

REDCAT INSTALLATION

BCMB/CHEM 8190, April 7, 2006

Installation note for instructors:

Download REDCAT from:

<http://tesla.ccrc.uga.edu/software/REDCAT>

While REDCAT is developed on and for Linux systems, versions are also available for SGI and Mac systems. Please contact Dr. Homayoun Valafar directly (homayoun@cse.sc.edu) to obtain non-Linux versions. You will find system requirements, installation/configuration instructions and a manual on the web site.

The laboratory exercise files are available on the course web site as:

<http://tesla.ccrc.uga.edu/courses/bionmr/labs/redcatData.tar>

The zip file contains:

- 1bq8.pdb
- 1bq8_1-25.pdb
- 1bq8_27-54_rotated.pdb
- Rub_fieldinduced_HN_RDC.txt
- Rub_fieldorient_HN_27-54.txt

- map.dat (*necessary for 2D plots*)
- map3D.dat (*necessary for 3D plots*)

REDCAT LABORATORY EXERCISE

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Introduction. REDCAT (REsidual Dipolar Coupling Analysis Tool), is a program that aids the interpretation of residual dipolar couplings (RDCs) in terms of structural models. It is primarily designed to test RDC data for consistency with structural models, but it can also aid in the assembly of structures from fragments by orienting each fragment in a common principal alignment frame. It incorporates a number of other useful tools such as graphical display of principal axis directions and prediction of averaging effects on RDCs for comparison with experimental data. It operates by taking in coordinates for various dipole interaction vectors (H-N bonds for example) and experimental RDCs, and then solving for an order tensor by singular value decomposition. It does this thousands of times using a random sampling of RDCs within defined error limits to produce a picture of reliability of results.

REDCAT has been installed on the computers you are using. However, it can be downloaded to most Linux, SGI or Mac computers from the NCCR supported website <http://tesla.ccrcc.uga.edu/software/REDCAT>. You will find a downloadable manual here as well. The instructions that follow are intended to supplement the manual by leading you through an exercise that manipulates data on a small paramagnetic protein, rubredoxin. In this exercise we will only use RDCs from H-N vectors. They are small in magnitude, and many are missing because they were collected using only natural field induced orientation of this paramagnetic protein. However, the data should suffice to illustrate principles of the program. You will first use data for the intact protein, and use REDCAT tools to evaluate consistency between RDC data and a crystal structure. You will then do a fragment assembly project in which the protein has been divided into two parts with one rotated from its original orientation. You will use REDCAT tools to reestablish the proper orientation.

Creating an input file:

Open an X-terminal (Mac: X11 window in the dock)

Go to the directory where your example files are stored, or set up a directory and copy the files to this directory.

Start REDCAT by typing **REDCAT.tcl**

Under the **File** menu select **Prepare Input**

Enter **1bq8.pdb** in the pdb file window

Enter a filename for the input file to be created (example: 1bq8.redcat)

Enter the starting residue number and end residue number (2 and 53 for this exercise)

Enter the type of data you intend to use in the first line of the table. For this exercise it will be: H, N, 0, 24350, 0.15 as shown in the figure below.

Atom 1	Atom 2	Gap	Max RDC	Error
H	N	0	24350	0.15

The atom names tell the program what coordinates to extract from the PDB file (these must be exactly as they appear in the PDB file), the 0 tells the program to find these in the same residue, the 24350 is the coupling that would be seen for a pair of atoms separated by 1Å and oriented rigidly along the magnetic field. The 0.15 is an estimated error for the RDC data that will eventually be entered.

Select **Run**. A message should tell you that the input file was written to disk.

Select **Done**. The prepare input window will close.

Loading the input file and RDC data.

- Select **File** and then **Load** from the Main Window. The input file you just created should be in the displayed directory. Select it. Note that the 999 entries indicate that no valid RDCs are yet in the file. The gray boxes at the left indicate that none of these data will be used in the calculations.
- Select **File** and then **Import RDC** from the Main Window. Select the file **Rub_fieldinduced_HN_RDC.txt**. Note that the entries in this text file must start at the starting residue indicated above and continue in order for all coordinate pairs selected. Some data should now replace the 999s in the input table and some boxes should turn red to indicate they are being used in the calculation.
- Leave default values for number of trials (10,000), number of null space values (10), and error search range (1).

Validating data and the model.

- Press **Run** and after a brief wait you should see a message window appear. This displays success of the calculation. Near the top you will note that 10,000 out of 10,000 tries were rejected. Each line lists how many times a particular RDC was the cause of a rejection. If you scroll down you will see a red line for **Rejections by equation 32: 10000**. This data point caused all possible solutions to be rejected. Check this piece of data or just eliminate it. This can be done by deselecting the checkbox by equation 32 in the Main Window. Clear the Message! output window by clicking on the **Clear All** button. Press **Run** in the Main Window.
- The run may still show 10,000 out of 10,000 rejections, but this time it cannot be traced to any one problematic data point. Go into the **Tools** menu on the main screen and select **Error Analysis > Perform Error Analysis**. Each equation highlighted in red has an error higher than the 0.15 error limit set. Change the error limits by selecting **Tools > Error Analysis > Get Estimated Errors for Violations**. This will increase the error for those RDCs in the output which have estimated errors higher than the error set in the Main Window. Select **Run**. You should get a thousand or more accepted solutions now.

Displaying and plotting results.

- Go to the **Tools** menu and select **Solution > Get Solutions**. You should see a hundred or so lines added to the message window displaying principal order tensor solutions and Euler angles for relating the molecule frame to the principal alignment frame. This large number of solutions is not easy to digest, but you can also ask for the single “best solution”.
- Plot the solutions to show the directions of the principal alignment axes in the molecular frame. This is done with the “Sauson-Flamsted plot” (**Tools > 2D SF Plot**).

Working with fragments.

- Now repeat the above (starting at **Create an input file**) using just a fragment of rubredoxin (1bq8). The appropriate files for the PDB coordinates and RDC input are: **1bq8_1-25.pdb**, **1bq8_27-54.pdb**, **Rub_fieldinduced_HN_RDC.txt** and **Rub_fieldorient_HN_27-54.txt**. For **1bq8_1-25.pdb** use a starting point of 2 and an ending point of 25 and **Rub_fieldinduced_HN_RDC.txt** for RDCs; for **1bq8_27-54.pdb** use a starting point of 27 and ending point of 53 and **Rub_fieldorient_HN_27-54.txt** for RDCs. The PDB file in the second fragment has been rotated into the principal alignment frame. When you look at the axes plot for this fragment you should see solutions fall on the axes of the globe.

- Now rotate the first fragment into its principal axis frame. To do this you need to record the Euler angles for the best solution. These are the a, b and c columns in the output window after you select **Tools > Solution > Get Best Solution**. In the Main Window under **Tools** select **Rotate > Rotate PDB**, enter the PDB filename, output filename and Euler angles (a, b, c) into the window that opens. The molecule in the newly created PDB file is in the principal alignment frame (PAF). You can recalculate order tensor solutions with this new PDB and view its Sauson-Flamsted plot to verify this operation.

Examining results:

- Open you favorite molecular graphics program and select PDB files for the 1-25 and 27-54-rot fragments of rubredoxin. You will see that they do not connect as a single protein. Now open your new PAF 1-25 fragment along with your 27-54_rot fragment. Just using translations see if you can make an intact molecule. Remember that RDC based orientations can be 4 fold degenerate (turn 180 about x, about y or about z). You may have to experiment with these alternate orientations to produce and intact molecule.