DSSR-enabled innovative schematics of 3D nucleic acid structures with PyMOL

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ABSTRACT

Sophisticated analysis and simplified visualization are crucial for understanding complicated structures of biomacromolecules. DSSR (Dissecting the Spatial Structure of RNA) is an integrated computational tool that has streamlined the analysis and annotation of 3D nucleic acid structures. The program creates schematic block representations in diverse styles that can be seamlessly integrated into PyMOL and complement its other popular visualization options. In addition to portraying individual base blocks, DSSR can draw Watson-Crick pairs as long blocks and highlight the minor-groove edges. Notably, DSSR can dramatically simplify the depiction of G-quadruplexes by automatically detecting G-tetrads and treating them as large square blocks. The DSSR-enabled innovative schematics with PyMOL are aesthetically pleasing and highly informative: the base identity, pairing geometry, stacking interactions, double-helical stems, and G-quadruplexes are immediately obvious. These features can be accessed via four interfaces: the command-line interface, the DSSR plugin for PyMOL, the web application, and the web application programming interface. The supplemental PDF serves as a practical guide, with complete and reproducible examples. Thus, even beginners or occasional users can get started quickly, especially via the web application at http://skmatic.x3dna.org.

INTRODUCTION

Sophisticated analysis and simplified visualization are crucial for interpreting complicated 3D structures of biomacromolecules, such as those deposited in the PDB (1). Unlike proteins and carbohydrates, nucleic acids possess unique structural features that require dedicated software tools for their in-depth analysis and effective depiction. The aromatic, planar bases are a defining feature of RNA and DNA, and they can pair and stack with each other in various ways. Together with the sugar-phosphate backbone, the bases participate in many tertiary motifs (2,3). Thus, bases have been simplified as slabs to emphasize base pairing and stacking interactions (4), or as sticks in ribbon representations (5).

With the exception of Jmol/JSmol (6), however, available visualization tools lack a sophisticated analysis engine for nucleic acid structures. They cannot select features such as hairpin/junction loops or noncanonical pairs, render minor grooves as an attribute, or simplify RNA/DNA representations beyond the level of individual bases or Watson-Crick WC pairs. As a salient example, none of them can automatically identify and highlight G-quadruplexes, an increasingly important class of noncanonical nucleic acid structures (7–9).

DSSR (Dissecting the Spatial Structure of RNA) (10) is an integrated computational tool that has been designed from the bottom up to streamline the analysis and annotation of 3D nucleic acid structures. Starting from an atomic-coordinate file in PDB or PDBx/mmCIF format, the program automatically identifies and characterizes numerous structural features, including modified nucleotides, arrays of stacked bases, (noncanonical) base pairs, higher-order base associations (multiplets), pseudoknots of arbitrary complexity, various types of ‘closed’ loops, canonical stems, coaxially stacked helices, and G-quadruplexes. DSSR has been extensively tested using all nucleic-acid-containing structures in the PDB and is continuously developed following user feedback. Over the years, DSSR has been widely cited in scientific literature (11–18) and adopted into many other structural bioinformatics resources (19–26).

The DSSR-Jmol integration (6) introduces a novel and powerful SQL-like selection mechanism for DSSR-derived structural features, and provides simplified representations of nucleic acids as step diagrams and base blocks in Jmol/JSmol. This work fills a gap in RNA/DNA structural bioinformatics, and serves as an example for incorporating DSSR-derived features into other molecular viewers. PyMOL (Schrödinger, Inc.) is an industry leader in 3D molecular visualization, and it is especially well-developed for protein structures. For nucleic acids, however, PyMOL is deficient in two main aspects. Firstly, it lacks a selec-
tion mechanism for RNA/DNA-specific features (including WC pairs or G-quadruplexes). Secondly, its representation styles of nucleic acids, most notably the planar nitrogenous bases, are limited. DSSR has unique features that can significantly enhance the selection and visualization of nucleic acid structures in PyMOL. Specifically, DSSR creates schematic block representations in diverse styles that can be seamlessly integrated into and complemented with other popular visualization options of PyMOL. DSSR dramatically simplifies the depiction of G-quadruplexes by automatically detecting G-tetrads and depicting them as large square blocks. The DSSR-PyMOL schematics are simple, informative, and appealing. They are especially effective for RNA and DNA structures with up to dozens of nucleotides (Supplementary Figure S1).

The idea of representing bases and WC-pairs as rectangular blocks came from the pioneering work of Calladine et al. (27,28) The block schematics were first implemented in the pair of SCHNAaP/SCHNArP programs (29,30) for rigorous analysis and reversible rebuilding of double-helical nucleic acid structures. The algorithms that underpinned SCHNAaP/SCHNArP laid the foundation of ‘analyze’ and ‘rebuild’, two core components of the 3DNA suite of programs (31–33). 3DNA also takes advantage of the standard base reference frame (34), and comprises quite a few other related programs. One of them is ‘blocview’, a script which calls several 3DNA utility programs to generate individual base blocks and set the view, MolScript (35) to produce backbone ribbons, and Raster3D (36) to render the composite image. The 3DNA ‘blocview’ schematics catch characteristic attributes of nucleic acid structures. They have gradually become popular and been adopted into the RCSB PDB (1) and the NDB (37), and then propagated into other bioinformatics resources (e.g., the ‘RNA Structure Atlas’ website hosted by the Leontis-Zirbel RNA group).

DSSR supersedes ‘blocview’ by eliminating all the internal and external dependencies of the 3DNA utility program. DSSR produces block representations, not only of individual bases but also WC-pairs and G-tetrads, that can be fed directly into PyMOL. The DSSR-PyMOL integration is easier to use, has more features, and produces better schematics than the original 3DNA-blocview approach. Users can easily create cartoon-block images via the command-line interface (CLI), the DSSR plugin for PyMOL, the web application, or the web application programming interface (API). Together, these four interfaces cover virtually all conceivable cases of usage. Moreover, the supplemental PDF has been diligently written to serve as a practical guide, with complete and reproducible examples. Thus, even beginners or occasional users can get started quickly, particularly via the web application at http://skmatic.x3dna.org.

MATERIALS AND METHODS

There are four ways to produce DSSR-enabled schematics with PyMOL: the CLI, the dssr block PyMOL plugin, the web application, and the web API. The CLI interface requires the installation of DSSR, as detailed in the supplemental PDF. In essence, users simply need to download one binary executable that is <2MB in size and available for macOS, Linux, or Windows. DSSR is self-contained, with zero runtime dependencies on third-party libraries. The dssr block plugin is available from the PyMOL Wiki page. The script can be directly executed, imported as a Python module, or installed with the ‘Plugin Manager’ of PyMOL. The simplest user interface is via the web application at http://skmatic.x3dna.org: it only requires a modern web browser with internet connection. Software developers can take advantage of the web API (http://skmatic.x3dna.org/api) programmatically, without installation of DSSR or the plugin.

In this section, we first define DSSR blocks (Figure 1), and then outline the major aspects of each of the four interfaces for creating DSSR-PyMOL cartoon-block images. See the supplemental PDF for complete and reproducible examples presented in a tutorial style.
Figure 2. Cartoon-block schematics enabled by the DSSR-PyMOL integration for six representative PDB entries. Note WC-pairs as long blocks and minor-groove edges rendered in black (A, B), G-tetrads as square blocks and the metal ion as sphere (C), the ligand as balls-and-sticks (D), and proteins as purple cartoons (E, F). Color code: A, red; C, yellow; G, green; T, blue; U, cyan; G-tetrad, green; WC-pairs, per base in the leading strand.

Definition of blocks

Currently available DSSR blocks are defined in Figure 1. The default purine (A, G) and pyrimidine (C, T, U) base blocks (Figure 1A) have already been used in the 3DNA-blocview program (31–33). The long WC-pair block, however, is new to DSSR. The standard base reference frame (34) has its positive $x$-axis pointing from the minor-groove edge to the major groove. The combined usage of WC-pair blocks and minor-groove edges reveals double-helical stems (10), their right- or left-handedness, and the alternations of minor- and major-grooves in unprecedented clarity (see Figure 2).

A brand-new feature of DSSR is the automatic identification and comprehensive characterization of G-quadruplexes (Figure 3, see also http://g4.x3dna.org). Details of the underlying algorithms and survey results on all G-quadruplexes available in the PDB will be reported elsewhere. Here we introduce the slim-guanine block that unambiguously highlights the H-bonding directionality in G-tetrads, and the large square G-tetrad block that greatly simplifies the visualization of G-quadruplexes (Figure 1B, see also Figure 3).

The command-line interface

The CLI is the most efficient and versatile way to explore the numerous features that DSSR has to offer. It also provides the basis for the other three interfaces that make DSSR-PyMOL schematics easily accessible to a wider audience. There are five options to set DSSR block types and PyMOL rendering parameters: (a) The block-file option turns on the block representation, with features specified as a list of keywords (Supplementary Figure S2A–C). For example, the list 'wc-minor-g4' (i.e., block-file=wc-minor-g4) leads to WC-pairs being rendered as long blocks, minor-grooves colored in black, and G-tetrads as square blocks (Figure 1, see also examples in Figures 2 and 3). The order of the keywords in a list does not matter. For instance, 'g4-wc-minor' would have the same effect as 'wc-minor-g4'. If a structure does not possess a feature, the corresponding keyword will be ignored. For example, if 'wc' is specified for a structure without WC pairs (e.g., Figures 2C and 3C), nothing will occur. (b) The block-color option is used to customize the colors of blocks or DSSR-derived features (Supplementary Figure S2D). (c) The block-depth option sets the thickness of blocks (Supplementary Figure S2D). (d) The cartoon-
block option combines PyMOL cartoon representations and DSSR block schematics with a selected set of style settings (Supplementary Figure S3A). (e) The blocview option is like cartoon-block but it also sets the structure into the most extended view and draws solid-colored blocks with black outlines (Figures 2 and 3, and Supplementary Figure S3B). The last two options can be used to automate the process of creating DSSR-PyMOL schematics. See Supplementary Figures S4–S6 and the corresponding commands listings for worked examples.

The dssr_block PyMOL plugin

The dssr_block plugin makes DSSR transparent to PyMOL users (Supplementary Figure S7). Essentially, it adds the dssr_block command to PyMOL for interactive creation of DSSR blocks in various styles (Figure 1, see also examples in Figures 2 and 3). Instead of working directly with coordinate inputs and block outputs on the command line, the plugin takes advantage of PyMOL’s atom selections and displays the results instantaneously. This way, it seamlessly integrates and complements the other visualization options in PyMOL.

The web application

The http://skmatic.x3dna.org site provides a cross-platform, web-based application for easy creation of DSSR-PyMOL schematics (Supplementary Figure S8). Implemented with standard web technologies (see the supplemental PDF), the client-side user interface works in all modern web browsers across a wide spectrum of platforms. The web application is intuitive, allowing even novices or occasional users to get started quickly.

The web application consists of two components: (a) pre-calculated schematics and summary information of PDB entries, a common use case (Figure 4, and Supplementary Figure S9); (b) the general application, necessitating the input of an atomic-coordinate file together with optional customizations.
RESULTS AND DISCUSSIONS

Cartoon-block schematics

Figure 2 showcases representative cartoon-block schematics enabled by the DSSR-PyMOL integration. The six PDB entries illustrated there cover different types of RNA, DNA, and their complexes with ligands or proteins: (a) 4kz2, a crystal structure of the phi29 pRNA three-way junction core (38); (b) 2lx1, the major conformation of the internal loop 5′GAGU/3′UGAG (39); (c) 6tzq, a DNA G-quadruplex/i-motif hybrid (40); (d) 2hoj, the crystal structure of an Escherichia coli thi-box riboswitch bound to thiamine pyrophosphate (41); (e) 6rjg, a high resolution cryo-EM structure of the St1Cas9-sgRNA-AcrIIA6-tDNA59-ntPAM complex (42); and (f) 6pj6, a high resolution cryo-EM structure of the E. coli 50S subunit (43). See the supplemental PDF for detailed instructions to reproduce these images.

Simplified representations of G-quadruplexes

G-quadruplexes are noncanonical nucleic acid structures formed from guanine-rich base sequences. The building block of G-quadruplexes is G-tetrad, a special type of higher-order, planar base associations (termed ‘multiplets’ in DSSR) (10). The four guanines of a G-tetrad are arranged in a cyclic manner via four consecutive G+G pairs (10,31), each held together by two H-bonds (Figure 3A-B, and Supplementary Figure S6). G-quadruplexes arise from stacking of G-tetrads and are stabilized by cations (Figure 3C, D, and also Figure 2C). The guanine base can be in anti or syn glycosidic torsion angle conformation, leading to polymorphic topologies and groove-width variations in the 3D structures of G-quadruplexes (Figure 3).

DSSR can detect and characterize G-quadruplexes, simply starting from an atomic-coordinate file. Please see http://g4.x3dna.org for experimentally determined G-quadruplexes that are auto-annotated by DSSR from the
PDB. A detailed description of this new DSSR module on G-quadruplexes will be reported in a separate article. Figure 3 showcases some of the structural features of G-quadruplexes that are made clear by the DSSR-PyMOL integration: panels A and B show the molecular images of two G-tetrads, overlaid with guanines as slim blocks. Switching a guanine from anti to syn conformation reverses the H-bonding directionality of the G-tetrads from counterclockwise to clockwise. The syn conformation (of the modified nucleotide BGM, 8-bromo-2’-deoxyguanosine) stands out by being colored teal instead of green for the anti guanines. Panel C illustrates the distinction between helix and stem and the definition of coaxial stacking interactions (10), in the context of G-quadruplexes. The meaning of the three terms is explicit in the simplified representation, using large square blocks for G-tetrads and different colors (red and green) for the two DNA chains. Panel D demonstrates the duplex-quadruplex transition using a combination of long blocks for WC-pairs and slim guanine blocks for G-tetrads. The solid-colored blocks make the three-layered G-quadruplex, including the syn guanines, easily discernible. In Figure 3, panels A and C are based on the crystal structure of a parallel DNA G-quadruplex with bulged cytosines (PDB entry 5ua3) (13), whilst panels B and D pertain to the (1+3) hybrid G-quadruplex structure with lateral-propeller-propeller loops and three syn guanines (PDB entry 69k) (44).

As shown in Figures 1B and 3A, B, the square-block representation is defined specifically for the G-tetrad of G-quadruplexes. By design, the square block for the G-tetrad parallels the long-block for WC pairs in canonical DNA and RNA double helices. Just as there are numerous types of noncanonical base pairs, many forms of non-G tetrads have been reported (9,45,46), including A-, C-, T- and U-tetrads, or mixed tetrads. Also, there are cases of pseudo-G-tetrads (47,48), where some of the G-G pairs are distinct from the one in the G-tetrad of G-quadruplexes (see above and Figure 3A, B). A recent survey of the PDB reveals that over 80% of all tetrads are the G-tetrad in G-quadruplexes (46). As noted at the beginning of this section, a tetrad (G-tetrad or otherwise) is a special type of ‘multiplets’ in DSSR (10), and it may belong to a larger context with more than four bases (pentad, hexad, heptad, and octad etc.) The WC-pair block schematics not only make the canonical duplexes stand out, but also highlight single-stranded regions and noncanonical pairs (e.g., Figure 2A, B). In a similar fashion, the G-tetrad block schematics introduced here allow the G-quadruplexes and nearby components (including other tetrads) to be immediately recognizable (e.g., Figures 2C and 3C).

The advantages enabled by DSSR for the visualization of G-quadruplexes in PyMOL are obvious (Figure 3). The DSSR-PyMOL integration, like the DSSR-Jmol case (6), exemplifies the critical roles that a domain-specific analysis engine may play in an otherwise general-purpose molecular visualization tool.

Pre-calculated PDB entries

A common usage of the DSSR-enabled schematics with PyMOL is for nucleic-acid-containing structures in the PDB. The web application (http://skmatic.x3dna.org) allows users to conveniently access pre-calculated PDB entries via a clean and clutter-free interface. The default image (main view) of a PDB entry was created with the two DSSR options block-file=wc-minor-g4 and blockview (see above), for the first model of an NMR-ensemble or the asymmetric unit of a crystal or cryo-EM structure.

As an example, Figure 4 shows screenshots for PDB entry 6jq6, a hatchet ribozyme structure solved by X-ray crystallography (49). The results include two parts: (a) Summary information and the primary citation (including abstract, if any) compiled automatically from the RCSB PDB (1) and the PubMed. DSSR-derived features in human-readable text and machine-friendly JSON formats are available. (b) Cartoon-block schematics in six orthogonal views (also accessible as a tarball): the main view (top-left) and the other five orientations derived from it via simple rotations. The PyMOL session file for the main view can be downloaded for further customizations. The corresponding PDB coordinate file for this view can also be downloaded or visualized directly online using 3Dmol.js (50). Furthermore, the main-view image is accessible programatically via the web API (see the supplemental PDF for details).

CONCLUSIONS

DSSR-enabled block schematics bring innovative, simplified visualizations of 3D nucleic acid structures into PyMOL. DSSR implements long blocks for WC-pairs, minor-grooves highlighting, and most notably, large square blocks for G-tetrads in G-quadruplexes. These features can be seamlessly integrated into PyMOL and complement its other popular visualization options. As demonstrated in Figures 2–4, the DSSR-PyMOL schematics are informative, aesthetically pleasing, and highly characteristic of nucleic acids. For small to mid-sized RNA- or DNA-containing structures, the base identity, pairing geometry, stacking interactions, double-helical stems, and G-quadruplexes are clear. The four interfaces allow users to easily create novel, publication quality schematics of 3D nucleic acid structures. Finally, all results reported here are completely reproducible (see the supplemental PDF). Any questions related to this work are welcome and will be openly addressed on the 3DNA Forum (http://forum.x3dna.org).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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