

RNAmapping 1.7.5 Tutorial

This tutorial introduces you to RNAmapping and its capabilities. It contains a step-by-step guide through the examples of most typical analyses. If you would like to read more details about the program and its options, please refer to the RNAmapping-manual document.

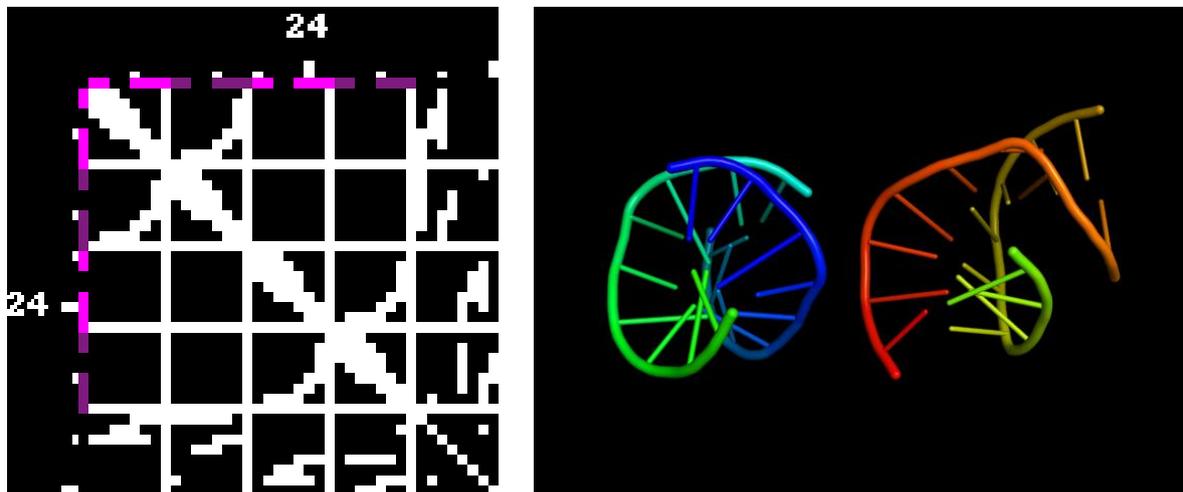
Getting started:

- Run the RNAmapping standalone executable (**RNAMAP2D.exe** file in Windows case, **RNAMAP2D.py.app** file in OSX case) or for Linux systems, type `./RNAMAP2D.py` in system console and hit enter to start the program.
- All files mentioned in this document are to be found in the “examples” directory distributed as an archive.

1. Calculation of a contact map, single RNA structure

In this example, you will create a contact map of a PDB file containing two double stranded RNA molecules (four chains in total) and several ions.

- Select **single model** from the job list in the main menu.
- Click **Browse...** button and select the file **1J9H.pdb**.
- Select **residue id – based (ungapped)** positioning type to keep the numbering of residues corresponding to that in the PDB file (*structure-based option for example, rennumbers all residues in file starting from 1 and thus compresses the eventual structural gaps*).
- Click **Load** button to load the structure. After uploading the coordinates, a new menu appears which allows you to define the way how distances are calculated and which features of the model to display.
- Click **Go** button to calculate distances. A contact map similar to the one shown below should appear in the RNAmap2D window within about 3 seconds.



Contact map of an RNA with two double stranded regions (left). The perpendicular white lines crossing in the picture indicate boundaries between the four chains. The smaller, fifth section on the bottom and right indicates contacts between the ions and RNA chains. Purple and violet dots indicate contacts forming RNA secondary structure. 3D rendering was shown on the right for comparison.

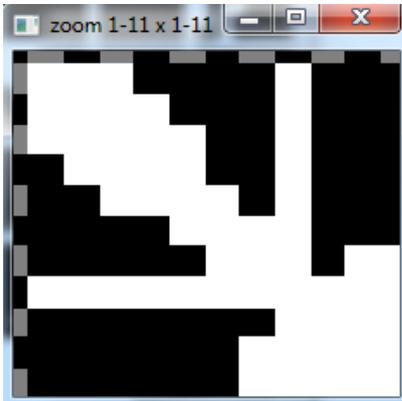
- To see more information about a particular contact, point the mouse cursor over any location on the map. An **INFO** window will appear, showing details about that point:
 - residues' names (resi)
 - indices of the residues (index)
 - contact type (Y – two residues are in the contact, N – two residues are not in the contact).

Important Notice: To enable INFO window refreshing, OSX users should press left mouse button while hovering mouse cursor over a contact map. This is because in OSX system an application window loses focus while mouse cursor leaves the application window area.

- You can zoom any part of the contact map by pressing the left mouse button and drawing a rectangle, then releasing the button. This will show you a zoomed portion of the map:

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Important Notice: To enable pop-up rectangle selection, OSX users should press left mouse button while hovering mouse cursor over a contact map. This is because in OSX system an application window loses focus while mouse cursor leaves the application window area.



- You can also enlarge the entire map by pressing **1con=9px** button.
- To color specific kinds of **contacts**, press **Option Panel** button. It allows you to color base pairing, stacking, ligand and ion contacts:

The screenshot shows the RNAmap2D v. 1.6.3 interface. The main window displays a contact map for 40 residues, with a 'Matrix view' selected. The map shows various colored contacts: magenta for S/S, blue for S/H, red for S/W, and green for H/H. The 'Option Panel' button is highlighted with a blue box and a blue arrow pointing to the 'Options' dialog box.

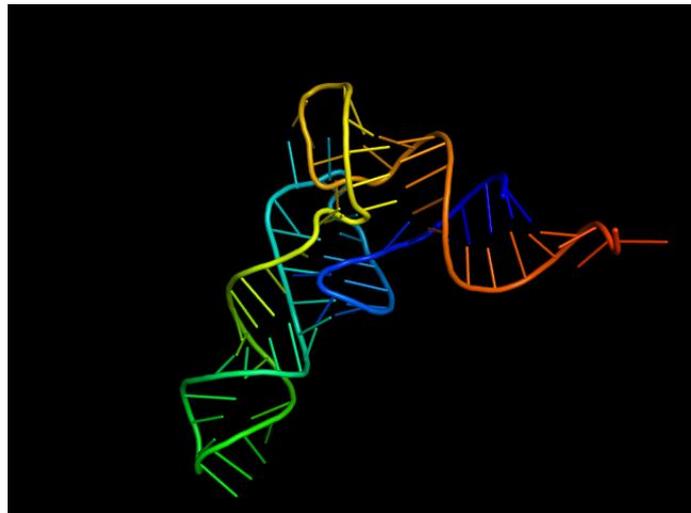
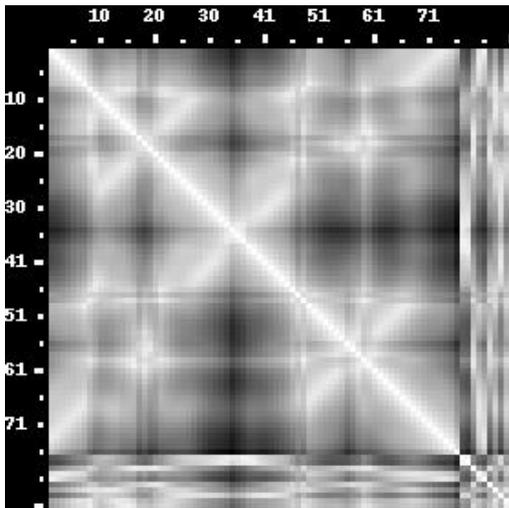
The 'Options' dialog box has the following settings:

Category	Option	Value
Base pairs	S/S	white
	S/H	white
	S/W	red
	H/H	white
	H/W	white
	W/W	white
	canon	white
	non-canon	white
	Wobble	white
Stacking	>>	blue
	<<	white
	<>	white
	><	white
Other ligands	ligands	white
	ions	light green
other	other	white
	background	black

2. Calculation of a distance map

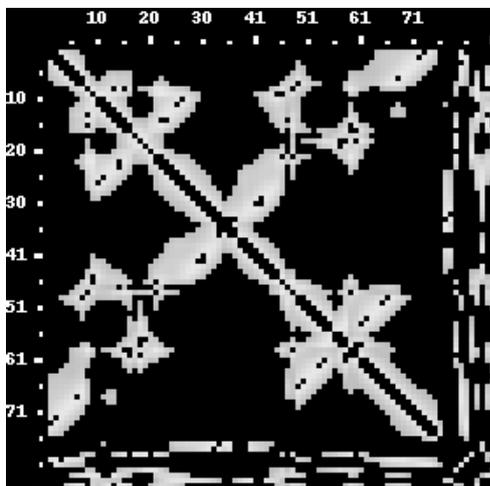
In this example, you will display distances between residues in a tRNA(Phe) molecule.

- Go back to the main menu, and choose **single model** from the job list.
- Click **Browse...** button and select the input file **1EHZ.pdb**.
- Select **structure-based (ungapped)** positioning type
- Click **Load** button.
- In the new menu choose **distance map** option from the matrix type list.
- Click **Go** button to run the calculation.
- A distance map similar to the one shown below should appear within a few seconds.



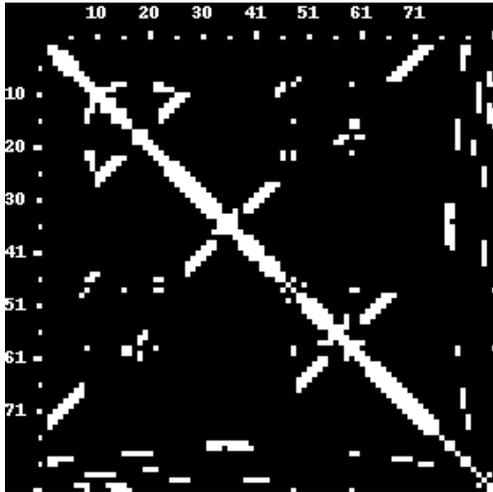
Distance map of a tRNA molecule (left). The three short diagonal lines from bottom left to upper right correspond to three helical stems of the tRNA. At the bottom left and upper right corner, a shorter diagonal for the acceptor stem is barely visible. 3D structure (no ligands or ions) was shown on the right for comparison purposes.

- To crop distances from the map, you can set two distance cutoffs in the **lower limit** and **upper limit** fields, e.g. to **7.0** and **20.0** respectively. This reduces the displayed distances to a smaller subset defined:



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- You can exclude residues close to one another in the sequence explicitly. First, set the **lower limit** value back to **0.0**. Then set the **separation** value, to 4, which excludes up to three neighbouring residues in sequence (not shown).
- You can toggle the view to a contact map by pressing **Contact** button (distance cutoff is set to 8 Ångströms by default). But before doing that, please reset cutoff values to lower – **0** and upper – i.e. **99**. After pressing Contact button, distance map gets stripped down to the essential contacts, thus becoming a contact map. The **distance cutoff value** can be changed with the mouse wheel move:

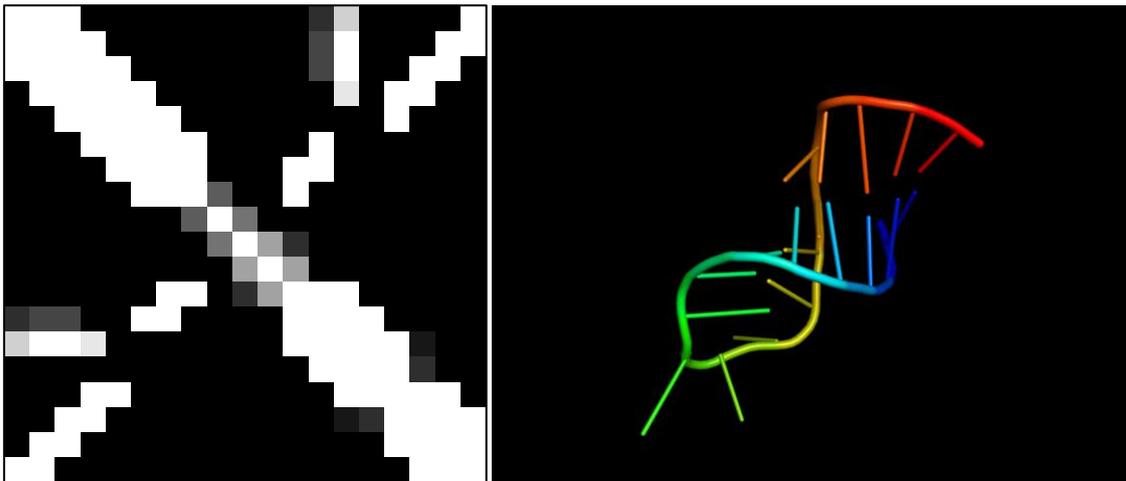


- You can switch back to the distance map view by pressing **Distance** button.

3. Model ensemble statistics

In this example, you will examine contacts over an ensemble of 20 models from the NMR structure of a short RNA hairpin.

- Go to the main menu and choose **model ensemble** from the job list.
- Click **Browse...** button, select the file **1ESY.pdb**, and click **Load** button.
- Uncheck the **sequence ruler** checkbox.
- Click **Go** button to run the calculations. A map of contact frequencies should appear within 3 seconds.



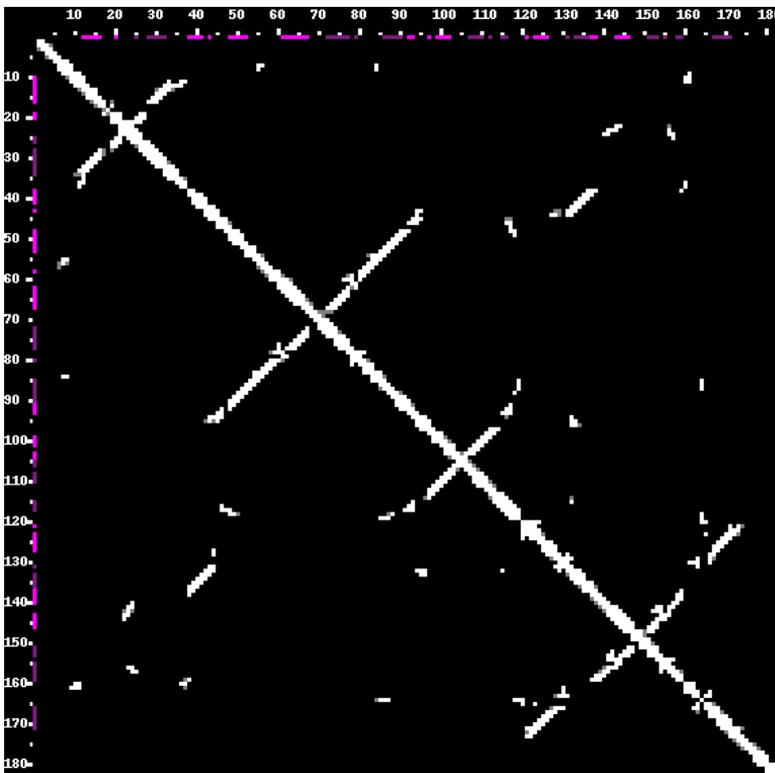
Contact map of an NMR structure ensemble (left). White contacts are present in all 20 models. Shades of grey indicate the percentage of the contact occurrence in the ensemble (the darker the fewer). In the picture, you can see that the loop region in the middle of the model is more flexible, because contacts are varying there. Single model 3D picture was shown on the right for comparison purposes.

4. Model vs. model

In this example, you will compare a tRNA(Cys) model obtained by structure prediction to the crystal structure.

1. Go back to the main menu and choose **model vs. model** from the job list.
2. Click the upper **Browse...** button and select the file with the crystal structure (**azo.pdb**).
3. Click the lower **Browse...** button and select the file for the theoretical model (**mod.pdb**).
4. Click **Load** button, and then **Go** button to run the calculations. A greyscale contact map similar to the one shown below should appear in a few seconds.

Please note that when RNAVIEW plugin is not present, calculation time can take longer. This is because the internal algorithm's complexity, if used, is larger than that of RNAVIEW. In such cases calculations can take several seconds because the program computes all pairings in order to derive and display RNA secondary structure, for both models.

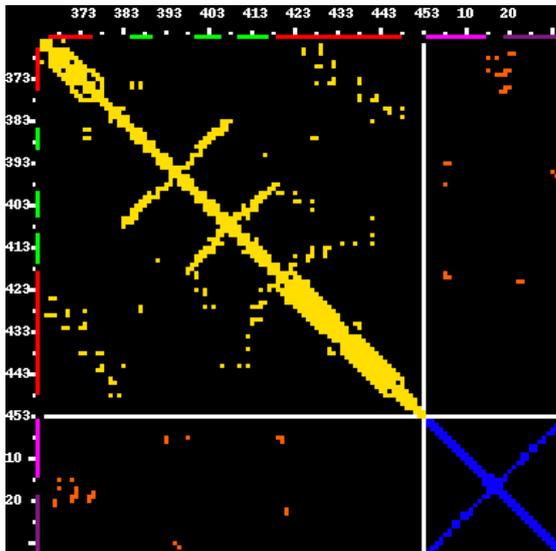


Contact map of a tRNA crystal structure vs. a theoretical model. The lower-left section of the map indicates contacts in the crystal structure, the upper right part indicates contacts in the model. White indicate contacts common to both models, grey dots are specific for either of the structures. Please note that despite minor structural differences, secondary structure differs.

5. Displaying a map of a protein-RNA complex

In this example, you will display contacts between RNA and a protein.

- Select **complex contact map** from the job list in the main menu.
- Click **Browse...** button and select the file **2LBS.pdb**
- Select **residue id-based (ungapped)** Click the **Load** button. After uploading the coordinates, a new menu appears which allows you to define the way how the distance is calculated and which features of the model to display.
- Click the **Go** button to run the calculation of intramolecular distances. A contact map similar to the one shown below should appear in the RNAmap2D window in about 4 seconds. On the option panel, you can color contacts by their type.



Contacts in a protein-RNA complex contact map. Protein-protein contacts are in yellow, RNA-RNA contacts in blue, and protein-RNA contacts in orange. Also, secondary structure for both protein and RNA molecule was denoted on the ruler (red for helices, green for strands in case of a protein). Below, 3D structure of the complex is shown – violet color: RNA, red and yellow: protein secondary structure (helices and strands, respectively)



6. Loading a contact map from a file

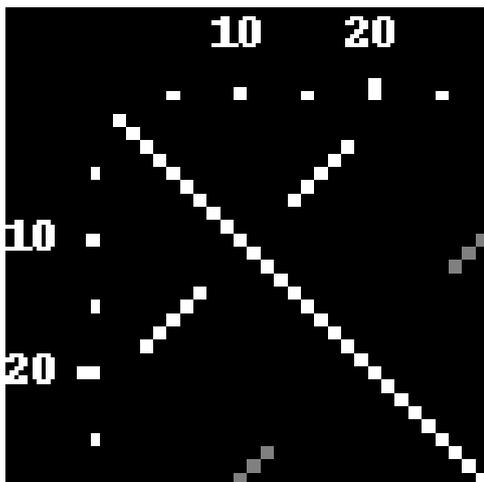
In this example, you will display a previously saved contact map.

1. Go to the main menu, and choose **contact map from file** from the job list.
2. Click the uppermost **Browse...** button and select the file **1di2_distancemap_phylip.txt** (a distance map in the PHYLIP format).
3. Click the **Load** button. A distance map should appear in 1 second (not shown).
4. You can also try the file **spreadsheet_17RA.xls** (a contact map in the MS Excel format). Remember to enable the “*MS Excel Workbooks*” file filter in the file dialog.

7. Loading a secondary structure file and displaying it as a contact map

In this example, you will display a previously saved secondary structure, as a contact map.

1. Go to the main menu, and choose **secondary structure** from the job list.
2. Click the uppermost **Browse...** button and select the file **1l2x_ss_vienna.txt** (a distance map in the PHYLIP format).
3. Click the **Load** button. A distance map should appear in 1 second.



RNA secondary structure file, encoded as a popular Vienna format, visualized as a contact map. Please note that not all RNA contact are forming secondary structure pairings. In this picture, grey contacts indicate RNA pseudoknots.