the exciton chirality rule.

- Solid line: 0.2 M NaCl
- Dotted line: after addition of more NaCl

- Right hand helix
- Left hand helix

(a) Positive chirality

Degenerate exciton coupling

+ chirality
- chirality
Zig Zag DNA?
Rich hexamer Z DNA structure (zdf002.pdb)

- Pitch = 45.6 Å
- 12 bp/turn
- 3.8 Å/bp

- No major groove!
- 18 Å deep minor groove
- 18 Å wide
- 3.7 Å wide minor groove
Z DNA structure forms from alternating CG sequence.
Forms a **dinucleotide repeat** (the C and G are in different conformations).

CpG step
CG-CG

-15° twist

GpC step
GC-GC

-50° twist

Interstrand base stacking

Intrastrand base stacking

All G’s are in a syn conformation

anti

syn

Normal Watson Crick base pair except that the G is syn
Flipping of Base Pairs in B to Z transition

**TOP VIEW**

- $5'$-down
- $5'$-up
- $3'$-up
- $3'$-down
- anti
- syn

**SIDE VIEW**

- glycosyl bond rotates
- whole nucleoside rotates

**Base pair flips by 180°**

**B DNA**

$3'$

- a
- GC
- CG
- GC
- CG

$5'$

- a
- a
- a
- a

**Z DNA**

$3'$

- s
- GC
- GC
- GC
- GC

$5'$

- s
- a
- s
- a
- a

**a = anti glycosyl**

**s = syn glycosyl**

Flipping of Base Pairs in B to Z transition
Alkylation of DNA by carcinogens can also enhance Z DNA formation thought to play a role in cancer induction

Dimethyl sulfate
Diazomethane

AAF, Acetoxyaminofluorene

Electrostatics (Z DNA has higher charge density)

Bad steric interactions in the anti conformation

Less severe steric interactions in the syn conformation which is the conformation at the purine site in Z DNA
Metal cations bind in the minor groove with water at $A_n$-tract and stabilize the B form. The minor groove at AT base pairs only has lone pairs and has a high negative electrostatic potential.

- Primary water layer light blue
- secondary layer magenta
- tertiary layer blue
- quaternary layer red.

Role of 2-amino group in disrupting the negative electrostatic potential and the spine of hydration in minor groove allowing A and Z DNA to form

<table>
<thead>
<tr>
<th>Polymer</th>
<th>C2-NH2 group</th>
<th>Helix</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(A)•d(T)</td>
<td>no</td>
<td>B</td>
</tr>
<tr>
<td>d(I)•d(C)</td>
<td>no</td>
<td>B</td>
</tr>
<tr>
<td>d(IIT)•d(ACC)</td>
<td>no</td>
<td>B</td>
</tr>
<tr>
<td>d(AG)•d(CT)</td>
<td>yes</td>
<td>B, A</td>
</tr>
<tr>
<td>d(AGC)•d(GCT)</td>
<td>yes</td>
<td>B, A</td>
</tr>
<tr>
<td>d(GC)</td>
<td>yes</td>
<td>B, A, Z</td>
</tr>
<tr>
<td>d(GT)</td>
<td>yes</td>
<td>B, A, Z</td>
</tr>
<tr>
<td>d(2AP-T)</td>
<td>yes</td>
<td>B, A, Z</td>
</tr>
</tbody>
</table>

http://nar.oxfordjournals.org/cgi/content/full/31/5/1536

Loss of O2 carbonyl of T disrupts spine of hydration
Divalent and trivalent ions better at stabilizing Z DNA - coordinate to phosphates

<table>
<thead>
<tr>
<th>Ion (mM)</th>
<th>poly d(G-C)</th>
<th>poly d(G-m5C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>2500</td>
<td>700</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>700</td>
<td>0.6</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>100</td>
<td>0.6</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>40</td>
<td>0.6</td>
</tr>
<tr>
<td>Co(NH₃)₆³⁺</td>
<td>0.02</td>
<td>0.005</td>
</tr>
<tr>
<td>EtOH 60% v/v</td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>Mg²⁺ + 10% EtOH</td>
<td>4 mM</td>
<td>-</td>
</tr>
<tr>
<td>Mg²⁺ + 20% EtOH</td>
<td>0.4 mM</td>
<td>-</td>
</tr>
</tbody>
</table>

chelation site for hydrated magnesium ion

hydrophobicity (methyl group occupies hydrophobic pocket)
Conformational and Electrostatic Factors Favoring A, B or Z DNA or RNA

A and Z DNA can better make use of sparse water under conditions of low water activity as results from the addition of alcohol solvent.

2′-OH stabilizes the A form of RNA, probably through H-bonding to the 3′-oxygen.

A: 5.7 A
B: 6.7 A
Z: 5.6 A

Z DNA intrastrand phosphate hydration

C₂-endo, 2E
C₃-endo, 3E

Steric interaction less severe
A nanomechanical sensor sensitive to metal ions based on B to Z transition

Donor and acceptor molecules (fluorescein and Cy3) are attached to a DNA molecule containing a (GC)_{20} section. When in B form the two dyes are close and show strong FRET, when in Z form, the DNA unwinds by about 3.5 turns, and extends about 6 Å, changing the distance by 20-60 Å, and greatly lowers the FRET.
If all the DNA in a human cell were stretched out, 2 meters long.

$$3 \times 10^9 \text{ bp} \times 0.34 \text{ nm/bp} = 10^9 \text{ nm} = 1 \text{ m} \times 2 \text{ copies} = 2 \text{ meters}$$

To fold up all the DNA into the nucleus of a cell (about 10 \text{ uM} in diameter), the DNA has to be bent to wrap around proteins called histones, which are then arranged into fibers, that are then further wrapped into chromosomes.
DNA bending and bendability

Persistence length – the average length for which DNA behaves like a rigid rod.

DNA Persistence length = 50 nm in solution

= 500 Å / 3.4 Å/bp

= 150 bp

= 15 turns of the DNA helix

Analysis of the contour length (L) vs the end to end length R, gives the persistence length

Atomic force microscopy

Nanoscale, 2017, 9, 11327–11337 | 11327
Euler buckling and nonlinear kinking of double-stranded DNA

A hairpin loop is synthesized with fluorescent donor and molecules in the stem and annealed to complementary strand. As it gets longer (h), the stiff duplex gets longer and unwinds the stem, separating the donor/acceptor fluorophores, until the DNA can spontaneously bend (buckle) allowing the stem to rewind and restore FRET.

\[ h = \text{length of ODN complementary to loop} \]

bending up to $7^\circ$/bp

Loop length \( L = 46 \)

\[ h = \{30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44\} \]
Discovery of sequence-specific bending at A-tracts

Certain sequences in kinetoplast DNA were discovered to bend because of anomalous electrophoretic mobility. A sequence isolated from an organism was found to migrate slower than expected based on the DNA length.

To locate the sequence involved the researchers circularized the DNA and then cut it at a specific location with restriction enzyme to produce the same length product, but with the bend locus in a different location (permuted sequences).

The sequence with the slowest migration was concluded to have the bending locus in the center.

Sequence discovered:

```
5' - AAAAAt gt c AAAAAAt aggc AAAAAAt gc c AAAAAAt gc c AAAAA- 3'
3' - TTTTTacagTTTTTTat ccgTTTTTTacggTTTTTTacggTTTTT- 5'
```
Phasing of Bends

To determine in general whether or not a sequence bends DNA a multimer assay was developed. The idea is that even a small bend can be amplified to the point where it causes retardation on a gel by polymerizing the sequence of interest.

Because DNA repeats approximately every 10 bp, if a 10 or 11-mer is polymerized the bends will reinforce each other and produce slow moving circular DNAs.

If a 15-mer or 16-mer is used, then the bends will be out of phase and cancel each other out and the DNA will move as expected based solely on its length.
Mobility of bend multimers as a function of phasing

The apparent length can be plotted against the actual length to assess the degree of bending.

Intermediately phased sequences will produce spiral structures with intermediate mobility.
The direction of a bend can be determined by referencing it to a known bend determined by X-ray crystallography.

Structure of the catabolite activator protein (cap) complexed to DNA (1cgp.pdb) shows an almost 90 degree bend towards the minor groove at the center.
Bending towards minor groove of A-tract determined by a phase sensitive experiment

To determine the direction of another bend, the unknown bend is put at various distances from the known bend.

The assumption is that the slowest moving sequence will be when the two bends are pointing in the same direction.

DNA Bending models

Like twisting of DNA, DNA can bend, either by a wedge or kink mechanism.

The bending for any length segment is defined as shown below. Straight DNA has a bending angle of 0°. Some sequences like An-Tn tracts are intrinsically bent.
Bending of DNA by proteins may be caused in part by neutralization of the negative charges on phosphate by positively charged arginine and lysine side chains.

146 bp (persistence length of DNA) wrapped around nucleosome twice or about 600°

Hud Pavlec junction model for DNA bending

B*-DNA A/T-tracts condense cations in minor groove and bend towards minor groove
A DNA G/C-tracts condense cation in major groove and bend towards major groove
wrapping of DNA around a nucleosome core particle of proteins causes DNA to bend preferentially towards the minor groove at A/T sequences and towards the major groove at G/C sequences.

Bending by proteins proposed to occur by a wedge/kink mechanism using a combination of roll and tilt angles at each base pair

angle expected for uniform bending per base pair  

<table>
<thead>
<tr>
<th>Base Pair</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-14</td>
<td>4.2</td>
</tr>
<tr>
<td>14-15</td>
<td>6.1</td>
</tr>
<tr>
<td>15-16</td>
<td>9.5</td>
</tr>
<tr>
<td>16-17</td>
<td>2.9</td>
</tr>
<tr>
<td>17-18</td>
<td>2.7</td>
</tr>
<tr>
<td>18-19</td>
<td>5.9</td>
</tr>
<tr>
<td>19-20</td>
<td>14.1</td>
</tr>
<tr>
<td>20-21</td>
<td>16.5</td>
</tr>
<tr>
<td>21</td>
<td>5.2</td>
</tr>
</tbody>
</table>

observed bend angle per base pair in nucleosome bp 13-21

- Roll
- Tilt
- Kinked bending
- Uniform bending
Integration host factor IHF from prokaryotes bends DNA by 160° using a kink/wedge mechanism to cause two 80° bends with linear DNA segments.