Supplementary Data

Web 3DNA 2.0 for the analysis, visualization,
and modeling of 3D nucleic acid structures

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by

Shuxiang Li, Wilma K. Olson*, and Xiang-Jun Lu*

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**S1 ‘Simple’ base-pair and step parameters for non-Watson-Crick pairs**

The relative position and orientation of the two bases in a base pair (bp) can be rigorously quantified by six rigid-body parameters—three translations (Shear, Stretch, and Stagger) and three rotations (Buckle, Propeller, and Opening) (see Fig. S1). Similarly, taking each bp as a rigid body, a set of six step parameters (Shift, Slide, Rise for translations, Tilt, Roll, and Twist for rotations) can be used to describe the spatial arrangement of neighboring bps. The numerical values of these bp and step parameters depend upon the choice of reference frames. For Watson-Crick (WC) pairs and DNA/RNA duplexes consisting of WC pairs, local 3DNA parameters make ‘intuitive’ sense (e.g., a Rise of around 3.4 Å and a twist of 36°). For non-WC pairs, however, the 3DNA local bp parameters could become cryptic, and the local step parameters may seem to be nonsensical (for example a Rise of 0, as reported on the 3DNA Forum. See also below).

![Fig. S1 Schematic diagrams of the six rigid-body parameters commonly used for the characterization of base pair geometry](image)

To address the increasingly common issues on the characterization of DNA/RNA structures with non-WC pairs, 3DNA (as of v2.3-2016jan01) introduced a set of ‘simple’ bp and
step parameters. Shown below are related results using PDB entry 1xvk as an example. This echinomycin-(GCGTACGC)$_2$ complex has a single DNA strand as the asymmetric unit. The biological unit is contained in an NMR-like MODEL/ENDMDL ensemble of two models, each corresponding to one chain of the duplex. This structure has two A+T Hoogsteen base pairs. In March 2013, questions were asked on the 3DNA Forum about the “zero or negative helical rise?” from a 3DNA analysis on 1xvk.

The related 3DNA commands for the analysis of 1xvk are shown below. Note the -symm option to include both strands of the duplex in the biological unit. The main output file is named ‘1xvk.out’.

```
find_pair -symm 1xvk.pdb1 1xvk.bps
analyze -symm 1xvk.bps
```

A comparison of the classic 3DNA local bp parameters and the new ‘simple’ ones is shown below. For the four WC pairs, the two sets are very similar. For non-WC pairs (no. 1, 4, 5, and 8 of M+N type pairs; prefixed with * in the list of ‘simple’ parameters), however, they are obviously different (e.g., in Shear and Stretch).

```
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<tr>
<th>Local base-pair parameters</th>
<th>bp</th>
<th>Shear</th>
<th>Stretch</th>
<th>Stagger</th>
<th>Buckle</th>
<th>Propeller</th>
<th>Opening</th>
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<td>2.64</td>
<td>0.32</td>
<td>17.27</td>
<td>5.36</td>
<td>52.16</td>
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</table>
```

The 'simple' parameters are intuitive for non-Watson-Crick base pairs and associated base-pair steps (where the above corresponding 3DNA parameters often appear cryptic). Note that they are for structural *description* only, not to be fed into the ‘rebuild’ program. See URL http://x3dna.org/highlights/details-on-the-simple-base-pair-parameters and related blogposts on the 3DNA home page for details.

This structure contains 4 non-Watson-Crick (with leading *) base pair(s)

http://web.x3dna.org
The comparison becomes more pronounced at the step level. The local TA/TA step (no. 4) has a Rise of zero, and a Twist of 180 degrees. A duplex containing Hoogsteen pairs with zero Rise surely does not make ‘intuitive’ sense, especially when visualized in an interactive molecular viewer like Jmol or PyMOL. This zero-Rise result is due to the symmetry of the biological unit of PDB entry 1xvk: the z-axes of the T+A (no. 4) and A+T (no. 5) Hoogsteen pairs are perfectly anti-parallel. As a result, the z-axis of the middle reference frame of the step (no. 4) is \[0, 0, 0\], which leads to zero Rise. On the other hand, the corresponding ‘simple’ parameters for step no. 4 has a Rise of 3.1 Å, and a Twist of 26°, which are more intuitively reasonable.
Bizarre as the local 3DNA bp and step parameters may appear for 1xvk, these are the parameters needed for rigorous reconstruction of the original structure, at the bp level (i.e., without considering the sugar-phosphate backbone). See the following list of commands for details. This `analyze/rebuild` reversibility is a defining feature of 3DNA and can be applied to DNA and RNA.

```
# The analysis step generates file `bp_step.par'
find_pair -symm 1xvk.pdb | analyze -symm
rebuild -atomic bp_step.par 1xvk-3dna.pdb

# 'analyze' of the 3DNA-rebuilt structure (1xvk-3dna.pdb)
# Note here the `-symm` option is no longer needed
# The main output file is `1xvk-3dna.out' with bp and step
# parameters very close to the original output in `1xvk.out'
find_pair 1xvk-3dna.pdb | analyze
```

Overall, bp geometry can be described in more than one way. The existing set of six local bp and step parameters in 3DNA is mathematically rigorous, allowing for an unambiguous characterization of any pair of interacting bases and serving as input for exact model building. The new set of six ‘simple’ bp and step parameters provides a more intuitive interpretation of structural variations in non-WC-containing structures. The term ‘simple’ is used to distinguish the new set of parameters from the traditional 3DNA local parameters. It should be emphasized that the ‘simple’ parameters are for structural description only, and are not suitable as input for the 3DNA `rebuild` program. The two sets of parameters complement one another and serve different audiences and/or purposes. Numerical values of both are readily obtained with 3DNA.

For more technical details, please see the blog post “Details on the simple base-pair parameters” and related posts on the 3DNA home page.
S2 Sample input files for the 3DNA rebuild program

The 3DNA rebuild program can be used for building DNA/RNA duplexes or single-stranded nucleic acid structures, given an input file containing base sequence and local bp and step parameters. The input file is most conveniently generated by running the 3DNA analyze program on a starting structure, and then modifying the resultant bp_step.par file. Two sample input files, one for a DNA duplex and another for an RNA single helix, are given below.

S2.1 Rebuilding of a double-helical DNA structure

The following data set is based on PDB entry 355d, the classical Dickerson-Drew B-DNA dodecamer with base sequence d(CGCGAATTCGCG)₂. It can be generated with the following 3DNA command, in a file named bp_step.par. The bases or associated parameters can be easily changed to build a customized duplex structure based on the starting one. Alternatively, such an input file can be created on purpose.

```
find_pair 355d.pdb | analyze
```

<table>
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<tr>
<th>#</th>
<th>base-pairs</th>
<th># <em><strong>local base-pair &amp; step parameters</strong></em></th>
</tr>
</thead>
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<td>C-G</td>
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<tr>
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<td>G-C</td>
<td>-0.244</td>
</tr>
</tbody>
</table>

http://web.x3dna.org  Li, Olson & Lu  5
S2.2 Rebuilding a single-stranded RNA structure

The following data set is based on PDB entry 1fir, the crystal structure of HIV reverse-transcription primer tRNA(Lys,3). It can be generated with the following 3DNA command, in a file named `bp_step.par`. The 15 modified bases (in lower cases) have been replaced by their standard counterparts, and the numerical values of the base-step parameters are rounded to two decimal places.

```
find_pair -s 1fir.pdb | analyze

# in file 'bp_step.par', replace modified bases with standard ones
# GCCCGgAUaGUAGUAgCUCAGuCGGuAGAGCAPCAGACtUUtUUaAPCUGAGGguccAGGGtPCAaGU CCCUGUUCCGGGC CGCA

# *** local step parameters ***
# Shift  Slide  Rise  Tilt  Roll  Twist
8  G  0.00  0.00  0.00  0.00  0.00  0.00
9  C -0.07 -1.62  2.99  7.24  3.05 24.71
10 C  0.34  1.73  3.33  0.06 12.16 33.11
11 C -0.02 -1.22  3.28  6.35  8.61 29.90
12 G -0.20 -1.71  3.41 -6.30 18.47 29.24
13 G -0.30 -2.41  2.90 -4.07  9.63 32.20
14 A  0.30 -2.14  2.92 -1.01 15.53 35.60
15 U 14.78 -2.10  8.45 -137.23 -1.76 153.31
16 A -7.40  4.51  4.65 27.02  5.03  48.56
17 G -7.48  1.52  5.26 -93.06 111.24 -17.94
18 C -0.33 -1.80  3.59  7.88 12.91 29.67
19 U  0.26 -1.42  3.86 -3.52  1.55 39.41
20 C  1.42 -1.85  2.00 20.65 -1.96 31.30
21 A -1.03 -2.90  3.65  5.63 10.81 20.03
22 U 14.78 -2.10  8.45 -137.23 -1.76 153.31
23 A -7.40  4.51  4.65 27.02  5.03  48.56
24 G -7.48  1.52  5.26 -93.06 111.24 -17.94
25 C -0.33 -1.80  3.59  7.88 12.91 29.67
26 U  0.26 -1.42  3.86 -3.52  1.55 39.41
27 C  1.42 -1.85  2.00 20.65 -1.96 31.30
28 A -1.03 -2.90  3.65  5.63 10.81 20.03
29 U 14.78 -2.10  8.45 -137.23 -1.76 153.31
30 A -7.40  4.51  4.65 27.02  5.03  48.56
31 G -7.48  1.52  5.26 -93.06 111.24 -17.94
32 C -0.33 -1.80  3.59  7.88 12.91 29.67
```

http://web.x3dna.org  
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<table>
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<th>#</th>
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<td>3.03</td>
<td>2.84</td>
<td>9.64</td>
<td>32.93</td>
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<td>0.78</td>
<td>-2.04</td>
<td>3.47</td>
<td>-3.42</td>
<td>7.62</td>
<td>26.84</td>
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<td>76</td>
<td>1.01</td>
<td>-2.12</td>
<td>3.17</td>
<td>3.03</td>
<td>2.10</td>
<td>29.20</td>
</tr>
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<td>3.19</td>
<td>9.58</td>
<td>-1.40</td>
<td>60.31</td>
</tr>
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<td>78</td>
<td>17.48</td>
<td>2.98</td>
<td>4.32</td>
<td>6.43</td>
<td>-13.77</td>
<td>135.87</td>
</tr>
<tr>
<td>79</td>
<td>-0.60</td>
<td>0.29</td>
<td>2.84</td>
<td>18.44</td>
<td>5.41</td>
<td>42.16</td>
</tr>
</tbody>
</table>

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S3 Two new backbone parameters

Two new parameters have been added in recent releases of 3DNA. One is a set of virtual backbone torsion angles, based on the positions of the phosphorus atom (P) and the origin of the standard base reference frame. The other is ssZp, single-stranded Zp, defined as the z-coordinate of the bridging P atom expressed in the standard reference frame of the preceding base (at 5′, see Figure 2 in the main text). Note that ssZp is sensitive to base conformation relative to the sugar moiety: for the syn conformation, ssZp is negative, while for the more common anti conformation, it is positive.

The following set of backbone torsion angles is based on 1xvk, the echinomycin-(GCGTACGC)$_2$ complex. The output file is generated with the command listed below.

```
analyze -symm -tor=1xvk -torsions.txt 1xvk.pdb1
```

The output list includes three sections, (1) “Main chain and chi torsion angles”, (2) “Pseudo (virtual) eta/theta torsion angles”, and (3) “Sugar conformational parameters”. Note the correspondence between syn/anti base conformations in section (1) and negative/positive ssZp values in section (3).
<table>
<thead>
<tr>
<th>base v0</th>
<th>v1</th>
<th>v2</th>
<th>v3</th>
<th>v4</th>
<th>tm</th>
<th>P</th>
<th>Puckering</th>
<th>ssZp</th>
<th>Dp</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 ...2A...6 [DC]C</td>
<td>80.0</td>
<td>36.2</td>
<td>-32.2</td>
<td>15.5</td>
<td>9.2</td>
<td>37.3</td>
<td>149.7</td>
<td>C2 'endo'</td>
<td>-1.15</td>
</tr>
<tr>
<td>82 ...2A...5 [DG]G</td>
<td>-20.6</td>
<td>-6.4</td>
<td>28.6</td>
<td>-41.4</td>
<td>38.8</td>
<td>41.9</td>
<td>47.0</td>
<td>C4 'exo'</td>
<td>1.68</td>
</tr>
<tr>
<td>83 ...2A...4 [DA]A</td>
<td>-37.3</td>
<td>35.2</td>
<td>-21.1</td>
<td>1.0</td>
<td>23.2</td>
<td>37.3</td>
<td>124.5</td>
<td>C1 'exo'</td>
<td>1.27</td>
</tr>
<tr>
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<td>19.2</td>
<td>-18.3</td>
<td>10.8</td>
<td>1.5</td>
<td>19.7</td>
<td>157.6</td>
<td>C2 'endo'</td>
<td>1.53</td>
</tr>
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<td>-33.2</td>
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<td>-4.6</td>
<td>33.8</td>
<td>169.1</td>
<td>C2 'endo'</td>
<td>---</td>
</tr>
<tr>
<td>90 ...2A...3 [DC]C</td>
<td>-20.5</td>
<td>-6.4</td>
<td>28.6</td>
<td>-41.4</td>
<td>38.8</td>
<td>41.9</td>
<td>47.0</td>
<td>C4 'exo'</td>
<td>1.68</td>
</tr>
<tr>
<td>91 ...2A...4 [DA]A</td>
<td>-37.3</td>
<td>35.2</td>
<td>-21.1</td>
<td>1.0</td>
<td>23.2</td>
<td>37.3</td>
<td>124.5</td>
<td>C1 'exo'</td>
<td>1.27</td>
</tr>
<tr>
<td>92 ...2A...5 [DG]G</td>
<td>-13.3</td>
<td>19.2</td>
<td>-18.3</td>
<td>10.8</td>
<td>1.5</td>
<td>19.7</td>
<td>157.6</td>
<td>C2 'endo'</td>
<td>1.53</td>
</tr>
<tr>
<td>93 ...2A...6 [DC]C</td>
<td>-20.5</td>
<td>-6.4</td>
<td>28.6</td>
<td>-41.4</td>
<td>38.8</td>
<td>41.9</td>
<td>47.0</td>
<td>C4 'exo'</td>
<td>1.68</td>
</tr>
<tr>
<td>94 ...2A...7 [DG]G</td>
<td>-39.9</td>
<td>39.3</td>
<td>-29.8</td>
<td>2.2</td>
<td>24.0</td>
<td>40.5</td>
<td>125.9</td>
<td>C1 'exo'</td>
<td>1.79</td>
</tr>
<tr>
<td>95 ...2A...8 [DC]C</td>
<td>-17.0</td>
<td>31.6</td>
<td>-33.1</td>
<td>24.4</td>
<td>-4.5</td>
<td>33.7</td>
<td>169.1</td>
<td>C2 'endo'</td>
<td>---</td>
</tr>
</tbody>
</table>

Sugar conformational parameters:

Note: v0: C4’-O4’-C1’-C2’
v1: O4’-C1’-C2’-C3’
v2: C2’-C3’-C4’-C’
v3: C2’-C3’-C4’-O4’
v4: C3’-C4’-O4’-C1’
tm: the amplitude of pucker
P: the phase angle of pseudorotation

Pseudo (virtual) eta/theta torsion angles:

Note: eta: C(i-1)-P(i)-O4’(i)-P(i+1)
theta: P(i)-C4’(i)-P(i+1)-C4’(i+1)
eta’: C(i)-P(i)-C1’(i)-C1’(i+1)
theta’: P(i)-C1’(i)-P(i+1)-C1’(i+1)
eta”: Borg(i-1)-P(i)-Borg(i)-P(i+1)
theta”: P(i)-Borg(i)-P(i+1)-Borg(i+1)

ssZp: single-stranded Zp, defined as the z-coordinate of the 3’ phosphorus atom (P) expressed in the standard reference frame of preceding base; the value is POSITIVE when P lies on the +z-axis side (base in anti conformation); NEGATIVE if P is on the -z-axis side (base in syn conformation)

Dp: perpendicular distance of the 3’ P atom to the glycosydic bond

(as per the MolProbity paper of Richardson et al. (2010))

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S4 Tutorials

S4.1 Analysis module

The analysis component of Web 3DNA 2.0 determines a wide range of conformational parameters — such as the rigid-body parameters of interacting nucleic-acid bases, base-pair steps of spatially adjacent base pairs, torsion angles of main chain and side groups, etc. — from a user-provided PDB/NDB ID (the structure identifiers used respectively in the Protein Data Bank and the Nucleic Acid Database) or a PDB-formatted file. The output files can be downloaded or viewed directly on a results page. Selected base-pairing, base-pair step, and torsion angle data are reported in interactive tables.

Example 1-1: Analyze a nucleic-acid-containing structure by using the PDB/NDB ID

Before conducting an analysis on the web server, users are encouraged to verify the PDB/NDB ID of the input structure at the PDB or NDB website and gain some familiarity with the structure. To use the analysis component, the user can enter a valid PDB/NDB ID in the input area, for example “355D”.

After clicking the ‘Analyze’ button, the user is directed to a results page containing five sections: 1. Structural summary, 2. Block Representations, 3. Interactive 3D view, 4. Output files of derived structural parameters, and 5. Interactive tables.

1. Structural summary

This section briefly describes the structure title, the author(s), the deposition date,
the experimental method used to determine the structure, the resolution (for X-ray and EM structures), and any external links related to the structure. If the input file is an NMR structure, the number of models in the structure will be counted and reported (see Example 1-2 for the analysis of a multi-model structure).

2. Block Representations

![Block representations](http://web.x3dna.org)

These figures are drawn with schematic block representations of the bases, tube representations of the strands, and atomic representations of the sugar-phosphate-backbones. Proteins (if any) are represented by violet ribbons, and small molecules by ball-and-stick images. The same representations are also used in the PDB and NDB websites. The image is enlarged when the mouse is hovered over a specific image.

3. Interactive 3D view

![Interactive 3D view](http://web.x3dna.org)

A 3D interactive visualization of the input structure is presented in this section. The user can click the “JSmol” label on the lower right-hand corner to choose more options provided by JSmol.
4. Output files of derived structural parameters

Nucleic acid structural parameter summary file. View • Download

This file gives a detailed listing of the DNA structural parameters including but not limited to:
1. H-bonding information
2. Origins and mean base-pair normal vectors in the coordinate system of the given structure
3. Local base-pair parameters [Pictorial definitions]
4. Local base-pair step parameters [Pictorial definitions]
5. Local base-pair helical parameters [Pictorial definitions]
6. ‘Simple’ base-pair and step parameters [Click for details]
7. Minor- and major-groove widths [Click for details]
8. Structure classification (A, B, Z forms)
9. Main chain and glycoyl (chi) torsion angles [Pictorial definitions]

Base-pair and base-pair step parameter file. View • Download

This file contains both base-pair parameters and base-pair step parameters.
1. It can be directly used for reconstruction of a double-stranded nucleic acid structure.
2. It can be modified to reconstruct a variant double-stranded structure.

Base step parameter file based on a continuous single-stranded chain. View • Download

This file contains base step parameters based on a continuous single-stranded chain.
1. It can be directly used for reconstruction of a single-stranded nucleic acid structure.
2. It can be modified to reconstruct a variant single-stranded structure.

Torsion angle parameter file. View • Download

This file gives a detailed listing of torsion angle parameters including:
1. Main chain and chi torsion angles [Pictorial definitions]
2. Pseudo (virtual) etatheta torsion angles [Pictorial definitions]
3. Sugar conformational parameters [Pictorial definitions]

The list of parameters given above corresponds to the 5’ to 3’ direction of strand I and the 3’ to 5’ direction of strand II.

This section includes four derived parameter files: the summary file, the base-pair and base-pair step parameter file, the ‘single-stranded’ step parameter file and the torsion angle parameter file. Each file can be viewed on the web page or downloaded.
5. Interactive tables (one of them shown here)

This section contains nine interactive tables embracing rigid-body parameters of base pairs and base-pair steps as well as torsion angles. Users can control the number of rows displayed on each page with a limit of 100 entries per page. Data in the table can be sorted by clicking the column headers. Each table can be downloaded in CSV format for further processing. The angles are expressed in degrees and the distances in Angstrom units.

**Example 1-2: Analyze a multi-model structure**

If the input PDB/NDB ID represents a structure with multiple models (for example the NMR structure “1HRZ”), the results page will contain extra information and options in the Structural summary section (see the following figure).

The user can click the “Analyze multiple models” button to go to a multi-model analysis results page. It should be noted that a maximum of 50 models will be analyzed. If the ensemble has more than 50 models, only the first 50 models will be analyzed. The user should modify the input file to analyze additional models, 50 at a time.
The multi-model results page contains block representations of all models (50 maximum). The user can move the mouse over the small icons at the bottom to display a magnified view of the fluctuations among the models. The user can download each of the images by clicking the corresponding links.

This section includes structural parameter and image files of all the models. The user can directly view the parameter file for each model and a download link is also provided. For the convenience of users, a compressed folder including all the output files in ‘.tar.gz’
format is offered for downloading.

**Example 1-3: Analyze a nucleic-acid-containing structure by uploading the PDB-formatted file**

The user can also upload a structural file, including a multi-model file, in PDB format for analysis.

![Upload a PDB-formatted file](e.g., example.pdb)

After clicking the ‘Analyze’ button, the user is redirected to a results page that is similar to Example 1-1, but without the Brief Description section.
S4.2 Visualization module

The visualization component of Web 3DNA 2.0 creates schematics images of molecular presentations. The server takes a PDB/NDB ID or a user-uploaded PDB-formatted file, and returns various representations of the structure or parts of it. In this component, three visualization styles are provided: 1) Schematic base block representation of a structure, 2) Block representation of a multi-model structure, and 3) Stacking diagram of the bases in a base-pair step.

**Example 2-1: Block representation of a structure**

This module generates composite block/ribbon/backbone representations of a single nucleic-acid-containing structure. The plotted image includes informative color-coding of the nucleic acid. The bases are color-coded by residue type following the NDB convention (C, yellow; G, green; A, red; T, blue; U, cyan). Minor-groove edges of the base pairs are shaded.

Clicking the button directs the user to an interface with two options: 1) enter a PDB/NDB ID, or 2) upload a PDB-formatted file.

After a file is provided (for example “2NP2”) and the ‘Next’ button is clicked, the structure will be analyzed and the user will be directed to an intermediate page, where
various questions will be asked depending on the nature of the structure.

In this page, the user has multiple options to customize the resulting schematic images: 1) whether to display both nucleic acid and protein, if protein is present, or only nucleic acid, or only protein; 2) whether to show only P and base blocks for the nucleic acid structure; 3) whether to show a double-helix ribbon along the nucleic acid structure; and 4) whether to rotate the structure as a whole. The default block view places the structure in a ‘best-view’ orientation, i.e. the DNA structure is shown in a view perpendicular to the shortest principal axis. The user can specify an arbitrary rotation about the X-, Y-, or Z-axis.

After clicking the ‘Visualize’ button, the user is directed to five generated images with downloadable links.

A 3D interactive visualization of the input structure is also shown.
Example 2-2: Block representation of an ensemble of structures

The ensemble visualization style creates a composite image for a multi-model structure, such as NMR or simulation-generated structures.

Clicking the button directs the user to an interface with two options: 1) enter a PDB/NDB ID, or 2) upload a PDB-formatted file.

After a file is provided (for example “2MOE”) and the ‘Next’ button is clicked, the structure will be analyzed and the user will be directed to a control page with the following options: 1) specify an ensemble of models by choosing the starting and ending models, 2) choose a specific base pair from the first model against which all other models will be aligned, or use the original alignment, and 3) indicate whether to display both nucleic acid and protein, if protein is present, or only nucleic acid, or only protein.
Then the user can click the ‘Visualize’ button to view the ensemble image and to download the coordinates of the aligned structures.

The user can also display all of the models in the 3D interactive view (after clicking the “JSmol” label on the lower right-hand corner and selecting “model 1/20” and “All”).
It should be noted that this module applies only to multi-model structures; otherwise error messages will appear.

**Example 2-3: Stacking diagram of base-pair steps**

The ‘stacking diagram’ component generates images of successive Watson-Crick base pairs in a nucleic-acid-containing structure. The images are plotted with H-bonds and filled base rings; the user has the option to label the bases.

Clicking the button directs the user to an interface with two options: 1) enter a PDB/NDB ID, or 2) upload a PDB-formatted file.

After a file is provided (for example “355D”) and the ‘Next’ button is clicked, the structure will be analyzed and the user will be directed to an intermediate page to choose a base-pair step in the initial plotting. The user can also specify whether to show the labels of base residues and where to place the labels before clicking the ‘Visualize’ button. The labels can be placed inside the six-membered ring of each base or next to the C8 atom of purine (R) bases and the C6 atom of pyrimidine (Y) bases.
The user may need to wait a few minutes for the calculation of all the stacking images of a long piece of DNA, such as that on the nucleosome. After viewing the stacking diagram of the selected base pairs, the user can navigate to other steps along the structure by clicking the “Previous step” and “Next step” buttons.

A 3D interactive visualization of the associated base pairs is also shown.
S4.3 Modeling module: customized rebuilding

The rebuilding component of Web 3DNA 2.0 allows the user to build: 1) 3D models of customized double-helical and single-stranded structures with base pairs and base-pair/nucleotide steps arranged according to user-supplied rigid-body parameters, 2) a mixed DNA structure comprised of canonical A-, B-, and C-type DNA fragments. The output webpage includes a schematic representation of the constructed model, a PDB-formatted file with full atomic coordinates and a downloadable link, and a 3D interactive visualization of the constructed model.

Example 3-1: Reconstruction of a customized model

Clicking the button directs the user to an interface for building a DNA/RNA model with customized base-pair/nucleotide step parameters.

The user must prepare a file containing the rigid-body parameters for the desired structure. Two example parameter files can be found at the “Test parameters” links. A single click of each button will automatically fill the corresponding content in the text area.

http://web.x3dna.org

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(one file for double-stranded DNA, the other one for single-stranded RNA). Note that the base-pair step parameters are null for the first base pair, but the parameter of the first base-pair can have non-zero values.

As an alternative, the user can also upload a parameter file in a valid format (please refer to the example parameter files: double-helix DNA and single-stranded RNA).

Next, the user needs to specify whether to construct a full atomic model and to select a sugar type: B-DNA (C2'-endo), A-DNA (C3'-endo), or RNA (C3'-endo). The user can also specify whether to optimize the backbone geometry.

After clicking the “Build Model” button, the user will be directed to a results page shown below.

http://web.x3dna.org
Example 3-2: Construction of a model that combines of A-, B-, and C-DNA conformational segments

The user can also construct a DNA model with a combination of A, B, and C double-helical components. Clicking the button directs the user to the following interface:

![Current choice: Combination of A-, B-, or C-form DNA](http://web.x3dna.org)

The user then can choose the desired number of segments in the model from a dropdown list (for example “3”). Clicking the “Next” button leads to an intermediate page where the
user can select the sequence and conformational form (A-, B-, or C-DNA) of each segment. Note that the input sequence is that of the leading strand.

![Image of sequence selection](http://web.x3dna.org)

After clicking the ‘Build’ button, the user will be directed to a results page shown below.

![Image of 3D DNA structure](http://web.x3dna.org)
S4.4 Modeling module: DNA ‘decorated’ with proteins

The composite component of Web 3DNA 2.0 allows the user to construct models of DNA ‘decorated’ with proteins and other molecules by providing the requisite DNA-ligand structural templates and the locations of the binding sites. The output webpage includes a schematic representation of the constructed nucleic-acid protein/ligand complex, a PDB-formatted file with full atomic coordinates of the constructed model, and a 3D interactive visualization.

Example 4-1: Construction of a protein-bound DNA model

First the user needs to select the number of bound ligands from a dropdown list (for example “2”), and then clicks the ‘Continue’ button.

Next, the user is asked to type in the DNA sequence bound by the proteins of interest. Here, we enter a test DNA sequence of 60 base pairs with poly dA:poly dT. Note that the input sequence is that of the leading strand.

Then, the user needs to specify the conformation of the unbound DNA (A- or B-form) and to supply, for each binding site, the binding position. The binding position corresponds to the location of the central base or base-pair step of the specified protein-bound DNA segment. Here, two DNA-protein complexes (PDB ID: 1LMB and 5Z2T) serve as templates for binding DNA at positions 20 and 40.
Clicking the ‘Continue’ button directs the user to a confirmation page, where the binding information can be verified. Warnings are given on this page if the binding input is inappropriate.

The user can choose whether to optimize the backbone geometry. After clicking the ‘Build’ button, the user will be directed to a results page that contains: 1) a ribbon-like representation of the DNA-protein complex, 2) downloadable links to the image and the atomic coordinate file, and 3) a 3D interactive visualization of the constructed DNA-protein complex.
Example 4-2: Construction of a chromatin-like model

The composite component also enables the user to build complicated protein-decorated DNA structures, such as a chromatin-like model. Here, we use a three-nucleosome model as an example.

First the user assigns the number of nucleosomes in the final model to 3.

Choose a binding site number to build a protein-decorated DNA model: 3

Next, the user enters a DNA sequence of poly dA:poly dT with the length of 520 base pairs. The user can just type in an “A” in the sequence text area and assign the repeating number to 520. Note that the input sequence is that of the leading strand.
Then, the user specifies the conformation of the unbound DNA to B-form and provides nucleosome structures (for example PDB ID: 4XZQ) as templates to bind DNA at positions 85, 260 and 435.

In the confirmation page, the user can verify all the binding information and then click the ‘Build’ button.
After waiting about 3 minutes, the user will obtain the constructed model: a chromatin-like structure.
S4.5 Modeling module: 56 fiber models

The fiber component of Web 3DNA 2.0 allows the user to build fiber-diffraction models of nucleic acids with arbitrary sequence and helical types. A total of 56 fiber models is available in the current web server, with six commonly used ones (RNA, A-DNA, B-DNA, C-DNA, Pauling triplex, and poly(I) quadruplex models) placed at the top of the list.

After selecting a fiber model from the list, the user can specify a sequence and/or a repeating unit number. The output webpage includes a schematic representation of the constructed model, a PDB-formatted file with full atomic coordinates of base and sugar-phosphate atoms, and a 3D interactive visualization.

<table>
<thead>
<tr>
<th>Commonly used fiber models</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short description</strong></td>
</tr>
<tr>
<td>RNA (generic sequence: A, C, G, U)</td>
</tr>
<tr>
<td>A-DNA (generic sequence: A, C, G, T)</td>
</tr>
<tr>
<td>B-DNA (generic sequence: A, C, G, T)</td>
</tr>
<tr>
<td>C-DNA (generic sequence: A, C, G, T)</td>
</tr>
<tr>
<td>Pauling triplex model (generic sequence: A, C, G, T, U)</td>
</tr>
<tr>
<td>poly(I) : poly(I) : poly(I) : poly(I)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Full list of available fiber models</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
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<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

The detailed parameters of 55 fiber models are listed in the following table, taken from the initial 3DNA publication. For the Pauling triplex model, please check the blogpost http://web.x3dna.org Li, Olson & Lu 32
“Pauling triplex model of nucleic acids is available in 3DNA” on the 3DNA homepage for further information.

Example 5-1: Construction of a generic B-form DNA fiber model

The user first selects the B-DNA (generic sequence: A, C, G, T) fiber model from the list of “Commonly used fiber models”.

http://web.x3dna.org
The generic DNA fiber models accommodate arbitrary base sequences of any length and only A, T, C, and G characters can be accepted in the input sequence. For a generic fiber, the user can type in a sequence and the number of repeating units as shown below. Note that the input sequence is that of the leading strand.

After clicking the ‘Build’ button, the user will be directed to a results page, which contains four sections: 1) a block representation of the constructed model, 2) a link to download a block image of the constructed model, 3) a link to download the coordinates of the constructed model, 4) a 3D interactive visualization of the constructed model.

Example 5-2: Construction of a Pauling triplex model

The fiber component also enables the user to build proposed DNA models. Here, we use the Pauling triplex model as an example.
After selecting the Pauling triplex fiber model (generic sequence: A, C, G, T, U) from the list of "Commonly used fiber models", the user can enter a desired sequence, for example “AAAACCCCGGGG”. The input characters are case-insensitive. Note that the input sequence is the same for all three strands.

After clicking the ‘Build’ button, the user is directed to a results page that contains four sections: 1) a block representation of the constructed model, 2) a link to download a block image of the constructed model, 3) a link to download the coordinates of the constructed model, 4) a 3D interactive visualization of the constructed model. The user should notice that the triplex model has phosphates in the center and bases on the outside of the structure.
S4.6 Modeling module: base mutations

The mutation component of Web 3DNA 2.0 allows the user to mutate any base(s) of a nucleic acid structure to desired one(s). The user can use a PDB/NDB ID, a user-uploaded PDB-formatted file, or any of the models constructed by the modeling components in the web server. The output includes: 1) schematic representations of the original and mutated structures, 2) a PDB-formatted file with full atomic coordinates of the mutated structure, and 3) 3D interactive visualizations of the original and mutated structures.

It should be noted that both the sugar-phosphate backbone conformation and base reference frame (position and orientation) are preserved in the mutation process. Therefore, the original and mutated structures share the same base-pair/step parameters.

Example 6-1: Mutate a nucleic-acid-containing structure by using the PDB/NDB ID

The user first enters a PDB ID (for example “355D”) and clicks the ‘List all bases’ button.

Then the user is directed to an intermediate page where an enumeration of all bases is listed.
After choosing base(s) for mutation (for example here we mutate base G in chain A/residue number 2 to A and base G in chain A/residue number 4 to U) and clicking the “Continue” button, the user will be directed to a results page containing five sections: 1) a block representation of the original structure, 2) a list of mutated bases, 3) a block representation of the mutated structure, 4) a 3D interactive visualization of the original structure.
structure, 5) a 3D interactive visualization of the mutated structure.

The text between the base-block images gives the number and identities of mutated nucleotides.
Example 6-2: Mutate a structure by uploading a PDB-formatted file

The user can also upload a PDB-formatted file to conduct base mutation(s). After uploading the structure and clicking the ‘List all bases’ button, the user follows a procedure that is similar to the example of mutating a structure by using the PDB/NDB ID.

Example 6-3: Construction of a G-quadruplex DNA model

The combination of mutation and fiber components can be used to build special DNA/RNA models, such as a G-quadruplex DNA model. The steps are as follows:

First, the user selects the poly(I):poly(I):poly(I):poly(I) fiber model from the list of "Commonly used fiber models".

For this non-generic fiber model, the user should enter the number of nucleotide repeating units, for example “12”.

After clicking the ‘Build’ button, the user is directed to a results page containing the constructed model.
To build a G-quadruplex DNA model, the user needs to mutate all hypoxanthine bases in the current structure to guanine (G). Clicking the link “Use this structure for mutation” leads the user to a mutation selection interface. The interface contains a list of mutable bases. The user then chooses the option “Mutate to All G” and clicks the “Continue” button. The user will be directed to a results page with the mutated structure: a G-quadruplex DNA model.
## Select base(s) you want to mutate:

<table>
<thead>
<tr>
<th>Index</th>
<th>Chain</th>
<th>Residue Num</th>
<th>Current Base</th>
<th>Mutate to</th>
<th>Clear all</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>1</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>2</td>
<td>I</td>
<td>All: A C G T U</td>
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</tr>
<tr>
<td>3</td>
<td>A</td>
<td>3</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>4</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>5</td>
<td>I</td>
<td>All: A C G T U</td>
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</tr>
<tr>
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<td>A</td>
<td>6</td>
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<td>All: A C G T U</td>
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</tr>
<tr>
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<td>7</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>8</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>9</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
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<td>10</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>11</td>
<td>A</td>
<td>11</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
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<td>12</td>
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<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>13</td>
<td>B</td>
<td>13</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
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<td>I</td>
<td>All: A C G T U</td>
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<tr>
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<td>15</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
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<td>16</td>
<td>B</td>
<td>16</td>
<td>I</td>
<td>All: A C G T U</td>
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<td>I</td>
<td>All: A C G T U</td>
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<td>B</td>
<td>19</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
<tr>
<td>20</td>
<td>B</td>
<td>20</td>
<td>I</td>
<td>All: A C G T U</td>
<td>Clear</td>
</tr>
</tbody>
</table>

### The mutated structure [48]

- chain A.1: I→G
- chain A.2: I→G
- chain A.3: I→G
- chain A.4: I→G
- chain A.5: I→G
- chain A.6: I→G
- chain A.7: I→G
- chain A.8: I→G
- chain A.9: I→G
- chain A.10: I→G
- chain A.11: I→G
- chain A.12: I→G
- chain B.13: I→G
- chain B.14: I→G
- chain B.15: I→G
- chain B.16: I→G
- chain B.17: I→G
- chain B.18: I→G
- chain B.19: I→G
- chain B.20: I→G
- chain B.21: I→G
- chain B.22: I→G
- chain B.23: I→G
- chain B.24: I→G
- chain C.25: I→G
- chain C.26: I→G
- chain C.27: I→G
- chain C.28: I→G
- chain C.29: I→G
- chain C.30: I→G
- chain C.31: I→G
- chain C.32: I→G
- chain C.33: I→G
- chain C.34: I→G
- chain C.35: I→G
- chain C.36: I→G
- chain D.37: I→G
- chain D.38: I→G
- chain D.39: I→G
- chain D.40: I→G
- chain D.41: I→G
- chain D.42: I→G
- chain D.43: I→G
- chain D.44: I→G
- chain D.45: I→G
- chain D.46: I→G
- chain D.47: I→G
- chain D.48: I→G
S5 Related external resources

- **3DNA Home** — the home page of the 3DNA software package
- **3DNA Forum** — online discussions of 3DNA-related questions
- **Web DSSR** — a webserver for DSSR (Dissecting the Spatial Structure of RNA)
- **do_x3dna** — software built on top of 3DNA for the analysis of DNA/RNA molecular dynamics simulations
- **3D-DART (3DNA-Driven DNA Analysis and Rebuilding Tool)** — a server for generating 3D structural models of double-stranded DNA
- **Phenix** — a software suite for the automated determination of molecular structures using X-ray crystallography and other methods. Web 3DNA 2.0 employs the ”phenix.geometry.minimization” command to optimize backbone geometry while keeping base atoms fixed
- **Curves+** — a popular approach for analyzing the structures of nucleic acids
- **NAB (Nucleic Acid Builder)** — a molecular manipulation language for constructing 3D structures of nucleic acids
- **make-na server** — a web-based automated tool for making nucleic acid helices powered by NAB
- **NUCGEN-Plus** — a web-server for generating sequence-dependent nonuniform nucleic acid structures
- **NUPARM** — a program for analyzing sequence-dependent variations in nucleic acid (DNA & RNA) double helices
- **RCSB PDB** — a database about 3D shapes of proteins, nucleic acids, and complex assemblies that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease
- **Nucleic Acid Database** — a database containing information about experimentally-determined nucleic acids and complex assemblies