

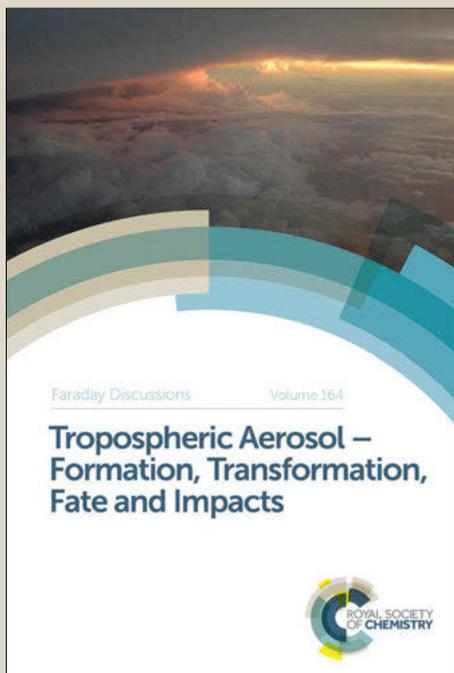
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RiboVision Suite for Visualization and Analysis of Ribosomes

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Abstract

RiboVision is a visualization and analysis tool for simultaneous display of multiple layers of diverse information on primary (1D), secondary (2D), and three-dimensional (3D) structures of ribosomes. The ribosome is a macromolecular complex containing ribosomal RNA and ribosomal proteins and is a key component to life responsible for synthesis of proteins in all living organisms. RiboVision is intended for rapid retrieval, analysis, filtering, and display of a variety of ribosomal data. Preloaded information includes 1D, 2D, and 3D structures augmented by base-pairing, base-stacking and other molecular interactions. RiboVision is preloaded with rRNA secondary structures, rRNA domains and helical structures, phylogeny, crystallographic thermal factors, etc. RiboVision contains structures of ribosomal proteins and a database of their molecular interactions with rRNA. RiboVision contains pre-loaded structures and data for two bacterial ribosomes (*Thermus thermophilus* and *Escherichia coli*), one archaeal ribosome (*Haloarcula marismortui*), and three eukaryotic ribosomes (*Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Homo sapiens*). RiboVision revealed several major discrepancies between 2D and 3D structures of 16S/18S and 23S/28S rRNAs. Revised structures mapped with the variety of data are available in RiboVision as well as in a public gallery (<http://apollo.chemistry.gatech.edu/RibosomeGallery>). RiboVision is designed to allow users to distill complex data quickly and to easily generate publication-quality images of data mapped onto secondary structures. Users can readily import and analyze their own data in the context of other work. This package allows users to import data from simple CSV format files and to map it directly onto 1D, 2D, and 3D levels of structure. RiboVision has features in rough analogy with web-based map services capable of seamlessly switching the type of data displayed and the resolution or magnification of the display. RiboVision is available at <http://apollo.chemistry.gatech.edu/RiboVision>.

Introduction

We present a tool devoted to the ribosome. This web-based portal, called RiboVision, is intended for rapid analysis, retrieval, filtering, and display of a variety of data types, simultaneously on three levels: primary (1D), secondary (2D), and three-dimensional (3D) structure, from ribosomes of six different species. RiboVision allows mapping and display of new and pre-loaded data, swapping of data between species, and quick generation of publication-quality images on any level of structure. RiboVision can maintain and display multiple layers of information, with controllable transparency. RiboVision allows users to import data from simple CSV format files and to map it directly onto all levels of structure. RiboVision has features in rough analogy with web-based map services capable of seamlessly switching the type of data displayed and the resolution or magnification of the display. RiboVision is available at <http://apollo.chemistry.gatech.edu/RiboVision>. We invite data for deposition, which would be made publically available.

The ribosome is a key component of life, found in every living cell, and is responsible for translation of genetic information from messenger RNA to coded protein.¹⁻³ The ribosome consists of several large ribosomal RNA molecules (rRNAs) and over fifty ribosomal proteins (rProteins). The ribosome contains over 250,000 atoms. Although eukaryotic ribosomes are larger and more sophisticated, many core functional regions of the ribosome are nearly identical in structure and sequence in all living organisms.⁴⁻⁶ Comparison of ribosomal components reveals information about phylogeny and evolution.^{7,8}

Ribosomes present special problems and opportunities for visualization and analysis. Ribosomes are exceedingly complex in terms of structure and function, highly abundant and widely distributed in biological systems, and are extremely dense in terms of available information. Easily accessible information on ribosomes includes (i) atomic positions of rRNA, rProtein, translation factors, associated ions, water molecules and antibiotics, obtained from x-ray and cryo-EM structures, (ii) molecular interactions between ribosomal components, inferred from 3D structures and from phylogeny, (iii) a very large and rapidly growing database of rRNA and rProtein sequences, (iv) phylogenetic relationships of various ribosomal components, (v) sites and types of modification of rRNA and rProteins, (vi) chemical mapping and reactivity data, (vii) functional and dynamical information, *etc.* Hundreds of secondary structures⁹⁻¹⁵ and tens of thousands of primary structures (sequences) of ribosomes have

been obtained.¹⁶ High-resolution x-ray or cryo-em 3D structures of ribosomal components are now available for species that represent the three major branches of the tree of life.¹⁷⁻²¹ This portal addresses the challenge of making accessible and integrating information on this key component of all biological systems. The basic features of RiboVision, such as selection of nucleotides, mapping the preloaded data onto 2D and 3D structures, and generating the figures, are intuitive. However, advanced features, such as mapping multiple custom data, manual coloring of nucleotides and interactions, asynchronous display of 2D and 3D structures, require reference to documentation available at <http://apollo.chemistry.gatech.edu/RiboVision/Documentation>.

A number of software packages have been designed to represent, edit, and manipulate secondary structures of RNA. This group includes RNA2DViewer/S2S,²² RNAMovies,²³ PseudoViewer,²⁴ NavRNA,²⁵ RNAPLOT/Vienna,²⁶ RNAVIZ2,²⁷ XRNA,²⁸ and VARNA²⁹. The functionalities of these viewers are summarized at <http://varna.lri.fr/index.php?lang=en&page=links&css=varna>. A specific secondary structure visualization tool has been recently developed for mapping structural and phylogenetic data onto 2D structures of ribosomal RNA [RNA2DMap].³⁰ In addition, three-dimensional structures can be visualized using either stand-alone applications e.g. PyMOL,³¹ Chimera,³² or VMD,³³ or java applets such as Jmol³⁴ and OpenAstexViewer.³⁵ Several RNA packages [*e.g.* Assemble2,³⁶ RNA2D3D³⁷] provide integration of 2D and 3D visualization.

We believe RiboVision is made distinctive by our attempt to integrate a broad variety of data at all levels of structure into a single intuitive and publically accessible portal, allowing users to visualize and archive any type of data, and to generate editable publication quality images. The RiboVision source-code is fully open to the public and released under the MIT license. RiboVision is fully functional without transfer of user data to remote servers, assuring confidentiality. RiboVision requires a modern browser with current Java. JSmol (to avoid Java) support is experimental, but causes degraded performance, so not currently recommended.

Features

RiboVision contains preloaded ribosomal structures along with preprocessed information related to these structures. The left menu (Figure 1a) controls data display, data import, and output. The right side toolbar controls data-visualization and website options. A

detailed description of the toolbar is given in the website manual (which opens in a separate browser tab upon clicking on the RiboVision logo).

User Interface

RiboVision's interface (Figure 1) is based on modern web technology (jQueryUI) and design principles. Simple features should be easy to discover, with more advanced functionality available, and described in detail in the online documentation.

Included Data

RiboVision contains pre-loaded structures and data for two bacterial ribosomes (*Thermus thermophilus* and *Escherichia coli*), one archaeal ribosome (*Haloarcula marismortui*), and three eukaryotic ribosomes (*Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Homo sapiens*). For each species, both Large Subunits (LSUs) and Small Subunits (SSUs) are provided, except for the omission of the SSU of *H. marismortui*, for which no 3D structure is currently available. LSUs and SSUs are visualized separately and independently of each other.

Secondary Structures

The 2D structures are composed of glyphs, one for each nucleotide. Each nucleotide glyph is a letter indicating the nucleotide type [A, C, G, U] centered on a circle. A user can map any desired quantitative of data to the colors of the letters, to the colors of the circles and to lines linking the glyphs. The data are either pre-loaded or supplied by the user. Both traditional and corrected versions of secondary structures for 16S/18S, 23S/28S/5.8S, and 5S rRNAs are available.

3D Structures

The coordinates of three-dimensional ribosomal structures were obtained from the Protein Data Bank (PDB). High resolution crystal structures were used for *E. coli*, PDB ID: 4GD1 and 3R8S,²⁰ *T. thermophilus*, PDB IDs: 2J00 and 2J01,¹⁹ *H. marismortui*, PDB ID: 1JJ2,¹⁷ and *Saccharomyces cerevisiae*, PDB IDs: 3U5B, 3U5C, 3U5D, and 3U5E.¹⁸ Cryo-EM structures were used for the three-dimensional coordinates of Metazoan species: *Drosophila melanogaster*, PDB IDs: 3J38, 3J3C, 3J39, 3J3E, and *Homo sapiens*, PDB IDs: 3J3A, 3J3D, 3J3B, and 3J3F.³⁸

Preloaded Data

Information in the data menus is populated with pre-computed data sets. The default database currently contains nucleotide attributes that fall into three categories: Nucleotide Data, Phylogeny Data, and Protein Contacts.

Nucleotide Data allows glyph coloring and selection by (i) nucleotide number, (ii) helix or domain, (iii) radial distance from a geometric center [known as the onion partition],⁴ (iv) crystallographic B-factor, and/or (v) proximity to a magnesium ion (within 2.4 Å, 2.6 Å, or 6.0 Å).

Protein Contacts allows glyph coloring and selection by molecular interactions with any desired subset of rProteins.

Phylogeny Data allows glyph coloring and selection by conservation statistics obtained from a preloaded multiple sequence alignment of rRNA sequences using the SILVA aligner.¹⁶ The multiple sequence alignment was performed on a subset of 128 species including major phyla from all three branches of the tree of life as reported elsewhere.^{39, 40} Phylogeny data are represented by pre-computed Shannon entropies (defined in a range 0-2).

Inter-Nucleotide Contacts allows visualization of various types of molecular interactions between nucleotides. The basic types of interactions include *base-pairing*, *base-stacking*, *base-phosphate*, and *base-sugar*. Each of these interactions types is further sub-divided using the data and nomenclatures of FR3D portal.⁴¹ This menu also contains data related to RNA-RNA interactions mediated by proteins or magnesium ions, organized in *protein interactions* and *Magnesium2.4A* options. The interaction data between rRNA and rProteins can be limited by the user to any selected subset of proteins. Secondary structures do not explicitly reflect tertiary interactions, which are inferred from 3D structures. Tertiary interactions are mapped and visualized by lines on 2D structures according to their Inter-Nucleotide Contact attribute.

ResidueTip

ResidueTip is activated by hovering the mouse directly over a nucleotide glyph. Various information about that nucleotide is raised in the tip. The name of the selected nucleotide is given at the top, followed by the name of the consensus nucleotide. Next, the "Selected Data" is displayed. Selected Data is associated with that residue in the currently selected layer. The Shannon entropy and a nucleotide frequency bar chart are also included. This information is

very useful to have at hand while exploring other mapped information. Holding down the Alt key activates the ResidueTip feature for interactions between glyphs. This function shows ResidueTips for both nucleotides, and elaborates on the type of interaction (Figure 1b).

Layer/Selection Manager

Multiple layers of data, of various types, can be simultaneously displayed and independently manipulated. Basic manipulation of data is implemented in the **Main** menu (Select and Display panels). The advanced display and output are controlled by the **Layer Manager** (Figure 1c) located in the toolbar. Sequence-related data is projected into the “Selected” Layer, controlled by the (S column of radio buttons).

Each layer has a type that determines how it is mapped onto the 2D structure. Data can be used to color a nucleotide letter, to color a nucleotide circle, or to draw lines between nucleotide glyphs. The layer type can be seen by clicking and expanding the properties of a layer. The default layers are displayed in the Main menu. In the “Letters” layer, data are represented by colors of the nucleotide letters. In the “Circles” layer, data are represented by the colors of nucleotide circles. The users can create additional layers using the **Layer Manager**. User-added layers cannot be of type letter. The layers will also appear in the Display panel of the **Main** menu. The ordering of the layers is controlled by dragging layer objects in the Display panel of the **Main** Menu or in the **Layer Manager**.

The color on the 3D structures can be made synchronous with any 2D layer by setting the “Linked” property (L column of radio buttons, Figure 1c) for that layer. A layer can be temporarily turned off and on by clicking on the “V” property (the eye icon), or permanently deleted from the menu by clicking the Trash icon.

Coordinated Nucleotide Selection and Coloring

Selection of nucleotides is coordinated across the 2D and 3D panels. The user can add nucleotides in the 2D panel view to the active selection group. Nucleotides can be selected individually or by using a click-and-drag selection box. A selected nucleotide is highlighted by a maroon circle. Multiple selection groups can be created and named using Selections tab of the Layers/Selection Manager. The user can re-activate a given group and add/remove nucleotide from the active selection group. In the 3D view, the selected nucleotides can be highlighted, or

the user can choose to hide the non-selected nucleotides. Custom selections can be saved in a session in Save Manager.

Importing User Data

The **Import** menu allows users to import their own data, such as conservation frequencies, SHAPE reactivity, foot-printing data, etc.). User data can be quickly and accurately mapped onto 1D, 2D, and 3D levels of structure. Data are imported as CSV files. User data does not go to the server; it is read and stored locally on the client. Pre-generated CSV input data templates are provided on the website. Differences in the numbering schemes between species require specific templates for each species, which are provided (see the detailed tutorial in the RiboVision Manual for additional information.) Users can also import data from the CSV files generated by the Save Manager. These files may contain not only the species sequences but also nucleotide and interaction data.

In the CSV template, the resNum column specifies the nucleotides in a format MoleculeName:NucleotideNumber(s) [*e.g.* 5S:35, 16S:(49-578), or 23S:271M]. Following convention, in some species NucleotideNumbers can consist of both digits and letter characters. The second column can have the heading DataCol, which contains numerical values corresponding to a desired property, or the heading ColorCol, with either hexadecimal color codes or supported color names (1700+ colors, see manual). When ColorCol is not provided, numerical information from DataCol will be mapped to a rainbow scheme, mapping the minimum value to blue, and the maximum value to red. Alternatively, the user can supply their own color scheme.

Saving Figures and Work

A number of options are incorporated to allow facile production of publication quality figures. Using the **Figures** tab in the **Save Manager**, images in the 1D, 2D, and 3D panels can be individually exported in a number of file formats. Specifically, images from the 1D panel can be exported in SVG format. Images from the 2D panel can be exported as PDF, SVG, JPG, or PNG. For the vector formats (SVG and PDF), either the currently visible layer or all layers can be outputted. 3D images from the Jmol applet can be exported as JPG images, or can be exported to a PyMOL script. The program exports a ZIP file that contains the PyMOL script with a description of the current state and the necessary PDB files.

The **Sequences & Data** tab allows easy export of the actual data used in figure generation. The whole sequence is exported, along with subsets of sequences stored in the user selections. Additionally, any data used to create figures is also exported as a table.

Users can save their current work to disk for later retrieval using the **Save/Restore Manager** tool from the Tool Bar or by clicking the **Session** tab inside the **Save Manager**. All layers and selections can be saved. The default save location is the local browser LocalStorage cache. Restoring will reset the display including layers, selections; loaded data types, *etc.* but requires re-loading the user input data. This feature ensures user data privacy. Alternatively, users can save their work to a text file. This method allows users to restore the work session without an internet connection, or to move between browsers and computers.

Programming Details

RiboVision is a cross-platform webapp that integrates several advanced web solutions. Processing of data is done with jQuery, jQuery plugins, and JavaScript. The interface is based on jQuery UI. The 1D drawing is done in SVG with d3 library. The 2D structure drawing is done using the Canvas element. The 3D drawing is handled by the third party Java applet, Jmol. Our data are stored in a MySQL database and retrieved by a PHP server. The PHP server is also used to export file formatting and writing. Images are converted by ImageMagick. RiboVision functions, with certain limitations, on devices such as phones and tablets. RiboVision is under active development and still needs both client and server optimizations. The code is available on <https://github.com/RiboZones/RiboVision>.

Examples

Here we demonstrate the basic functionality of RiboVision using examples. We highlight the major functions of RiboVision rather than describe the actual data used for the examples. A fully detailed description of features, as well as explanations of the methods used to generate preloaded data sets, is contained in the manual (<http://apollo.chemistry.gatech.edu/RiboVision/Documentation>)

Example 1. Mapping 1D data onto 2D and 3D structures

RiboVision allows users to map various data simultaneously onto all three levels of structure (1D, 2D, and 3D). In example 1 (shown in Figure 2), we assign a color to each

nucleotide glyph of the *E. coli* 23S rRNA. The color corresponds to the Shannon entropy precomputed for a set of species sampled over all phylogeny. This example, which in practice takes two steps and less than 30 seconds to accomplish, shows how to visualize, at nucleotide resolution, the degree of conservation of rRNA. The input data (as well as the data used in Example 2) are a feature of RiboVision. The data contains the nucleotide number and a conservation score describing variability over a multiple sequence alignment using 128 representative species that sparsely represent the Woese tree of life. The 1D display (Figure 2a) shows the phylogenetic Shannon entropy of each nucleotide, illustrating that rRNA nucleotides from 1400 to 1600 of the 23S rRNA of *E. coli* exhibit high variability. The 2D display (Figure 2b) shows that the central loop of Domain V and other non-duplex rRNA are generally conserved (dark blue); while double-stranded nucleotides are variable (red, paired nucleotides co-vary). The 3D panel (Figure 2c) indicates that the central core of the LSU is highly conserved (blue) and the surface regions are variable (red).

To generate Figure 2, select LSU rRNA of *E. coli* from the **Species/Subunit** section of the Main Menu (Figure 1a). Drag Shannon Entropy from **Phylogeny Data** and drop it to the Circles layer object in the Display panel of the Main Menu. In a similar fashion, essentially any type of data can be mapped simultaneously onto the 1D, 2D, and 3D structures. For example, RiboVision contains crystallographic data, in the form of B factors, which can be mapped in the same way. The pre-loaded database in RiboVision will grow over time.

Example 2. Mapping and visualizing the protein interactions in combination with other structural data

In this example, we illustrate a simultaneous mapping of the rRNA domain structure (from **Nucleotide Data**) and rRNA-rProtein molecular interactions (from **Protein Contacts**) onto the SSU rRNA of *T. thermophilus*. Figure 3a depicts the 2D structure of the 16S rRNA of *T. thermophilus* with nucleotide letters colored by domain (Domain 5' is light blue, Domain C is brown, Domain 3'M is pink, and Domain 3'm is green). The same 2D structure contains the information about the molecular interactions of rProteins with rRNA marked with colored circles. Nucleotides within 3.4 Å of an rProtein are enclosed by circles that are color-coded by protein (S1, S2, etc.).

To generate Figure 3, select SSU rRNA of *T. thermophilus* from the **Species/Subunit** section of the Main Menu. Drag Domains from **Nucleotide Data** and drop it on the "Letters"

layer object of the Display panel of the Main Menu. This action colors rRNA nucleotide letters by domain. Then select all proteins from the **Protein Contacts** menu, drag Protein Contacts object to the Circles Layer of the Display panel of the Main Menu. The name of the specific protein(s) interacting with a particular nucleotide can be seen in the **ResidueTip** window, which will automatically pop up upon hovering the cursor on top of a nucleotide glyph in the 2D structure.

Any given region of the ribosome can be represented in isolation and at high resolution. Figure 3a shows isolated Helix 23, and indicates that it interacts with rProtein S11 (light green circles). Figure 3b contains a selected region of the 2D structure of Helix 23. The nucleotides are selected using tools in the **Select** menu and marked by maroon circles. Nucleotides of Helix 23 that are in contact with rProtein S11 are highlighted by green circles. The same selected fragment, along with rProtein S11, appears on the 3D structure (Figure 3c).

Example 3. Visualizing user data and nucleotide interactions

RiboVision allows one to not only import and display data associated with single nucleotides but also to visualize pairwise nucleotide interactions, drawing lines between them on the 2D structure. The interactions can be direct (*e.g.* base pairs or base stacking) or mediated (*e.g.* by proteins or magnesium ions).

In Example 3, we demonstrate visualization of base pairing interactions in the 18S rRNA of *S. cerevisiae*. The interactions are indicated by gray lines and assign a color to each nucleotide according to type: A-green, G-black, U-red, and C-blue (Figure 4). The color definition is not preloaded to RiboVision but supplied by an external file (File Example3.csv is given in the Supplementary Data).

To achieve the display in Figure 4, select the SSU rRNA of *S. cerevisiae* from the **Species/Subunit** section of the Main Menu. First, import the Example3.csv containing assigned colors of each residue according to its identity [A, G, U, C], (available in the Supplementary Data) by opening **Import** section of the main menu and selecting the file from a location on the local workstation. Then, drag the “User Data” object from the **Import** section of the main menu to the “Circles” layer object. Finally, choose the Base Pairs data set from the **Inter-Nucleotide Contacts** menu to visualize Base Pairs interactions between rRNA nucleotides. The base pairs interaction data are automatically loaded into the Interactions

layer of the **Layer Manager** (Figure 1c). For this example, we further filtered the base pair interactions by choosing only a few sub-types. By default, the lines appear in gray. Colored lines are an advanced feature. Additional details about connected nucleotides and the interaction type is shown in the **ResidueTip** window upon hovering a mouse over a line while holding the ALT key (the key is required to distinguish selection of nucleotides from selection of lines).

Conclusion and Future Perspectives

RiboVision was originally conceived as an in-house package for internal use in the Center for Ribosomal Origins and Evolution at Georgia Tech. We faced a bottleneck in visualization and analysis of a variety of data-types on 1D, 2D, and 3D levels of structure, and in the rate of production of publication-quality figures. As the package matured, it was suggested that others might find it useful.

RiboVision has shown significant utility in our laboratories. This software enabled us to detect long-standing discrepancies between 2D and 3D structures of rRNAs. We have substantially revised the 2D structures of 23S/28S rRNAs⁴⁰ to reflect a double-stranded region (Helix 26a) formed between the left and right segments of the central loop in the traditional 2D structure. RiboVision enabled more modest revisions of 2D structures of 16S/18S rRNAs,⁴² providing accurate representation of the triple helical structure of the central pseudoknot and numerous non-canonical base pairs distributed throughout the 2D structure. We used RiboVision to create a public gallery of rRNA secondary structures, mapped with a variety of data (<http://apollo.chemistry.gatech.edu/RibosomeGallery>).

RiboVision is open source and is based on modern website technology. The version released here is focused primarily on manipulation and presentation at the 2D structural level with more limited options in 1D and 3D Panels. Visualization is limited to one subunit of one species at a time. The scope of the program will be extended in the future versions.

Ribosomes are not the only large assemblies with a high level of complexity and extensive available data. We believe that the framework here can be further generalized to protein structures, other macromolecular assemblies such as viruses and bacteriophages, and to organelles and cells.

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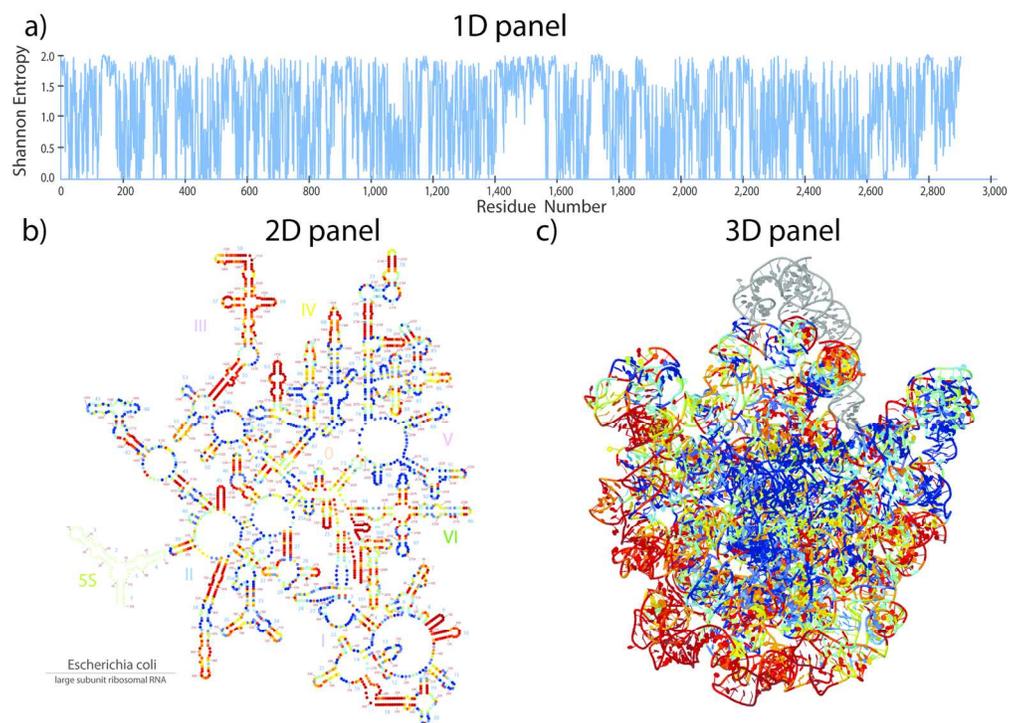
Figure Legends

Figure 1. Main menu. The **Species/Subunit** menu offers selection of the LSU or SSU from six species. **Nucleotide Selection** provides options for selection and display of specific fragments of rRNA. **Nucleotide Data** contains nucleotide-specific data from previous structural analyses of ribosomes. **Phylogeny Data** contains the Shannon entropies of each nucleotide. **Protein Contacts** allows users to map interactions between rProteins and rRNA. **Inter-Nucleotide Contacts** allows the users to display interactions between nucleotides by type. **Import** allows users to upload data for mapping onto each level of ribosomal structure. **Display** contains layer objects to which data can be loaded by dragging and dropping from the Data menus. **Save** allows export of figures, along with additional saving and exporting options. a) **Species and subunit selection.** b) **ResidueTip.** Hovering the mouse over data mapped on the 2D structure produces a ResidueTip, a pop up box containing nucleotide-specific data. Hovering over an interaction line with the Alt-key gives a ResidueTip with data on both nucleotides. c) **Layer/Selection Manager.** The Circles layer and the Interactions layer options panels are opened here, revealing advanced functionality.

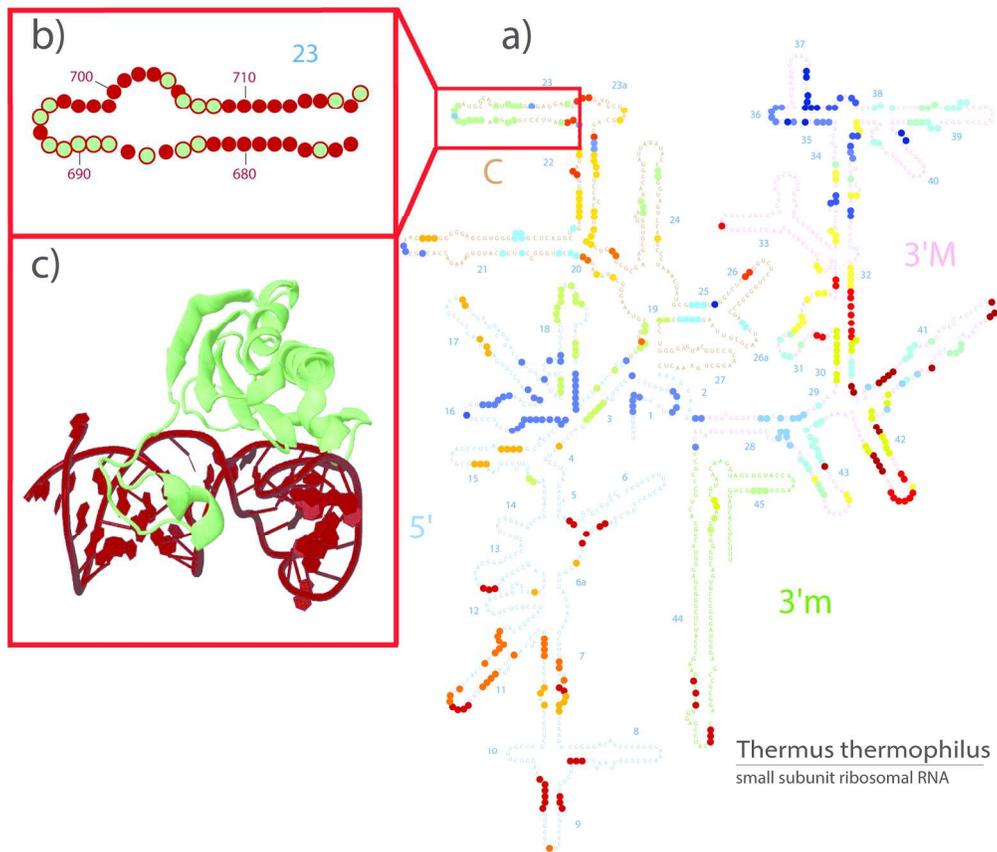
Figure 2. Mapping of Shannon entropies simultaneously onto 1D, 2D, and 3D structures of the *E. coli* 23S rRNA. Each nucleotide is assigned a color based on its Shannon entropy (the lowest values are blue; the highest values are red). The pre-computed Shannon entropies are plotted by nucleotide number in the a) 1D Panel, and mapped onto b) the 2D structure, and c) the 3D structure. The 23S rRNA nucleotides are numbered from 1 to 2904 and the 5S rRNA are numbered from 2905 to 3024 (Shannon entropies are not shown for 5S rRNA). Virtually any quantitative, nucleotide resolution data can be quickly mapped in this way.

Figure 3. Visualizing interactions between ribosomal proteins of the small subunit of *T. thermophilus* and the 16S rRNA using RiboVision. a) The nucleotides in the 2D structure of 16S rRNA are colored by Domain (Domain 5' is light blue, Domain C is brown, Domain 3'M is pink, and Domain 3'm is green), while nucleotides contacting ribosomal proteins are overlaid with colored circles; each protein is assigned a distinct color. Interactions of rProtein S11 (green) with Helix 23 (maroon) of the SSU rRNA b) projected onto 2D structure and c) shown as cartoon representation of 3D structure.

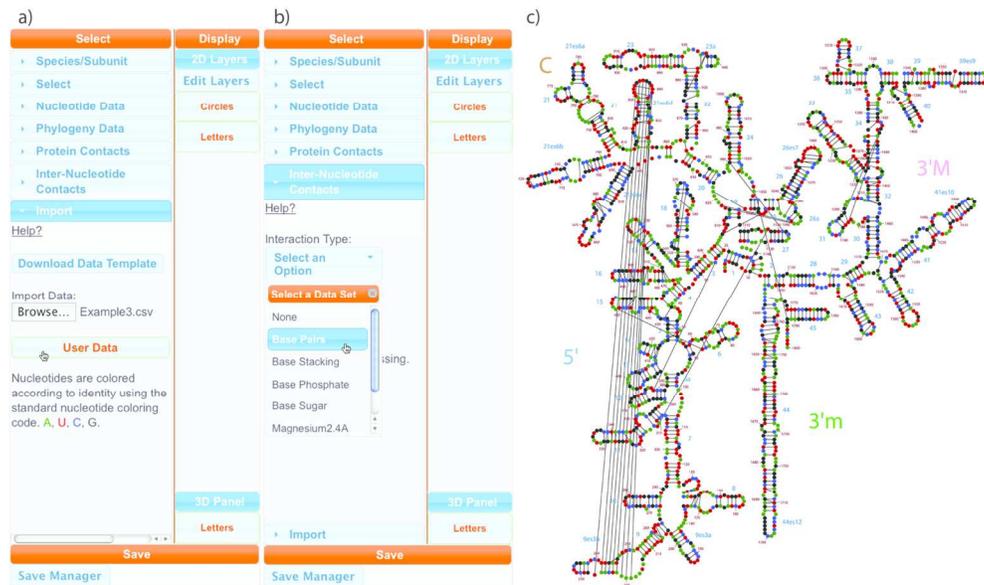
Figure 4. A subset of base pair interactions in *S. cerevisiae* 18S rRNA visualized along with imported user data. a) The user data file "Example3.csv" was loaded into RiboVision. A description of the file is visible under the "User Data" data object. b) Base Pairs were selected as the Interaction Type, from **Inter-Nucleotide Contacts**. Additional filtering was performed through selection of interaction subtypes. c) The resulting 2D display illustrates the standard nucleotide color code. Each nucleotide is assigned a color according to its identity. In addition, the gray lines connect nucleotides that form Watson-Crick / Watson-Crick interactions.



81x59mm (600 x 600 DPI)



96x82mm (600 x 600 DPI)



66x38mm (600 x 600 DPI)