QUANTA 2006

Simulation, Search, and Analysis

MAY 2006

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Contents

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Simulation, Search, and Analysis

Who Should Read This Book

QUANTA Simulation, Search, and Analysis is designed primarily for first-time users. If you are in this category, read it to understand how to simulate molecular motion, search molecular conformations, and analyze the data generated from these procedures within QUANTA.

If you are an experienced user, read this book to review information on functionality and procedures for executing building and editing tasks of QUANTA or to update yourself on changes in QUANTA.

What This Book Contains

This book contains information on simulating motion in a molecular system, applying molecular constraints during CHARMm calculations, generating sets of molecular conformations, evaluating similarities among molecules, and analyzing data from these operations. Information provided in progressive chapters is based on the assumption that you are familiar with basic software operations and with material in previous chapters and in the other volumes in the QUANTA introductory documentation set.

Since this book is designed primarily for new users, neither the descriptions nor the tutorial exercises comprehensively document all aspects of using QUANTA.

The book is written assuming that you are familiar with:

- Basic UNIX commands and file and directory structures
- Windowing software on your QUANTA workstation

- The difference between a shell window and a console window on your QUANTA workstation
- How your workstation mouse works

Assumptions also are made that you have a home directory where you can create subdirectories, and a licensed copy of QUANTA installed on your workstation.

How to Use the QUANTA Introductory Documentation Set

This book is part of a documentation set that introduces users to QUANTA. The set consists of:

- Basic Operations
- Generating and Displaying Molecules
- Simulation, Search, and Analysis

Although each book is self-contained, information within the set flows in a logical sequence. You must be familiar with basic operations to successfully complete building and editing tasks; molecule preparation in the building and editing phase must precede simulation, search, band analysis.

Within each book, exercises are step-by-step procedures that may be linked across chapters. Data files are frequently modified or reused by several exercises.

Because of the sequential nature of the documentation set and of the exercises in each book, you will get the most coherent and complete view of the software if you go through the books in the proper order.

Documentation Set Roadmap

The table below lists key topics and where to find information about them in the introductory set of QUANTA books. Book names are abbreviated:

- Ops — *Basic Operations*

- Gen — *Generating and Displaying Molecules*
- SSA — *Simulation, Search, and Analysis*

Each book contains a master index covering topics in all three books. Since aspects of a topic may be covered in a number of places, use the index as an important resource for locating all the information on a topic.

For information about	Look at
Understanding program layout and design	Ops Chapter 1
Using operating principles and procedures	Ops Chapter 2
Manipulating molecules and displays	Ops Chapter 2, Gen Chapters 6 And 7
Building structures	Gen Chapters 1,2,4, and 5
Editing structures	Gen Chapters 1, 2 And 8, SSA Chapter 4
Applying constraints for CHARMm calculations	SSA Chapter 2
Importing and exporting files	Gen Chapter 8
Executing calculations using CHARMm or external programs	Gen Chapters 2, 3, 4, 6, And 9, SSA Chapter 1
Handling data - visual displays, tables, graphs	Ops Chapter 2, Gen Chapters 6 and 7 SSA Chapters 8 and 9
Performing experimental simulations	Gen Chapter 3, SSA Chapters 1, 3-5, 7
Analyzing data	SSA Chapter 6

Related Documentation

In addition to the QUANTA introductory books, other books in the QUANTA documentation set include:

- *QUANTA Installation Guide*
- *CHARMm Principles*
- *CHARMm Dictionary, Parts I and II*

Other QUANTA-related documentation is included with specific software application packages. This documentation includes:

- *Protein User's Reference*
- *Protein Homology Modeling Tutorial*
- *X-Ray Structure Analysis User's Guide*
- *NMR Structure Determination User's Guide*
- *MCSS/Hook User's Guide*

Restarting QUANTA

This book assumes you have already run QUANTA. If you have not, refer to the configuration and start-up instructions in *QUANTA Basic Operations*.

For this and subsequent start-ups, enter the command:

```
> cd ~/subdirectory
```

Then enter the command:

```
> quanta
```

QUANTA opens, displaying the structure you have worked on most recently in the current directory. The Molecule Management Table displays the structure's name and status.

Conventions

Typographical conventions used in this book are shown below:

This ...	Looks like this ...
Commands you type, dialog box choices, information in the Textport or on the Message line	Courier font
Menu and palette names and choices	**Bold** text
File and directory names	*Italic* text

1. Executing CHARMm Dynamics

This chapter describes CHARMm dynamics calculations and the Dynamics Animation application that is used for viewing and analyzing CHARMm dynamics trajectories.

CHARMm dynamics simulates the natural motion of a molecular system and produces a set of coordinates and velocities that describes the atomic motions of the system over time. The resulting dynamics trajectory can be viewed in the Dynamics Animation application and analyzed in the Analysis application. A typical molecular dynamics run involves heating, equilibration, and simulation

All dynamics calculations begin with an initial set of Cartesian coordinates that are derived from experimental data or selected parameters. (See citation 1 under *References*). The energy of a molecular structure is minimized to remove bad contacts and to relax strained torsion angles before a dynamics simulation is started. It is not necessary to minimize to an absolute minimum, since the conformation of the structure changes during heating. Structures that do contain bad contacts between atoms or highly strained torsion angles can cause abnormally large forces on some atoms and unrealistic simulation results.

With the Dynamics Animation application, you can interactively display a dynamics trajectory calculated by CHARMm, CNX, or X-PLOR. Displays can include a graphical representation of hydrogen bonding, interatomic distances, atomic positions, molecule energy, or other atomic properties.

The dynamics trajectory calculated in CHARMm consists of datasets of atom coordinates written at periodic intervals. If the trajectory is thought of as a movie, then each individual dataset may be considered a frame. You can display the trajectory frame-by-frame at any desired speed. Single frames may be saved to individual MSFs that can be compared in detail using the Molecular Similarity application.

Commands in the CHARMm pulldown

Table 1. CHARMm Menu in QUANTA

Command	Sub Option	Function
Select CHARMm Host		Allows the user to specify the host machine to execute a CHARMm job and point to the appropriate executable
CHARMm Initialization Options		Set up initialization options for CHARMm
	Bomb Level	Specify checking level for termination errors
	Warning level	Specify checking level for warnings
	Print Level	Specify level for verbosity
	CHARMm log destination	Location of output
	CHARMm Stream file for additional initialization commands	Location of CHARMm script with miscellaneous, user-defined initialization options.
CHARMm Process		Specify CHARMm initialization options
pullright	Initialize interactive	Initialize interactive QUANTA/CHARMm communication
pullright	Terminate interactive	Terminate interactive QUANTA/CHARMm communication
pullright	Interactive status	Show status of interactive QUANTA/CHARMm communication
Energy Terms		Specify which energy terms will be used
Update Parameters		Specify update parameters
		Frequency of updates
		Cutoff distances
		Smoothing function
		Dielectric model
		Image update parameters
		Ewald summation options
Minimization options		Options for CHARMm minimization
Dynamics options		Options for CHARMm dynamics
	Setup Heating	Options for heating
	Setup Equilibration	Options for equilibration
	Setup Simulation	Options for production simulations
	Setup Detailed Dynamics	Detailed options for MD simulation
Constraint Options		
pullright	Set Atom Constraints	Create/setup CHARMm constraints
pullright	Atom Constraints On	Turn on constraints
pullright	Atom Constraints Off	Turn off constraints

Table 1. CHARMm Menu in QUANTA

Command	Sub Option	Function
pullright	Dihedral/Distance	Create/setup dihedral and distance constraints see Applying Constraints (page 29)
pullright	Dihedral on	Turn on constraints
pullright	Dihedral off	Turn off constraints
pullright	Distance on	Turn on constraints
pullright	Distance off	Turn off constraints
Shake Options		Setup SHAKE options
Parameters		CHARMm parameter options
pullright	Read Parameters	Location of *.prm file
pullright	Save Parameters	Location to save custom parameter file
pullright	Set Options	Parameter options
CHARMm Mode		Set CHARMm operation mode
pullright	PSF	PSF mode
pullright	RTF	RTF Mode
pullright	MMFF	MMFF mode
pullright	MMFFS	MMFFS mode
pullright	PSF Terms	Select PSF terms
pullright	RTF options	RTF settings
Get Fourth parameter		Setup forces and scalar parameters
pullright	Forces	Force options
pullright	Scalars	Scalar options
Optimize hydrogens		Optimize hydrogens using CHARMm hbuild
Solvate structure		
pullright	15A Radius Sphere	Solvate molecule in sphere of radius 15 A
pullright	15A Length Box	Solvate molecule in periodic box of waters with edge length 15A
pullright	30A Length Box	Solvate molecule in periodic box of waters with edge length 30A
pullright	Solvate residues 8A	Solvate residues to 8A
pullright	Solvate residues 10A	Solvate residues to 10A
pullright	15A methanol sphere	Solvate in a sphere of methanol with radius 15A
pullright	Water image 15A	Turn on 15A water image file
pullright	Water image 30A	Turn on 30A water image file
pullright	Turn of Water image	Turn of image options
Periodic boundaries		Setup periodic boundary conditions
Send CHARMm command		Console for entering CHARMm commands
Stream CHARMm file		Location of CHARMm standalone stream file

Table 1. CHARMm Menu in QUANTA

Command	Sub Option	Function
Capture CHARMm commands		Options to turn on/off capture of CHARMm commands
pullright	On	Select file to capture CHARMm commands
pullright	Off	Turn off capturing
pullright	Suspend	Suspend capture of CHARMm commands
pullright	Restart	Restart capture of CHARMm commands
Settings		Options for capturing settings
pullright	Show	Examine various setup options
pullright	Save	Save CHARMm setup file to specified location
pullright	Restore	Read CHARMm setup file from specified location

Running CHARMm dynamics calculations

A typical molecular dynamics run involves heating, equilibration, and simulation. Parameters are set for each stage in a run. Table 2 lists parameter categories and provides a brief description of each. Structures must be minimized before they are used in dynamics runs.

Heating

A minimized structure represents a molecule at a temperature close to absolute zero (0 K). Molecular dynamics simulations are usually run at room temperature (300 K). Kinetic energy is slowly and uniformly added to the system over time intervals by randomly assigning velocities to each atom to achieve the final temperature.

Dynamics heating occurs over several picoseconds, depending on the size of the molecular system. The recommended heating rate is 0.1 K per step. Small systems can be heated at a faster rate. Some large systems may require heating at a slower rate.

Table 2 . CHARMm Dynamics Setup dialog box

Option	Description
Dynamics Steps	Determines the total number of steps of dynamics to calculate for this stage of basic dynamics. The number of steps that may be specified for equilibration and simulation depends on the nature of the study. The heating stage requires that the number of steps be large enough to allow a reasonably smooth increase in temperature in the molecular system**.**
Restart Read File **Restart Write File**	Specifies filenames used for restarting dynamics calculations. Restart files store the final velocities and coordinates of a molecular structure at the end of a stage of dynamics. The **Restart Write File** of one stage is typically the **Restart Read File** of the next. Restart files are automatically given a .RST extension.
Coordinate Trajectory File	Specifies the name of the file to which the dynamics trajectory is written at the end of this stage of the calculation. The number of sets of coordinates in this file is the number of steps of dynamics divided by the output frequency. Trajectory files are automatically given a .DCD extension.
Energy Values File	Contains the energy values corresponding to each set of coordinates saved in the coordinate trajectory file. The saved energy information includes time step, total energy, potential energy, kinetic energy, and temperature. Energy value files are automatically given a .ENE extension.
Output Frequency	Specifies, in steps, the frequency with which coordinates and energies are saved to the coordinate trajectory and energy files.

Table 2 . CHARMm Dynamics Setup dialog box (Continued)

Option	Description
Time Step	Specifies the integration time step for the dynamics calculation. The time step must be sufficiently small to maintain energy conservation and trajectory accuracy. A large time step reduces the total time required to calculate a trajectory.
Initial Temperature **Final Temperature**	Specifies the temperatures at the beginning and end of dynamics heating. In a typical dynamics study, a molecular system is slowly heated from absolute zero (0 K) to room temperature (300 K) to raise the kinetic energy with minimal conformational changes. It is then equilibrated for a time at 300 K to stabilize the temperature. These two steps are typically performed once. Thereafter, multiple simulation stages may be run to show conformational changes over time. If the heating and equilibration stages are omitted, the dynamics simulation may be run directly at the desired temperature. In this case, the initial and final temperatures are given the same values.
Start ... from the beginning	Specifies that the calculation is to start without a predetermined set of energies. If all three stages of basic dynamics are to be run, this option is selected in the heating setup dialog box only. If heating and equilibration are not to be run, this option is selected in the simulation setup dialog box.
Restart... from the Restart File	Specifies that the calculation is to start using initial velocities and coordinates stored in the **Restart Read File**. If all three stages of dynamics are to be run, this option is selected in the equilibration setup and simulation setup dialog boxes. The restart from **Restart File** option is rarely selected in the heating setup dialog box.
Do Not Run	Specifies that this stage of dynamics is not to be run.

Equilibration

When the structure reaches the temperature at which the dynamics trajectory is to be computed, it must be equilibrated. Dynamics equilibration is achieved by allowing the molecular system to evolve spontaneously for a period of time, integrating the equations of motion until the average temperature and structure remains stable. Equilibration is facilitated by periodically scaling velocities appropriately for the desired temperature. The procedure is continued until the statistical properties of the system become independent of time.

The equilibration temperature *must* correspond to the final temperature used in heating. If you want an equilibration temperature different from

the final heating temperature of the heating phase, the heating step must be repeated.

The Restart Write File generated during heating is used as the Restart Read File for the equilibration phase. Although a separate trajectory file is created, the equilibration trajectory is a continuation of the heating trajectory.

Simulation

The final step of a dynamics run is dynamics simulation. Simulation takes an equilibrated structure as its starting point and produces a dynamics trajectory. In a typical simulation, the trajectory traces the motions of the macromolecule for a specified time.

Dynamics heating and equilibration are usually performed only once on a molecular structure. Simulation can be run several times to show changes in the conformation of the molecule over time.

The simulation temperature *must* correspond to the equilibration temperature. The Restart Write File generated during equilibration is used as the Restart Read File for simulation. Although a separate trajectory file is created, the equilibration trajectory is a continuation of the heating and equilibration trajectories.

Saving dynamics output

At the completion of dynamics simulation, the final frame of the calculated trajectory is displayed in the viewing area. The final frame's energy is displayed in the upper right corner. In the Modeling palette, **Undo Changes, Save Changes,** and **Reject Changes** become active because the displayed conformation is different from the starting conformation. One of these tools must be selected before further modeling can be carried out on the displayed structure.

In most cases, select **Reject Changes** from the Modeling palette to restore the last saved set of coordinates for the structure. Otherwise, initial coordinates are not saved, since these coordinates are not part of the trajectory.

All coordinates calculated during a dynamics run are stored in the coordinate trajectory file specified in the dynamics setup. Each set of coordinates in the dynamics trajectory can be viewed using the Dynamics

Animation application. You also can read data into an individual MSF. The final set of coordinates is stored in CHARMm external format at the end of the calculation. Table 3 lists file types and describes each briefly.

Table 3. Dynamics output files

File Type	Description
Dynamics Trajectory File (.DCD)	Contains all the coordinate sets defining the dynamics trajectory for the calculated stage of dynamics, in CHARMm binary format. May be viewed in the Dynamics Animation application or analyzed in the Analysis application.
Energy File (.ENE)	Contains energies stored periodically throughout the dynamics calculation, in ASCII format. May be viewed using **Plots** or **Graphs** from the **File** menu.
Restart File (.RST)	Contains final velocities and coordinates and can be used as a starting file for additional dynamics calculations. ASCII format.
Velocity Trajectory File (.DVL)	Contains velocities at intervals throughout the dynamics calculation. Available only from detailed dynamics. Binary format.
Coordinate File (.CRD)	Contains the final coordinates at the end of the calculation of a particular stage of dynamics. Stored in CHARMm coordinate format (ASCII).
CHARMm Log File	Contains input and output from modeling the structure in CHARMm and running the dynamics calculation. Contains energy and dynamics-related summaries written periodically throughout the calculation. ASCII format. The actual filename, including extension, is specified in the charmm.cis file; default is CHARMm.LOG.

Complete the following exercise to become familiar with the procedures for executing a dynamics run. This exercise uses mypeptide.msf, which is generated in Chapter 4 of *QUANTA Generating and Displaying Molecules*. Complete Chapter 4 before proceeding.

1. Open an MSF

Display the **File** menu. Select **Open**. A File Librarian dialog box opens. Select mypeptide.msf from the scrolling list. Select the **Open** button. The structure is displayed in the viewing area.

Make sure the **Shake** option is on. Display the **CHARMm** menu. Select **Shake Options**. Accept the default settings:

Run dynamics with Shake **ON.**

Shake Tolerance: **1e-09**
Maximum number of iterations: **500**
Use Parameter-specified Geometry

Select the **OK** button.

2. Select heating parameters and heat the structure.

Display the **CHARMm** menu. Select the **Dynamics** option. The CHARMm Dynamics Setup dialog box is displayed offering options for the selection of heating, equilibration, and simulation conditions.

Select **Setup Heating.**

Select the **OK** button. A dialog box is displayed offering the ability to change heating conditions from their default values. Enter the values:

Dynamics Steps: 600
Restart Write File: mypep_h
Coordinate Trajectory File: mypep_h
Energy Values File: mypep_h
Output Frequency: 10
Time Step: 0.00100
Initial Temperature: 0.00
Final Temperature: 300.00

Selecting these parameters produces a heating trajectory of 0.6 ps (600 steps x 0.001 ps/step). Every tenth step is saved into a trajectory file that is 60 frames long.

Also select the option **Start Heating From The Beginning.**

Select the **OK** button. The specifications are defined and the CHARMm Dynamics Setup dialog box is redisplayed.

3. Select equilibration parameters and equilibrate the structure.

From the CHARMm Dynamics Setup dialog box, select the option:

Setup Equilibration

Select the **OK** button. A dialog box is displayed offering the ability to adjust the equilibration specifications.

Enter the values:

Dynamics Steps: 600
Restart Read File: mypep_h
Restart Write File: mypep_e
Coordinate Trajectory File: mypep_e
Energy Values File: mypep_e
Output Frequency: 10
Equilibration Frequency: 10
Time Step: 0.00100
Temperature: 300.00

Then select:

Restart Equilibration From The Restart File

Select the **OK** button. The specifications are defined and the Dynamics Options dialog box is redisplayed.

4. Select the simulation parameters.

From the CHARMm Dynamics Simulations Setup dialog box, select the option:

Setup Simulation

Select the **OK** button. A dialog box is displayed, offering the ability to adjust the simulation specifications.

Enter the values:

Dynamics Steps: 600
Restart Read File: mypep_e
Restart Write File: mypep_s
Coordinate Trajectory File: mypep_s
Energy Values File: mypep_s
Output Frequency: 10
Time Step: 0.00100
Temperature: 300.00

Select the option:

Restart Simulation From The Restart File

Select the **OK** button. The specifications are defined and the dialog box containing the dynamics options is redisplayed.

Select the **DONE** button. The dialog box is cleared from the screen.

5. **Execute the dynamics calculation.**

   Display the Modeling palette. Select **CHARMm Dynamics**. The selection is checked and highlighted.

   CHARMm automatically starts the dynamics calculation, using the defined specifications. Status information is printed in the message line, indicating the progress of the CHARMm calculation. The calculation can take 10 min or more.

   Each frame of the dynamics trajectory and the energy for that conformation are displayed in the viewing area as the calculation proceeds. The time and temperature of the simulation are also displayed in the message line.

   The results of the dynamics calculation are automatically stored in the output files specified in the dynamics setup.

6. **Do not save the results of the calculation to an MSF.**

   From the Modeling palette, select **Reject Changes.** The structure using the last saved set of coordinates for mypeptide.msf is displayed in the viewing area.

7. **Display hydrogen bonds for mypeptide.msf.**

   From the Modeling palette, select **Hydrogen Bonds.** Bonds are displayed as white dashed lines. They also are displayed when the dynamics trajectory is animated.

Executing dynamics animation

The procedure for displaying a dynamics trajectory in the Dynamics Animation application involves four steps:

1. Assemble a list of coordinate trajectory files that make up the trajectory.
2. Specify datasets from the collection of trajectories files to be displayed and the information to be graphically represented in the display.

3. Download the selected set of datasets into memory for display as frames to create the animation.

4. Interactively display the selected set of frames.

When **Dynamics Animation** is selected from the **Application** menu, the Dynamics Animation palette is displayed over the Modeling palette. In addition, a Dynamics dial emulator is displayed in the Dial Emulator window. Table 4 lists the selections in the palette and provides a brief description of each. The table also provides a description of the dynamics dial.

Table 4. Dynamics Animation palette and dials

Selection	Description
Select Trajectories	Assembles the list of coordinate trajectory files from which the dynamics frames to be downloaded are specified.
Set Up Animation	Specifies the frames that are to be downloaded and the graphic items or display features that are to be generated for downloaded frames. Also sets the rate of animation display.
Color By Velocity	Allows a velocity file to be specified; the animation is then created using these velocities to color each atom in each frame. The colors range from white (slowest) to red (fastest).
List Settings	Lists the setup specifications in the textport.
Create Animation	Downloads dynamics frames into memory as specified by **Set Up Animation**.
Open Energy File	Selects a new energy file for display in the energy trace.
Display Energy Trace	Toggles on and off the display of the energy trace.
Clock	Continuously displays dynamics frames in one direction.
Cycle	Continuously displays dynamics frames in both directions alternately.
Select Frame	Specifies a new atom reference frame.
Fix Atom Position	For all dynamics frames, fixes the atom that you select as the display origin.
Release Atom	Returns the display origin for all dynamics frames to the center of the structure.

Table 4. Dynamics Animation palette and dials (Continued)

Selection	Description
Delete Dynamics Frames	Deletes dynamics frames from memory.
Write Frame to MSF	Writes the coordinates of a dynamics frame to an MSF.
Exit Dynamics Animation	Exits Dynamics Animation and returns control to Molecular Modeling.
Dynamics Dial	Horizontal dial scans frame-by-frame through the trajectory.
Speed Dial	Vertical dial controls the frame display rate of the continuous display selections (**Clock** and **Cycle**).

A list of one or more coordinate trajectory files must be defined before a dynamics animation can be displayed. A single trajectory is assembled using **Select Trajectories** on the Dynamics Animation palette. Table 5 lists and describes the options in the dialog box that is displayed when **Select Trajectories** is selected.

Table 5. Select Trajectories Options

Option	Description
Initialize Dynamics Files	Displays the File Librarian with a list of available dynamics files. By selecting a dynamics file from the File Librarian, you initialize a new list with the chosen file as the first entry. The first file in the list is used to set appropriate default values for the first and last animation frame and the step size between animation frames.
Change a File	Displays a scrolling list dialog box showing the current files in the list. Select the file to be replaced, then the File Librarian lists dynamics files in your current directory. Selection of a coordinate trajectory file from the File Librarian causes that file to replace the previously selected file in the list.

Table 5. Select Trajectories Options

Option	Description
Add a File	Displays a File Librarian listing available dynamics files. Selecting a file adds that file to the end of the current list. Dynamics files must be added to the list in the order in which they are to be displayed.
Delete a File	Displays a File Librarian showing the current files in the list. Selecting one of these files causes that file to be removed from the list. Subsequent files in the list are renumbered.
List Current Files	Displays names of currently used coordinate trajectory files in the textport. The files are numbered sequentially in the order in which they are to be displayed.
Show File Headers	Displays the header block of the specified coordinate trajectory files in the textport.
Binary or Hexadecimal Files	Specifies whether the trajectory to be loaded was written in binary or hexadecimal format. The latter format is useful when producing trajectories on a remote compute server for display on the graphics workstation.

Multiple files from the trajectories list are combined into a single animation. The composite trajectory from the datasets in these files also is specified for graphic display and analysis.

The file header in each coordinate trajectory file contains critical information including:

- The number of datasets in the file.
- The starting dataset of the file.
- The number of time steps between successive datasets in a dynamics trajectory.

This information is required to specify the range of datasets to be viewed. The application automatically uses the dataset information contained in the first trajectory file. It does not automatically combine dataset information contained in a series of trajectory files. The trajectory files must be reviewed to determine:

- The **Dataset Range From** value — the starting dataset value in the header of the first trajectory.
- The **Dataset Range To** value — the ending dataset value in the header of the last trajectory.
- The **Step Size** value in the header of each trajectory

Additional information that may be graphically represented in the animation includes:

- Atom labels
- Distances
- Hydrogen bonds

Specifications for this information are established using **Set Up Animation** in the Dynamics Animation palette. Each frame of the dynamics trajectory is loaded into memory before an animation is displayed. **Set Up Animation** governs the creation process. Table 6 lists options in the Set Up Animation dialog box and provides a brief description of each.

Table 6. Set Up Animation dialog box

Option	Description
Use CHARMm Header	Retains the dataset numbers contained in the dynamics file headers. This is the default. It can be used for animations composed of a single dynamics file or of several files created through the use of **Restart Files**.
Index Sequentially	Assigns sequential numbers 1 through *n*, where *n* is the total number of datasets in the animation, to the datasets for constructing the animation. This method must be used for animations composed of dynamics files from different runs.
Dataset Range	Consists of two fields, **From** and **To**, defining the first and last datasets of the range to be displayed. The lower limit for the **From** field is the first dataset in the animation. The upper limit for the **To** field is the last dataset in the animation.
Step Size	Specifies the step interval, which must be an integral multiple of the output frequency.
Clock Speed	Determines the initial rate at which the program cycles through the display of the frames in the dynamics animation when **Clock** or **Cycle** is selected from the Dynamics Animation palette. The default speed is 100. Interactive control of the speed is provided in the Dynamics emulator dial set (dial set 8).
Number Steps to Average Over	Controls the averaging of the displayed molecule from the trajectory files and is a simple mechanism for removing high-frequency motions in an animation. This allows more extensive and concerted motions to be visualized.
Regenerate Hydrogen Bonds	Causes hydrogen bonds to be generated for each frame of a subsequently created animation.

Table 6. Set Up Animation dialog box (Continued)

Option	Description
Regenerate Selections and Hydrogen Bonds	Controls current display and color selections applied to each frame of a subsequently created animation. Selecting this option also calculates hydrogen bonds on a frame-by-frame basis during creation of the animation. This option is needed, for example, for coloring by atom property.
Pack Atoms into Cell	Specifies that a molecular structure created by the Amorphous Builder application to be displayed in a unit cell.
Display Atom Labels	Specifies that labels are to be generated for each frame of an animation according to the currently defined atom labeling regime.
Display Geometry Monitors	Specifies that distance, angle, and torsion monitors are to be displayed for each frame of a dynamics animation. The distances, angles, and torsions are designated before creating the animation, with the tools on the Geometry palette.
Display Trails	Causes trails to be generated in frames of a subsequently created animation. Trails are vectors representing the last three positions of each atom in a frame of an animation. The vectors are drawn in display color 14 and appear as traces of atom movements.
Display Energy Trace	Specifies that a trace of the energy and temperature of the molecule throughout the dynamics simulation be displayed in the animation.
Do Not Display Dipole	Specifies that no dipole be displayed during animation.
Display Dipole for Whole Molecule	Specifies that a dipole in the form of a vector arrow be displayed for the molecule in each frame of the animation.
Display Dipole for Each Residue	Specifies that a dipole in the form of a vector arrow be displayed for each residue in each frame of the animation

An animation may be displayed frame-by-frame or continuously (like a movie). As each frame is displayed in the viewing area, its frame number is shown above the Dynamics dial. Frames may be displayed either forward or backward.

Sample procedure — Dynamics animation

Complete the following exercise to become familiar with procedures in the Dynamics Animation application. The exercise uses the trajectory files generated in the previous section of this chapter.

1. Start Dynamics Animation.

Display the **Applications** menu. Select **Dynamics Animation**.

The Dynamics Animation palette and **Dial emulators** are displayed. The filename of the current MSF (mypeptide.msf) is displayed in the top left corner of the viewing area. However, the structure is not displayed until the animation is created.

2. Select coordinate trajectory files.

From the Dynamics Animation palette, select **Select Trajectories.** A dialog box is displayed offering options for the management of coordinate trajectory files to be displayed.

Select the option:

Initialize Dynamics Files

Select the **OK** button. A File Librarian dialog box is displayed.

Select the file mypep_h.DCD, created during the dynamics heating phase.

Select the **Open** button. Information contained in the .DCD file is displayed in the textport. The dialog box offering options for the management of coordinate trajectories is redisplayed.

Select the option:

Add a File

Select the **OK** button. A File Librarian dialog box is redisplayed.

Select the file mypep_e.DCD, created during the equilibration phase of the dynamics run.

Select the **Open** button. Information contained in the .DCD file is displayed in the textport, and the dialog box offering options for the management of coordinate trajectories is redisplayed.

Select the option:

Add a File

Select the **OK** button. The File Librarian dialog box is redisplayed.

Select the file mypep_s.DCD, created during the dynamics simulation phase.

Select the **Open** button. Information contained in the .DCD file is displayed in the textport. A dialog box offering options for the management of coordinate trajectories is redisplayed.

Select the option:

List Current Files

Select the **OK** button.

The three .DCD files are listed in the textport:

Dynamics file: 1 mypep_h.DCD
Dynamics file: 2 mypep_e.DCD
Dynamics file: 3 mypep_s.DCD

The dialog box offering options for the management of coordinate trajectories is redisplayed.

3. Display the header information for the selected .DCD files.

Select the option:

Show File Headers

Select the **OK** button. A dialog box is displayed allowing the selection of trajectory files to be read.

Enter the values:

Enter the first number in range: 1
Enter the last number in range: 3

Select the **OK** button. The header for each of the three trajectory files is displayed in the textport.

```
file no. 1 is of type CORD
The icntrl array is:
60 10 10 600 10 0 0 558 0 0.02045
number of datasets: 60
starting dataset: 10
ending dataset: 600
step size: 10
no of fixed atoms: 0
*** TITLE CARD READ FOR FILE UNIT - 3
* MINIMIZED COORDINATESFROM CHARMM
* DATE: 6/17/91 9:47:31 CREATED BY USER:emily

file no. 2 is of type CORD
The icntrl array is:
60 610 10 600 10 0 0 558 0 0.02045
number of datasets: 60
starting dataset: 610
ending dataset: 1200
step size: 10
no of fixed atoms: 0
*** TITLE CARD READ FOR FILE UNIT - 3
* COORDINATES AFTER HEATING DYNAMICS
* DATE: 6/17/91 9:56:26 CREATED BY USER:emily

file no. 3 is of type CORD
The icntrl array is:
60 1210 10 600 10 0 0 558 0 0.02045
number of datasets: 60
starting dataset: 1210
ending dataset: 1800
step size: 10
no of fixed atoms: 0
*** TITLE CARD READ FOR FILE UNIT - 3
* COORDINATES AFTER EQUILIBRATION DYNAMICS
* DATE: 6/17/91 10: 8:1 CREATED BY USER:emily
```

A dialog box offering options for the management of coordinate trajectories is redisplayed. Select the **Exit** button in the dialog box. The box is removed from the screen.

4. Select animation specifications.

From the Dynamics Animation palette, select **Set Up Animation.** A dialog box is displayed that is used to define how the trajectory is displayed.

Select the option:

Use CHARMm Header

Enter the values:

From: 10
To: 1800
Step Size: 10
Clock Speed: 100.0
Number Steps to Average over: 0

Select the options:

Regenerate Hydrogen Bonds
Display Trails
Do not display dipole

Select the **OK** button. The dialog box is removed from the screen.

5. Create the animation.

From the Dynamics Animation palette, select **Create Animation**. The selection is checked and highlighted. The dynamics trajectory is displayed frame by frame. Hydrogen bonds for the conformation are calculated and displayed in the frame.

The frame number is displayed above the dynamics emulator dial in the Dial Emulator window. The progress of the creation is displayed in both the message line and the textport.

When the creation is finished, the first frame is displayed in the viewing area. The dynamics emulator dials are activated, and frame number 10 is displayed over the Dynamics emulator dial.

6. Step through several frames.

Place the cursor on the plus sign (+) in the Dynamics dial emulator box. Press and hold the left mouse button. Each trajectory frame is displayed in ascending order until the mouse button is released or the end of the trajectory is reached. The frame number in the Dynamics dial changes to reflect the frame in view.

Use the minus (-) button in the Dynamics dial box to display the frames in descending order.

7. Display frames continuously

From the Dynamics Animation palette, select **Clock**. The selection is checked and highlighted. The animation movie is continuously displayed in the forward direction until the last frame is reached. The

movie immediately jumps back to the first frame and repeats the display until **Clock** is deselected.

8. Adjust the speed of the display.

Place the cursor on the **D** of the Speed dial emulator. Press and hold the left mouse button. Release the mouse button. The speed value stops changing. and the display step rate increases to a higher value.

Press and hold the left mouse button. Release the mouse button. The speed value stops changing and the display step rate decreases to a lower value.

9. Turn off the Clock tool.

From the Dynamics Animation palette select **Clock.** The tool is turned off and the continuous display of the trajectory is stopped.

Create an animation with an energy trace

An energy trace plots the energy and temperature of the molecule throughout a dynamics simulation. The following exercise sets up an energy trace for the mypeptide animation.

1. Select animation specifications.

From the Dynamics Animation palette, select **Set Up Animation.** A dialog box is displayed offering options for displaying the trajectory.

Select the option:

Use CHARMm Header

Enter the values:

From: 10
To: 1800
Step Size: 10
Clock Speed: 100.0
Number Steps to Average over: 0

Select the option:

Regenerate Hydrogen Bonds
Display Trails

Display Energy Trace
Do not display dipole

Select the **Create** button. A dialog box is displayed offering choices for the energy values to display.

Select the options:

Total Energy
Temperature

The animation is created. As each frame of the dynamics trajectory is displayed, the corresponding energy trace is displayed in a graph near the top of the viewing area. **Energy Trace** is checked and highlighted.

2. Cycle through all of the frames.

From the Dynamics Animation palette, select **Cycle**. The trajectory is continuously displayed: first forward until the last frame is reached, then backward until the first frame is reached, then forward again until the selection is turned off. When each frame of the trajectory is displayed, the energy trace graph and the frame number are updated.

Select **Cycle** again. The selection is turned off and the continuous display of the trajectory is stopped.

3. Examine structures associated with energy trace values.

Move the mouse so the cursor is over any point in the energy trace. Click the left mouse button. The energy trace cursor is moved to this location and the conformation corresponding to the selected energy trace values is displayed in the viewing area.

4. Exit Dynamics Animation.

From the Dynamics Animation palette, select **Exit Dynamics Animation.** The palette, energy trace, and animation graphics are removed from the display. The structure mypeptide.msf is redisplayed in the viewing area.

Summary

CHARMm dynamics simulates the natural motion of a molecular system and produces a set of coordinates and velocities that describe the atomic motions of the system over time. The resulting dynamics trajectory can be viewed in the Dynamics Animation application and analyzed in the Analysis application.

A typical molecular dynamics run involves heating, equilibration, and simulation. Parameters are set for each step in a run. Molecular dynamics simulations are usually computed at room temperature.

Equilibration is achieved by allowing the molecular system to evolve spontaneously for a period of time, integrating the equations of motion until the average temperature and structure remain stable. The procedure is continued until the statistical properties of the system become independent of time. Equilibration temperature *must* correspond to the final temperature used in heating.

Simulation takes an equilibrated structure as its starting point and produces a dynamics trajectory. In a typical simulation, the trajectory traces the motions of the macromolecule for a specified time. The simulation temperature *must* correspond to the equilibration temperature.

Dynamics heating and equilibration are usually performed only once on a molecular structure. Simulation may be run several times to show changes in the conformation of the molecule over time.

All coordinates calculated during a dynamics run are stored in the coordinate trajectory file specified in the dynamics setup. Each set of coordinates in the dynamics trajectory can be viewed using the Dynamics Animation application. You also can read data into an individual MSF. The final set of coordinates is stored in CHARMm external format at the end of the calculation.

With the Dynamics Animation application, you can interactively display a dynamics trajectory calculated by CHARMm, CNX, or X-PLOR. Displays can include a graphical representation of hydrogen bonding, interatomic distances, atomic positions, molecule energy, or atomic properties.

The dynamics trajectory calculated in CHARMm consists of datasets of atom coordinates written periodically. If the trajectory is thought of as a movie, then each individual dataset may be considered a frame. You can

display the trajectory frames at any desired speed. Single frames may be saved to individual MSFs that can be compared in detail using the Molecular Similarity application.

References

1. Charles Brooks III, Martin Karplus, and B. M. Pettitt. 1988. Proteins: A Theoretical Perspective Of Dynamics, Structure And Thermodynamics. *Advances in Chemical Physics*. John Wiley and Sons, New York.

2. Applying Constraints

This chapter describes the options in QUANTA used to define and apply various constraints for CHARMm calculations. Read this chapter if you want to apply constraints to structures undergoing CHARM-based operations.

For information on the definition and analysis of constraints used specifically for NMR structure determination, consult another volume in the QUANTA documentation set *NMR Structure Determination*.

Four kinds of constraints are described in this chapter:

- Atom constraints — Used to fix or harmonically constrain an atom or set of atoms to their current positions during energy minimization or dynamics calculations.
- Distance constraints — Used to apply a square-well constraining potential between one atom or set of atoms and a second atom or set of atoms to limit the distance between them to specified bounds. Where the constraint involves a collection of atoms (protons of a methyl group, for example), distances are measured from an average position.
- Dihedral constraints — Used to apply a harmonic constraint to restrict a dihedral (torsion angle) to a specified value.
- Stochastic boundary constraints — Used to simulate forces arising from average properties of the system outside a specified boundary.

Access to the tools for defining and applying constraints is through the **CHARMm** menu. From the **CHARMm** menu, select **Constraints Options** to display a pull-right menu containing selections for each type of constraint option. Table 7 lists the selections and provides a brief description of each.

Table 7. Constraints Options Menu

Selection	Description
Select Atom Constraints	Provides palette choices for defining atom constraints and for selecting atoms to which the constraints are applied.
Atom Constraints On	Turns on the atom constraints selected for the current session and stored in an *.atc* file.
Atom Constraints Off	Turns off the atom constraints selected for the current session and stored in an *.atc* file.
Dihedral/Distance	Provides palette choices for defining dihedral and distance constraints and preparing a Constraints Table.
Dihedral On	Turns on the dihedral constraints selected for the current session and stored in the Constraints Table.
Dihedral Off	Turns off the dihedral constraints selected for the current session and stored in the Constraints Table.
Distance On	Turns on distance constraints selected for the current session and stored in the Constraints Table.
Distance Off	Turns off the distance constraints selected for the current session and stored in the Constraints Table.
Stochastic Bdry Settings	Provides choices for defining deformable boundary forces used to study an active site.
Stochastic Bdry Settings On	Turns on stochastic boundary settings selected for the current session.
Stochastic Bdry Settings Off	Turns off stochastic boundary settings selected for the current session.

Defining and applying atom constraints

Atom constraints are first defined, then applied. To define a constraint:

1. Set the force constant.
2. Select the atoms to which this particular force constant applies.

Start the process by selecting **Constraints Options** from the **CHARMm** menu. Then select **Select Atom Constraints** from the pull-right menu. Two palettes open, the Set Atom Constraints and Atom Constraints Utilities palettes. These palettes are similar to those used throughout QUANTA for atom selection. For example, see the description of the Active Atoms and Active Atoms Utilities palettes in Chapter 2 of *QUANTA Basic Operations*.

Table 8 and Table 7 list the selections in the Set Atom Constraints and Atom Constraints Utilities palettes, respectively, and provide a brief description of each selection.

Table 8. Set Atom Constraints palette

Selection	Description
All MSFs	Selects all MSFs that have been chosen on the Molecule Management Table if each MSF has <500 atoms. This option is turned off if one MSF has >500 atoms.
One MSF...	Selects the atoms contained in the specified MSF. Only the selected MSF is displayed.
Apply to All MSFs	Applies specified selections of one MSF to all active MSFs.
Color by Value	Assigns a color to each atom according to the force constant that is associated with the atom. Helps ensure that constraints are properly set.
Show Force Constants	Labels each atom with the force constant assigned to it. Helps establish proper constraints.
Harmonic Force...	Opens dialog box to set a force-constant value. Default value is 100.00. The force constant remains in effect until it is changed.
Fix	Constrains selected atoms to their current position.
Free	Sets force constant to zero.
All Atoms	Selects all atoms in the structure. Can be used to restore a structure after using other atom selection options.
All Non-Hydrogen Atoms	Selects all atoms except hydrogen atoms.
Solvent	Selects all residues of type: WAT, HOH, TIP, TIP3, SOL, SOLV, ST2, NON, H2O, TIP4.
Alpha-Carbon Atoms	Selects only protein alpha-carbon atoms.
Protein Backbone	Selects only protein backbone atoms.
Nucleic Acid Backbone	Selects only nucleic acid backbone atoms.
Residue Range...	Active residue range selected by completing information in a dialog box. The process can be repeated.
Residue Type...	Active residue type selected by completing information in a dialog box. The process can be repeated.
Atom Type...	Active atom type selected by completing information in a dialog box. The process can be repeated.
Pick Molecule	Using the mouse, selection of a molecule is made from the active MSFs on the screen.
Pick Segment	Using the mouse, selection of a segment is made by picking an atom in the segment from the active MSFs on the screen.

Table 8. Set Atom Constraints palette (Continued)

Selection	Description
Pick Residue	Using the mouse, selection of a residue is made by picking an atom in the residue from the active MSFs on the screen.
Pick Residue Range	Using the mouse, selection of a residue range is made by picking two atoms from the active MSFs on the screen.
Pick Atom	Using the mouse, selection of an atom is made by picking it from the active MSFs on the screen.
Proximity Tools	Opens the Proximity Tools palette containing multiple choices for selecting a central atom and nearby portions of a molecule.
Type in a Selection	A dialog box is used to enter Selection-Commands.
Undo	Returns the viewing area to the state before the previous command.
Clear	Deletes all selections. Resets to defaults.
Revert	Returns the viewing area to the initial state when **Select Atom Constraints** was selected.
Quit	Exits **Select Atom Constraints** without saving changes and reverts to the original .atc file.
Finish	Exits **Select Atom Constraints** and writes the changes to a temporary *.atc* file. At least one atom of the active molecule must be selected to use this option.

Table 9. Atom Constraints Utilities palette

Selection	Description
Retrieve Labeled from MSF	Recovers labeled constraints from MSF created in QUANTA 3.3 or older. Atom constraints for QUANTA are stored as a set of commands in an .atc file.
Read Selection-Commands	Reads command from file.atc and updates the viewing area to select the new set of commands.

Table 9. Atom Constraints Utilities palette (Continued)

Selection	Description
Append Selection-Commands	Appends the current file.atc with a set of commands from another file2.atc and updates the viewing area to reflect the new combined set of commands.
Save Selection-Commands	Saves the current set of commands to a new file.
List Selection-Commands	Lists the current set of commands in the Textport window.
Edit File Manually	Allows an .atc file to be manually edited. A window is opened, which uses the vi editor for editing the file[a].
Help on Commands	Provides a list of all commands and their functions in the Textport.

[a]You can change the default editor by using the **setenv** command before QUANTA is started. The syntax is: **setenv** ***EDITOR*** (name of system editor to be used).

To set a particular force constant, select **Harmonic Force...** from the **Set Atom Constraints** palette. Or select **Fix** or **Free** to fix atoms or free them, respectively.

When you have completed the task of setting a force constant, select the set of atoms to which this force is to apply, using one of the selections in the **Set Atom Constraints** palette. Selections are automatically written to a *filename*.atc file.

The Atom Constraints Utilities palette provides tools to save a particular selection scheme to an .atc file. This file can then be read and used in subsequent sessions.

To use your constraints selections in CHARMm energy calculations, select **Finish** from the Set Atom Constraints palette. The current set of selection commands in the .atc file is translated, and a value for each atom is stored in the appropriate MSF. A status flag is set on, and the constraints are communicated directly to CHARMm for all subsequent energy calculations. These constraints remain active until explicitly turned off using the **Atom Constraints Off** selection in the **Constraints Options** pullright menu or until the QUANTA session is ended.

If a second molecule is added to an ongoing session, the .atc file is updated to include the new molecule. But you must apply the **Atom Constraints On** selection to inform CHARMm of the change.

Sample procedure — Defining and applying atom constraints

Complete the next exercise to become familiar with the procedures for defining and applying atom constraints. This exercise uses mypeptide.msf generated in Chapter 4 of *QUANTA Generating and Displaying Molecules.* If you have not done so, complete Chapter 4 before proceeding.

1. Open an MSF.

From the **File** menu, select **Open**. A File Librarian dialog box is displayed.

Select the file mypeptide.msf. Select **Replace** then the **Open** button. The structure is displayed in the viewing area.

2. Define atom constraints.

From the **CHARMm** menu, select **Constraints Options.** From the pull-right menu, select **Select Atom Constraints.** The Select Atom Constraints and Atom Constraints Utilities palettes are displayed over the Geometry palette. The Modeling palette is removed. The molecule is displayed in light green

From the Set Atom Constraints palette, select **Show Force Constants. Color by Value** and **Fix** are default selections**.** The message line reports the force constant as -1.00.

From the Set Atom Constraints palette, select **Alpha-carbon Atoms.** The alpha carbons are displayed in light green, and the force constant -1.00 is displayed in a label on each carbon. The rest of the molecule is displayed in blue.

3. Exit from Select Atom Constraints.

From the Set Atom Constraints palette, select **Finish**. The palette is removed and the Modeling palette is redisplayed. The structure returns to standard colors. A textport message reads:

```
The file mypeptide.msf contains the following extra informa-
tion: Next energy calculation will use constraints.
```

4. Apply constraints.

From the modeling palette, select **CHARMm Energy**. An energy value is calculated and displayed in the upper-right corner of the viewing area.

From the **CHARMm menu,** select **Minimization Options.** A dialog box is displayed allowing you to select a minimization method and to specify associated parameters controlling the calculation

Select the option:

Steepest Descents

Enter the values:

Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.00001
Energy Value Tolerance: 0.000
Initial Step Size: 0.020
Step Value Tolerance: 0.000

Select **OK**. The minimization technique and associated parameters are defined, and the dialog box is cleared from the screen.

From the Modeling palette, select **CHARMm Minimization.** The cursor changes from an arrow to a watch while the minimization calculation runs.

At the end of the calculation, the structure is displayed with new coordinates. The energy is displayed in the upper- right corner of the viewing area.

5. Rerun minimization without constraints.

From the **CHARMm** menu, select **Constraint Options.** From the pull-right menu, select **Atom Constraints Off.**

Rerun an energy minimization exactly as in Step 4.

Compare the data from the calculations in Steps 4 and 5.

6. Reject changes.

Select **Reject Changes** from the Modeling palette.

Defining and applying distance constraints

Distance constraints are used most frequently to represent interatomic distances determined experimentally by measurement of the nuclear Overhauser effect. NOE interactions or general distance constraints used in CHARMm calculations can be defined as described below. The NMR Constraints Editor and NMR Structure Determination applications of QUANTA provide additional tools to be used with the X-PLOR interface. They are described in the book *NMR Structure Determination*.

A subset of the options used in the NMR application is available for use in general modeling problems. As with atom constraints, distance constraints first must be defined then applied.

The subset of distance constraints described in this section is accessed using the Edit Constraints palette that opens when **Distance/Dihedral** is selected from the **Constraints Options** pull-right menu that is opened from the **CHARMm** menu. This palette is also used to apply dihedral constraints. Table 8, lists and briefly describes the palette selections.

Table 10 . Edit Constraints palette

Selection	Description
Read Constraints	Reads a Constraints Table created in a previous session or in NMR Workbench.
Import Constraints...	Provides an interface to alternative constraint file formats. The CHARMm format reads and writes constraints in a format that can be read into CHARMm when used in the stand-alone mode. The Xplor format reads or generates constraints suitable for the XPLOR program. Several other constraint formats are supported for the NMR-Structure Determination package and are described elsewhere.
Export Constraints...	Provides an interface (same as import) to move files into alternative constraint file formats.
Define Dihedral Constraint(s)	Defines a dihedral constraint when four connected atoms are picked. These atoms are taken to be definitions of the end atoms of the constraint. A constraint thus defined is added to the Constraints Table. There is no provision to define a constraint for unconnected atoms.
Propagate a Dihedral Pick	Attempts to find all possible occurrences of the dihedral defined by the atom names of the four picked atoms. This selection is valid only for polymers in which atom names repeat from residue to residue.
Dihedral Families	Opens a dialog box where dihedral family constraints are defined.

Table 10 . Edit Constraints palette (Continued)

Selection	Description
Generate Dihedral Constraints	Used in conjunction with the NMR workbench. Reads a table of experimentally determined NMR coupling constants of the type JNH_HA and JHA-HB and converts these to constraints for torsion angles Phi and Chi1, respectively. Operation of this selection is described in the book *NMR Structure Determination.*
Dihedral Options	Sets defaults to use in defining dihedral constraints.
Define Distance Constraint(s)	Defines a constraint by picking two atoms from the active molecule on the screen. There are six picking modes (listed below) available.
Individual Atom	Picks just the atom that is selected. Default mode.
Protons Bound to Picked Atom	Defines a constraint to a pseudo atom whose position is defined as the average of the positions of all protons bound to the picked atom. For example, the protons of a methyl group can be simultaneously picked by picking the methyl carbon atom.
Atoms of Picked Residue	Defines a constraint for a pseudoatom whose position is determined by the average of all atoms in the picked residue.
Protons of Picked Residue	Defines a constraint to a pseudoatom whose position is determined by the average of all protons in the picked residue.
Atom of Picked Segment	Defines a constraint for a pseudoatom whose position is determined by the average of all atoms in the picked segment. Limited to 500 atoms.
Protons of Picked Segment	Defines a constraint for a pseudoatom whose position is determined by the average of all protons with the average taken over the entire segment. Limited to 500 atoms.
Distance Options	Opens a dialog box containing distance constraints defaults, and options for changing those values. The options assign values to constraint types, class, upper and lower bounds, activity status, and constraints display.
Select Constraints	Allows selection of a subset of constraints to be active and displayed. Additional selections can be made using table selection mechanisms.
Delete Selected Constraints	Allows an entire set of selected constraints to be deleted. A single constraint can be deleted by selecting it then deleting it.

Table 10 . Edit Constraints palette (Continued)

Selection	Description
Show Violation Labels	Displays constraints on the screen using a color code to indicate the extent to which each constraint satisfies boundaries imposed upon it. For those constraints that are not satisfied, the violation label reports the extent to which the bound is violated.
Show Constraints	Toggles the display of constraints on the structure in the viewing. Constraints are also listed in the Constraints table.
Save Constraints	Saves the current constraints in table form and supplies a .con file extension as a means to identify it as a Constraints table. This is the recommended format to use for communicating with NMR Workbench.
Save Constrains As...	Saves the current constraints in table form and supplies a .con file extension to identify it as a Constraints table. Allows new filename to be assigned. This is the recommended format to use for communicating with NMR Workbench.
Exit Edit Constraints	Exits the Edit Constraints palette for distance and dihedral constraint definition and application. Returns to molecular modeling mode where CHARMm energy calculations can be carried out. Contacts displayed on the model are removed, but the table remains displayed. The table must be present in order to send Constraint commands to CHARMm.

When the **Define Distance Constraints** selection is active, any two picks are taken to be definitions of the end atoms of the constraint. A constraint thus defined is added to a table of constraints labeled Constraints_Database.con. This table opens in a separate window over the window for the Molecule Management table. It is stored in a .con file when the Edit Constraints palette is exited.

As each constraint is defined, a colored dashed line on the screen connects the end atoms of the constraint. If the actual interatomic distance in the model falls within specified bounds, the contact line is displayed in blue. If the distance exceeds the upper bound, it is drawn in red, and if less than the lower bound, in yellow.

Constraint properties are assigned current default values. These defaults can be changed using the dialog box that is displayed when **Distance Options** is selected from the Edit Constraints palette. After a constraint is defined and appears in the table, its properties can be edited directly in the table using table edit procedures. The atom identifiers in columns End 1 and End 2 of the table cannot be edited.

The options defined in this dialog box include:

- **Type** —A code used primarily to distinguish distance constraints (defined by two atoms) from torsion constraints (defined by four atoms). For distance constraints in most applications, set the type to NOE.
- **Class** — Four-letter code used to distinguish various kinds of distance interactions from one another. Its primary use is in the NMR Structure Determination application, where different potential functions are assigned to different types of interactions. The class assigned here is useful only as a tag for selection.
- **Targeted Distance (Target)**— The optimal distance between two picked atoms.
- **Upper Bound** and **Lower Bound** —Values that define the range for which a constraint is satisfied. These correspond to R_{MAX} and R_{MIN} parameters, respectively, in the CHARMm NOE potential function. They determine the width of the square well.
- **CHARMm Force Constant KMIN** and **KMAX** — Force constants that determine the steepness of the potential outside the R_{MIN} and R_{MAX} ranges.
- **Activity Setting** — Determines if a constraint in the Constraints table is sent to CHARMm. If the toggle is set to **Inactive**, the constraint can be defined but not used in CHARMm calculations.

The Constraints table contains columns for each parameter described above, plus several that are specific to the NMR Structure Determination application. Full column display is achieved in an expanded window.

The Constraints table also includes its own set of menus that can be used to manipulate table data and edit constraints. These pull-down menus are accessed from the menu bar at the top of the table and include:

- **File** — Manages import and export of table files. Limited use recommended. Instead use the **Import**, **Export**, and **Save** selections in the Edit Constraints palette.
- **Edit** — Manages constraint subsets. Also provides tools to define and edit rows.
- **Format** — Customizes appearance of the table.
- **Data** — Manipulates table entries.
- **Special Tools** — Defines and manages constraint subsets.

Initially, all constraints are selected and highlighted in blue, both in the Constraints table and on the screen,. When many constraints are defined, it may be useful to select or delete a subset of them as an aid to viewing. Constraints can be selected by using options in the Select Constraints palette. This palette is displayed when you select **Select Constraints** in the Edit Constraints palette.

You also may select constraints from the Constraints table. Selections in the table are made by clicking or double-clicking the row number of the constraint you want to select, by using **Select All** in the table **Edit** menu, by using **Find** in the table **Data** menu, or by using selections in the **Special Tools** menu.

If the <Shift> key is depressed as a row is selected, the row is added to the current constraints list. If the <Ctrl> key is depressed as a row is selected, all rows between the current row and the previously selected row are selected.

In the CHARMm **Constraints Options** pull-right menu, **Distance On** and **Distance Off** are used to communicate selected constraints to CHARMm. In addition, the **Status** column (activity setting) in the Constraints table can be used to toggle individual constraints on or off. When changes are made in the table, turn all constraints off then on again to make sure the changes are sent to CHARMm.

Each time constraints are sent to CHARMm, a dialog box appears requesting values for a scaling factor to be used in the CHARMm NOE constraint potential function. The functional form of this potential is described in the CHARMm documentation.

Defining and applying torsion constraints

A torsion (dihedral) constraint is defined by picking four connected atoms after selecting **Define Dihedral Constraint** in the Edit Constraints palette. No provision is made to define a constraint for unconnected atoms.

The atom identifiers that appear in the Constraints table are the end atoms of the torsion. The constraints that you define are listed in the table and also displayed on the model as a small ring around the middle bond of the torsion. The ring is blue if the value of the torsion in the model lies within

the specified upper and lower bounds. Red indicates a violated upper bound and yellow indicates a violated lower bound.

Default values for constraint parameters can be modified by selecting **Dihedral Options** in the Edit Constraints palette. The parameters in the dialog box that opens include:

- **type** — Used do distinguish dihedral constraints from distance constraints when interacting with CHARMm.
- **Constrain to** — Provides options for defining the optimal constraint value.
- **Current value** — Constrains the torsion to stay at or near its current value.
- **Specified Target** — Defines the optimal value and its upper and lower bounds.
- **Solve Karplus Equation** — Each time a dihedral is picked, activates a dialog box that presents coefficients for the Karplus equation and solutions that would be generated by a particular value of an experimentally determined coupling constant. Both the coefficient and the coupling constant can be changed.
- **CHARMm Force Constant** — Sets a default value that appears in the Constraints table for the force constant The CHARMm potential function used for torsion constraints is a simple harmonic function containing force constant *K*.
- **Activity Setting** — Determines if a constraint in the Constraints table is sent to CHARMm. If the toggle is set to **Inactive**, the constraint can be defined but not used in CHARMm calculations.

The Edit Constraints palette selection, **Propagate a Dihedral Pick**, attempts to find all possible occurrences of the torsion defined by the atom names of the four picked atoms. If, for example, the atoms C, N, Cα, and C are picked in a residue of a polypeptide chain, all occurrences of this torsion are found and defined. This selection is valid only for polymers in which atom names repeat from residue to residue.

As with **Propagate a Dihedral Pick,** the **Dihedral Families** selection is useful only in molecules that contain repeating subunits. **Dihedral Families** allows quick definition of commonly occurring dihedrals such as phi, psi, and omega in proteins. For example, the selection could be useful in setting up simulations of proteins in which all omega torsions are restricted to the *trans* configuration.

In the CHARMm **Constraints Options** pull-right menu, **Dihedral On** and **Dihedral Off** are used to communicate selected constraints to CHARMm. In addition, the **Status** column (activity setting) in the Constraints table can be used to toggle a single constraint on or off. When changes are made in the table, turn all constraints off then on again to make sure the changes are sent to CHARMm.

Sample procedure — Defining and applying distance and torsion constraints

Complete the following exercise to become familiar with the procedure for defining and applying distance and torsion constraints. Continue using mypeptide for this exercise.

1. Define distance constraints.

From the **CHARMm** menu, select **Constraints Options.** Then select **Dihedral/Distance** from the pull-right menu that opens. The Edit Constraints palette opens and the Modeling palette is removed.

From the Edit Constraints palette, select **Define Distance Constraints**. In the palette, **Individual Atom** is highlighted as a default selection.

Pick any two pairs of atoms as end atoms in the mypeptide molecule to define two distance constraints. A dashed line appears connecting each pair of atoms. If the distances are within default bounds, the line is blue. If not, the line is either red (too far) or yellow (too near).

The Constraints table entitled Constraints_database.con is displayed in a separate window. The distance constraints are listed in the table.

2. Define dihedral constraints.

From the Edit Constraints palette, select **Define Dihedral Constraints**.

Pick any four connected atoms to define a torsion angle in the mypeptide molecule. The torsion constraint data is added to the Constraints table.

3. Exit the Edit Constraints palette.

From the Edit Constraints palette, select **Finish**. The palette is removed and the Modeling palette is redisplayed. The Constraints table remains on display. A File Librarian dialog box opens.

Enter:

mypeptide

Select the **Save** button. The distance constraints are saved to a .con file.

4. Apply constraints.

From the **CHARMm** menu, select **Constraint Options.** From the pull-right menu that opens, select **Distance Constraints On** and **Dihedral Constraints On**.

From the Modeling palette, select **CHARMm Energy**. An energy value is calculated and displayed in the upper right corner of the viewing area.

From the **CHARMm** menu**,** select **Minimization Options.** A dialog box is displayed allowing you to select a minimization method and to specify associated parameters controlling the calculation

Select the option:

Steepest Descents

Enter the values:

Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.00001
Energy Value Tolerance: 0.000
Initial Step Size: 0.020
Step Value Tolerance: 0.000

Select **OK**. The minimization technique and associated parameters are defined, and the dialog box is cleared from the screen.

From the Modeling palette, select **CHARMm Minimization.** The cursor changes from an arrow to a watch while the minimization calculation runs. The distance and torsion constraints that you defined are applied during the calculations.

At the end of the calculation, the structure is displayed with new coordinates. The energy is displayed in the upper right corner of the viewing area.

From the Modeling palette, select **Reject Changes.** The molecule is redisplayed using the most recently stored coordinates.

5. **Rerun minimization without constraints.**

From the **CHARMm** menu, select **Constraint Options.** From the pull-right menu that opens, select **Distance Off** and **Dihedral Off.**

Rerun an energy minimization exactly as in Step 4.

Compare the visual and written data from the calculations in Steps 4 and 5.

Select **Reject Changes** from the Modeling palette.

Applying stochastic boundary constraints

Deformable boundary forces are used in studying small localized regions of solvent (the reaction zone) around an active site. The program assumes that either the entire molecule or the region of the active site is solvated. Outside the reaction zone, atoms of the solvent and solute are either fixed or ignored. Boundary forces are applied to the solvent atoms inside the zone and serve to contain the reaction zone.

In QUANTA, boundary forces generally are computed from the deformable boundary method of Brooks and Karplus. (See References) These forces are computed in 1-Å increments for solvent spheres 8–25 Å in diameter.

To select and apply these forces, select **Constraint Options** from the **CHARMm** menu. From the **Constraints Options** pull-right menu that opens, select **Stochastic Bdry Settings.** A dialog box opens from which you select options and specify parameters.

Summary

This chapter describes the options in QUANTA used to define and apply various constraints for CHARMm calculations. Access to the tools for defining and applying constraints is through the **CHARMm** menu.

Four kinds of constraints are described:

- Atom constraints — Used to fix or harmonically constrain an atom or set of atoms to their current positions during energy minimization or dynamics calculations.
- Distance constraints — Used to apply a square-well constraining potential between one atom or set of atoms and a second atom or set of atoms to limit the distance between them. Distance constraints are used most frequently to represent interatomic distances determined experimentally by measurement of the nuclear Overhauser effect
- Torsion constraints — Used to apply a harmonic constraint to restrict a torsion angle to a specified value.
- Stochastic boundary constraints — Used to simulate forces arising from average properties of the system outside a specified boundary. In QUANTA, boundary forces generally are computed from the deformable boundary method of Brooks and Karplus.

Initially all constraints are selected and displayed both in the Constraints table and on the screen. The Constraints table contains columns for each constraint parameter. It also includes its own set of menus that can be used to manipulate table data and edit constraints.

References

1. C. L. Brooks III and M. Karplus. 1983. *J. Chem. Phys.* 79, 6312.

3. Defining Geometric Properties

This chapter covers methods to define torsions and other geometric properties that are used for the Conformational Search and the Analysis applications.

Conformational Search uses torsion driving as the primary mechanism for sampling the conformation space accessible to a molecule. Since torsions must be defined before a search can be performed, read this chapter first if you want to use Conformational Search in your modeling work.

When a conformation or group of conformations have been generated, torsions and other geometric properties provide useful measures for filtering and selecting subsets of structures. These tasks are performed in the Analysis application, so this chapter is also relevant as an introduction to Analysis.

Torsion driving in cyclic modeling presents some problems for maintaining closed rings during a search procedure. This chapter includes a section to describe techniques for handling cyclic structures in Conformational Search.

The chapter also describes tools for defining and applying other (non-torsion) geometric properties uses in Analysis including general distance, angle, and dihedral monitors and some specialized methods for defining distances.

For additional information on Conformational Search see Chapters 4 and 5. For additional information on Analysis, see Chapter 6.

Understanding Torsion Manipulations

Torsion angles are primary conformational search variables. The first step in establishing a search procedure is to define torsions in the displayed structures. During the search, these angles are varied to generate new conformations. A special palette selection, **Elastic Bond**, can be used to limit the effects of torsion manipulation on a molecule. In particular, this tool is used to study cyclic structure**s.**

Each torsion is identified by a sequential number, the torsion family name, (for example, tor1) the name of the atoms composing the torsion (for example, C1, C2, C14, C15), and an indicator identifying if the torsion is a backbone or side-chain torsion.

Tools are available for defining, selecting, listing, or saving torsion angles. These tools are accessed by selecting **Torsions** from the Conformational Search palette that is displayed when you select **Conformational Search** from the **Applications** menu. For more information on the Conformational Search palette, see Chapters 4 and 5.

When you select **Torsions** from the Conformational Search palette, the Torsions palette is displayed. Table 11 lists palette selections and provides a brief description of each. When this palette is displayed, the Conformational Search palette is closed. It is automatically redisplayed when you select **Exit Torsions** from the Torsions palette.

Table 11. Torsions Palette

Selection	Description
Define All Torsions	Defines all non-ring torsions in the molecular system.
Use Default Names	Automatically generates torsion names when **Define All Torsions** is employed. This toggle is turned off if **Use Names from Template** is selected.
Use Names from Template	Derives torsion names from a specified template file when **Define All Torsions** is employed. A prompt appears for the name of the torsion template file. This toggle is turned off if **Use Default Names** is selected.
Define Peptide Backbone Torsions	Automatically defines and selects all the backbone torsion angles of a peptide. This tool detects if the peptide is linear or cyclic and displays the information in the Message Line.
Pick Torsions	Allows the interactive selection of atoms to define torsion angles.
Pick Torsion Sequences	Allows the interactive selection of atoms to define torsion angles where a sequence of bonded atoms is picked and all the torsions involved in the sequence are defined.
Read Torsions from File	Reads an ASCII file (*filename*.tor) containing torsion angle definitions.
Read Torsion Template	Reads an ASCII file (f*ilename*.trn) containing torsion angle family definitions and defines all the torsions in the molecule belonging to the specified families.
List Torsions	Displays a list of currently defined torsions and their values for the current conformation. This list is displayed in the Textport.

Table 11. Torsions Palette (Continued)

Selection	Description
Save Torsions	Saves the atoms and torsion name defining each torsion to a torsions definition file *filename*.tor).
Save Torsion Template	Saves the torsion family names of all defined torsions to a torsion template file (*filename*.trn).
Select Torsions	Selects a subset of defined torsions. The selected torsions are used as variables in a search procedure. This selection brings up a new palette used to make the torsion selection.
Clear All Torsions	Clears all torsions specified during the current session.
Elastic Bond	Restricts the effects of rotation about a bond to a localized region of a molecule. The connectivity of each elastic bond is broken during the torsion perturbation phase of a search procedure, but the energy term for the bond is retained. The bond's energy term closes the ring during energy minimization. See *Dealing with Cyclic Structures* on page 54.
List Elastic Bonds	Displays a list of defined elastic bonds for the current conformation. This list is displayed in the textport.
Exit Torsions	Closes the Torsions palette and returns to Conformational Search.

Defining Torsion Angles

Torsions defined in Conformational Search are used both to modify and analyze structures. Since they are used to modify conformations, they must be defined by four connected atoms. If you want to use a more general (non-driveable) dihedral angle in analysis, see the information on Geometry Monitors under *Defining Other Geometric Properties* in this chapter.

Define All Torsions is the selection in the Torsions palette used to define non-ring torsions in a molecular system. It provides the simplest way to define torsions about all rotatable bonds in molecules. When you use this selection, there are two ways to name torsions. If you select **Use Default Names** from the Torsions palette, **Define All Torsions** assigns default names for the torsions.

If you select **Use Names from Template**, the **Define All Torsions** function prompts for the name of an existing torsion template file. In this case, whenever a defined torsion has the same four atom names defined as a torsion family in the specified torsion template file, the family name from

the file is assigned to the torsion. If such a torsion is not found in the file, a default name is assigned to that torsion.

Using **Define All Torsions** is the fastest way to define rotatable torsions, but it may not always be the best way. This selection defines *all* torsions, but, in some cases, it may be desirable to define only some of the torsions.

In addition, the specification of a torsion angle about the bond B-C requires the identification of four connected atoms, A-B-C-D. For a given bond B-C, there may be several ways of measuring the torsion value depending on the choice of the pendant (rotating) atoms A and D. When you use **Use Default Names**, there is no control over the choice of atoms A and D for a rotation about the bond B-C.

Interactively Defining Torsion Angles

The **Pick Torsions** selection in the Torsions palette defines torsions by enabling interactive selection of four connected atoms from the viewing area. When this tool is selected, the Pick Torsions palette is displayed, providing tools that aid in defining torsions.

If torsions are already defined when **Pick Torsions** is selected, a dialog box is displayed. This box offers the option of adding to the existing variable torsions list or canceling all previously defined torsions and starting a new variable torsions list.

Groups of four atoms are picked interactively until all desired torsions are defined. **Undo** removes the most recently picked atom from the torsion definition or, after completing a four-atom sequence, **Undo** cancels the most recently defined torsion angle.

When four atoms are selected to define a torsion, a dialog box is displayed for entering a prefix name to assign the torsion. Assigning a meaningful name to a torsion (for example, alpha or omega) is useful for identifying the torsion at a later time.

After torsion angles are defined, select **Finish** in the Pick Torsions palette. **Finish** cannot be chosen until a four-atom sequence is selected to define a torsion. In contrast, **Quit** removes all torsion definitions specified during the current **Pick Torsions** session, then exits.

Pick Torsion Sequences is a selection similar to **Pick Torsions,** but it makes it possible to pick a sequence of four or more connected atoms from which a set of torsions is defined at one time. If *n* connected atoms

are picked, *n*-3 torsions are defined. A dialog box with proposed torsion names is presented. For example, if a sequence of eight connected atoms, A-B-C-D-E-F-G-H, is picked, five torsions angles, A-B-C-D, B-C-D-E, C-D-E-F, D-E-F-G, E-F-G-H, are defined.

The selections **Pick Torsions** and **Pick Torsion Sequences** define only the torsion angles interactively picked. They do not define all the torsions of any given family. The selections are particularly suited to those cases where only a small number of torsion angles need to be defined.

Specifying Torsion Angles and Families

As many as 2,000 torsions can be defined in the Conformational Search and Analysis applications. To enable easy referencing of various torsion angles, a naming convention is employed. The naming system makes it easier to deal with torsion angles belonging to various families.

Each torsion is assigned a name that consists of a user-supplied prefix name and the residue number to which the torsion belongs. The residue number is automatically appended to this prefix to generate a name of the form: name(residue_number). The prefix name can have as many as six characters.

In many molecular systems, identical or nearly identical subunits are repeated, giving rise to repetition of the same type of torsion angle. For example, in a polypeptide chain, the torsions phi, psi, and omega occur repeatedly in the backbone chain. If the prefix names chosen for these angles are phi, psi and omega, a particular torsion angle can be conveniently referred to, for example, as phi (7), psi (12), or omega (3).

All torsions of the same type have the same prefix name and are said to belong to the same torsion family. All torsions belonging to a particular family may be identified by specifying a torsion template. Torsion templates may be generated interactively or read from a torsion template file, *filename*.trn.

A torsion template file is an ASCII file composed of one or more records, with each record defining one torsion family. In the following example, three torsion families are defined and identified as being part of a molecular backbone:

```
phi      C       N      CA      -C      back
psi      N      CA       C       N      back
omega   CA       C       N      CA      back
```

The first field in the record is a six-character field that assigns a symbolic name to the torsion family. The next four fields are six-character fields containing atom names defining the atom sequence of the torsion family. The sixth field is a four-character field designating whether the torsion belongs to the main chain or side chain of a structure This field is relevant only to polymeric systems such as proteins.

For a given torsion family name, it is acceptable to have more than one template declaration in a file. No two torsion angles may have the same family name within the same residue. Also, torsions that are part of a ring system or cyclic structure should not be assigned in a torsion template file.

A library of sample template files for some commonly occurring biopolymers is provided in QUANTA. This library can be accessed using the environment variable $QNT_TORTEMPLT. Templates are available for defining protein, polysaccharide, and DNA torsion angles. It must be emphasized that these are sample template files. For any specific molecule, the torsion angle definitions should be used with care. For instance, make sure that no two torsion angles have the same family name within the same residue. To handle specialized molecules, you are encouraged to construct your own template files.

Saving and Restoring Torsion Angle Definitions

Torsion selections employ two different kinds of ASCII files for saving and retrieving torsion angle definitions:

- .tor files — ASCII files containing torsion angle definitions. A *.tor* file consists of several lines; each line is made up of four integers separated by spaces, with each integer specifying an atom number. Therefore, each line in this file makes up a torsion angle definition. The full name of the torsion angle may be optionally included at the end of each line.
- .trn files — Torsion template files described on page 51.

The following selections in the Conformational Search palette are involved in saving and retrieving torsion angle definitions:

Read Torsions from File reads an existing .tor file and defines these torsion angles. A File Librarian dialog box is displayed listing all torsion definition files in the current directory having the proper filename format. This selection checks the four atom numbers for a correctly defined torsion by employing atom connectivity information. It also checks for duplicate specifications. If two torsion definitions have the same two atom numbers in the middle, the entire torsion definition file is rejected. A message is displayed in the message line stating that the torsion definition file was ignored.

Save Torsions writes the current torsion definitions to an ASCII file. A File Librarian is displayed so that you can enter a base filename for the torsion definition file or select an existing torsion definition file. When a base filename is entered, a .tor file extension is automatically appended to it.

Read Torsion Template reads an ASCII file (*filename*.trn) that defines torsion angle families. A File Librarian displays a list of torsion template files in the directory $QNT_TORTEMPLT. One of these files can be selected, or the directory can be changed to select a user-defined torsion template file. After the file is read, all torsion angles belonging to the specified families are defined.

Save Torsion Template creates a torsion template file (*filename*.trn) corresponding to the torsions currently defined. An output template filename must be specified.

List Torsions displays a list of currently defined torsions and their values for the current conformation. This list is displayed in the Textport.

Selecting Torsions

Select Torsions in the Torsions palette is used to make specific torsion subset selections. When torsions are defined, all the defined torsions are automatically selected. All selected torsions are considered to be variables in any search procedure.

The option of selecting a subset of torsion angles enhances the flexibility of search procedures. For example, the torsion template file $QNT_TORTEMPLT/protein.trn defines all torsions in a peptide or protein. After reading this template file, you can use **Select Torsions** to specify a torsions subset that includes only sidechain torsions of a particular residue

type. This subset becomes the variable torsion list during a search procedure.

The selected torsions list is modified by selections in the Select Torsions palette that is displayed when **Select Torsions** is selected from the Torsions palette.Table 12 lists and briefly describes each selection.

As selections are made, structures displayed in the viewing are modified to show the selected torsions using color 2 (default blue) for selected bonds. Atom coloring is reset when you leave the **Select Torsions** facility.

Include and **Exclude** are mutually exclusive toggles. One of them is active in the Select Torsions palette at all times. Combined with **Include** or **Exclude, All Torsions** includes or excludes all the torsions from the selection list. **All Backbone Torsions** includes or excludes all the backbone torsion angles. **All Sidechain Torsions** includes or excludes all sidechain torsion angles.

Double and **Peptide Torsions** examines the atom types of the central two atoms in the torsion to determine if the bond between them is a double or peptide bond. If there is a bond of either type, selection includes or excludes the torsion angle. This selection is used to deal rapidly with peptide and double bonds during conformational search.

Select a Subset of Torsions allows you to make torsion selection from a dialog box listing all the defined torsions in a structure. This selection method is ideally suited for a small molecule with a few torsion angles.

Make a Custom Selection is primarily used with molecules made up of several residues such as a synthetic polymer or a biopolymer chain. Selecting this tool brings up a dialog box that permits specification of a set of torsions within a residue range, within a set of residue types, and within a set of torsion family types.

List Torsions lists the defined torsions in the textport. Those torsions belonging to the current subset selection are easily identified by the asterisk in the last column of the listing.

Dealing with Cyclic Structures

Four selections and options aid in searching conformations of cyclic structures. Two selections, **Define Peptide Backbone Torsions** and

Table 12. Select Torsions Palette

Selection	Description
Include	Includes subsequently identified torsion angles in the torsions selection subset. Used in conjunction with **All Torsions, All Backbone Torsions,** and **All Side Chain Torsions.**
Exclude	Excludes subsequently identified torsions from the torsions selection subset. Used in conjunction with **All Torsions, All Backbone Torsions,** and **All Side Chain Torsions**.
All Torsions	Includes or excludes all torsions from the current variable selected torsions list.
All Backbone Torsions	Includes or excludes *only* backbone torsions in the variable torsions list. The classification of a torsion as a backbone or side chain is specified in the torsion template file.
All Side Chain Torsions	Includes or excludes *only* side chain torsions in the variable torsions list. The classification of a torsion as a backbone or side chain is specified in the torsion template file.
Double & Peptide Torsions	Includes or excludes double and peptide bond torsions.
Select a Subset of Torsions	Displays a dialog box listing all available torsions and allows selections from this list to be included or excluded to create a subset of torsions.
Make a Custom Selection	Selects a subset of torsions by specifying a residue range, residue types, and torsion family types in a dialog box.
List Torsions	Displays a Textport list of all currently available torsions. An asterisk designates selected torsions.
Quit	Exits the Torsion Selection palette without making any changes in the torsion selections.
Finish	Exits the Torsion Selection palette, saving the torsion subset selections in the variable torsions list.

Elastic Bond, are selections in the Torsions palette. The remaining two, **Initialize Go Scheraga Cyclization** and **Find Go Scheraga Cyclization Type**, are options displayed in a dialog box when **Cyclization** is selected from the Conformational Search palette.

Define Peptide Backbone Torsions automatically defines and selects all backbone torsion angles of a peptide. It detects if the peptide is linear or cyclic, and displays this information in the message line. Cyclization detection is limited to cyclization through the backbone atoms. This selection is the only proper way to pick the backbone torsion angles for cyclic peptides before the Go-Scheraga cyclization method is employed.

Define Peptide Backbone Torsions temporarily opens the cyclic peptide by manipulating the atom connectivity information. It defines the backbone torsions, then resets the connectivity information. This procedure automatically makes one of the backbone torsions elastic and enables the backbone torsions to be correctly manipulated. When both sidechain and mainchain torsions are defined, the program requires selection of this tool before reading or interactively selecting sidechain torsion angles.

Elastic Bond properly defines torsion angles in cyclic structures to allow conformational search procedures to execute. Normally, if an attempt is made to rotate about a torsion defined in a ring system, the entire structure tumbles in space and the conformation remains unchanged. This problem is circumvented by making one or more bonds in the ring temporarily breakable but hiding the break from the force field. For example, if the bond C-D is made elastic in the atom sequence A-B-C-D, a rotation about bond A-B will affect atom C but leaves atoms D and E unchanged. Since C is moved and D is not, the bond C-D will be stretched relative to its equilibrium length. However, the connectivity break is hidden from the force field so it sees only a stretched bond. Subsequent energy minimization is used to restore the bond it its equilibrium length.

When you select **Elastic Bond** from the Torsions palette, the bond you choose flashes momentarily when it is selected. Information about the bond is printed in the Textport. **Elastic Bond** must be selected each time a bond is picked. When **Elastic Bond** is selected, existing torsion definitions are automatically cleared. Therefore, torsions must be defined after elastic bonds are designated.

If the molecule under investigation is a cyclic peptide, and **Define Peptide Backbone Torsions** is selected, an elastic bond is automatically defined.

In addition to torsion manipulations in cyclic molecules, **Elastic Bond** can be used in non-cyclic situations to confine deformations to a specific region of the molecule.

Since atom connectivity lists are used to define bonds for CHARMm energy calculations, calculate an initial energy before creating an elastic bonds.

The **Initialize Go Scheraga Cyclization** option sets up the Go-Scheraga cyclization procedure. **Define Peptide Backbone Torsions** must be used to detect a cyclic structure before the **Initialize Go Scheraga Cyclization** option is employed.

Initialize Go Scheraga Cyclization establishes the Go-Scheraga cyclization solution type. See citation 1 in the *References* section for a description of this technique. The selection is only available when **Define Peptide Backbone Torsions** detects a peptide as being cyclic.

The conditions for cyclization are satisfied when six dependent backbone torsion angles are suitably adjusted as other independent backbone torsion angles vary during a search procedure. The six dependent torsion angles are the last three phi-psi pairs in a peptide sequence.

If the independent torsion angles are given a specific set of values, cyclization may or may not be achieved, depending on how values for the six dependent torsion angles are found to satisfy the cyclization condition. If no solutions are found for the given independent torsion angle values, a cyclization failure is reported. In contrast, there may be multiple solutions to close the ring for the same independent torsion angle values.

The algebra used for the cyclization solution necessitate entering two-integer solution types. There are four of these types, referred to as [1,1], [1,2], [2,1], and [2,2]. The solution type is identified in the Textport.

Only torsions are modified when cyclization type is determined. **Initialize Go Scheraga Cyclization** does not vary bond lengths or bond angles during cyclization attempts. This tool assumes the conformation in the viewing area is cyclic.

The method of solving for a cyclization condition is iterative. There are two solution-seeking methods imbedded in the procedure: a global solution and a local solution

The global solution searches for all solutions for the six dependent angles for a given set of independent torsion angle values. The local solution starts from the values of the six dependent torsion angles of the previous cyclic structure, then varies each independent torsion angle iteratively to find the next set of values.

Go-Scheraga cyclization is especially useful whenever small changes in the independent torsion angles are made on an already perfectly cyclized structure. The local solution in most cases involves small random perturbations of torsion angles in the search procedure. In the Boltzmann Jump search procedure, failure after several local solution attempts automatically causes a global solution attempt. If successful, the cyclization type change is displayed in the textport.

Find Go Scheraga Cyclization Type reports the Go-Scheraga solution type in the Textport. This option will work only if **Initialize Go Scheraga Cyclization** has been used to determine the cyclization solution types.

Defining Other Geometric Properties

Torsion definition must be carried out prior to conformational search because torsion driving is used to alter conformations. Other geometry properties such as interatomic distances, additional torsions, and general dihedral angle monitors can be defined either during a search or from within the Analysis application. Use of these properties is described below.

To define specific interatomic distances in a displayed structure, select **Interatomic Distances** from the Conformational Search palette. A dialog box opens offering distance handling options.

When you select **Pick Distances** from the dialog box, the Interatomic Distances palette is displayed. Table 13 lists and briefly describes palette selections. Up to 200 distances can be defined.

Table 13. Interatomic Distances Palette

Selection	Description
Pick Distances	Defines specific distances using atom picking.
End Picking Atoms	Ends the distance selection process by ending atom picking.
Ignore Hydrogens	Excludes any subsequently specified interatomic distances that contain a hydrogen atom.
Only C-alpha Atoms	Includes only subsequently specified interatomic distances between C-alpha atoms.
All Distances	Defines all pair-wise distances in a structure.
Atom<>Atom	Defines distances between interactively selected atom pairs.
Res<>Res	Defines distances between all atom pairs in selected residues.
Finish	Exits the palette, saving the distance definitions.

Use **Finish** to exit the palette. Confirm distance definitions by again selecting **Interatomic Distances** from the Conformational Search pal-

ette. From the dialog box, select **List Distances.** Distances are listed in the textport.

Read Distances reads a previously defined set of distances from a distance file *(filename*.dis) created and saved during a previous session.

Save Distances in the dialog box saves a set of defined distances to a file. The file can be read during subsequent sessions. A distance file extension is automatically appended to the base filename to generate a file named *filename*.dis.

You can access the CHARMm constraints facility from the Interatomic Distances dialog box by selecting **Constraints Table Distances.** The constraints facility provides a mechanism for defining or importing tables of distance constraints. You can extract previously defined tables and use them in your analysis. For more information on this facility, see Chapter 2.

In the Geometry palette, available at the modeling level, you can select **Distance, Bond, Angle,** and **Dihedral Monitors** to define and monitor these parameters. Defined monitors can be carried into analysis and used in the same way as other geometry measures. **Dihedral Monitors** allows you to define general dihedrals composed of any four atoms (connected or not).

Other properties that depend on geometry or internal coordinates are defined using **Define Property** in the Conformational Search palette. When you make this selection, a dialog box is displayed with the options:

Radius of Gyration
Dipole Moment
Number of Hydrogen Bonds
Fractional Free Volume

There properties can be calculated for each conformation during a search procedure. They can be analyzed using the Analysis application.

Sample Procedure — Defining Torsion Angles

Complete the following exercise to become familiar with the procedures for defining torsion angles. This exercise uses *search1.msf* created in Chapter 2 of *QUANTA Generating and Displaying Molecules.* If you have not completed Chapter 2, do so before proceeding.

1. Open *search1.msf*.

Display the **File** menu. Select the **Open** function. A File Librarian dialog box is displayed.

Select search1.msf from the scrolling list.

Select the **Open** button. The structure search1 is displayed in the viewing area.

2. Start Conformational Search.

Display the **Applications** menu. Select **Conformational Search** and the Conformational Search palette replaces the Modeling palette.

The Geometry palette remains displayed. The textport reports the following information about the displayed structure, search1.msf:

```
   Number of Fragments = 1
Number of Ring Bonds= 6
No of rotatable bonds= 6
```

3. Display atom labels for all atoms name.

Display the **Draw** menu. Select **Label Atoms**.

From the pull-right menu that opens, select **Selection Tools**. The Label Atoms and Label Components and Utilities palettes are displayed.

From the Label Components and Utilities palette, select **Atom Name, Residue ID,** and **Show Labels.**

From the Label Atoms palette, select **Include** and **All Atoms.** A label containing the atom name is displayed for all atoms. These labels permit identification of the atoms composing the available torsions.

From the Label Atoms palette, select **Finish.** The Conformational Search palette is redisplayed

4. Define torsions in the displayed structure.

From the Conformational Search palette, select **Torsions.** The Torsions palette is displayed.

From the Torsions palette, select **Define All Torsions.** The command line displays:

```
No of Torsions= 6 Families=6
```

Select **List Torsions** and the following torsion definitions are reported in the textport:

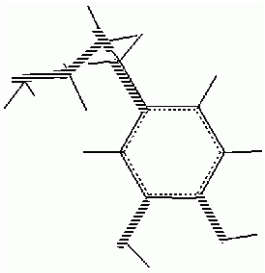
```
1    1:tor1 [PHEN1] C1C2C14C15 -77.9 back *
2    2:tor2 [PHEN1] C3C4O9H131 179.5 back *
3    3:tor3 [PHEN1] C4C5O11H121 -147 back *
4    4:tor4 [PROP2] C2C14C15C161 -63 back *
5    5:tor5 [PROP2] C14C15C16N211 -60 back *
6    6:tor6 [PROP2] C15C16N21H221 179.9 back*
```

Actual atom names may differ, depending on the sequence in which bonds and atoms were placed when the structure was built.

5. Specify a torsion subset.

From the Torsions palette, select **Select Torsions**. The Torsion Selection palette is displayed.

Selected torsion bonds in the displayed structure are colored blue and all other bonds are colored red.



Torsion Bonds in search1

The message line reads:

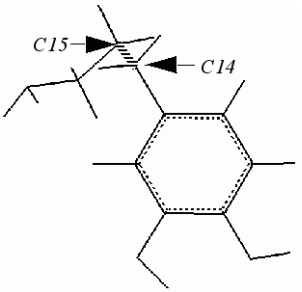
```
Torsion Selection: INCLUDE on 6 Torsns SLCTD out of 6.
```

From the Torsion Selection palette, select **Include** and **Select a Subset of Torsions.** A dialog box is displayed offering options for the selection of one torsion from all torsions currently available.

Select the options:

Include
and **Exclude all others**
4 tor4(2) C2-C14-C15-C16

Select the **OK** button. The dialog box is cleared from the screen. The torsion named tor4(2), comprised of the bond between the carbons that are alpha and beta to the *para* ring carbon, is selected. This bond remains displayed in blue and all others bonds are displayed in red.



Selected Bond in Search1

The message line reads:

```
Torsion Selection: INCLUDE on 1 Torsns SLCTD out of 6
```

From the **Torsion Selection** palette, select **Finish.** The palette is no longer displayed. The search1 structure is redisplayed in the original colors. The message line reads:

```
1 of the 6 torsions selected
```

From the Torsions palette, select **Exit Torsions.** The palette is removed from the screen. Defining torsions for the search procedure is completed.

Summary

Conformational Search is an application that is used to explore the conformational space of a molecular system. In a search, torsion angles are modified to generate various conformations and each conformation is processed according to criteria you define. In addition, when two or more molecules make up the system under study, spatial orientations of one molecule with respect to the other can be explored.

Torsion angles are the primary conformational search variables. The first step in establishing a search procedure is to define torsions in the displayed structures. During the search, these angles are varied to generate new conformations.

Torsions defined in Conformational Search are used both to modify and analyze structures. Since they are used to modify conformations, definition must be carried out prior to conformational search, and all torsions must be defined by four connected atoms.

Define All Torsions in the Conformational Search palette is the fastest way to define rotatable torsion. **Pick Torsions** is also available to define torsions by interactive selection of atoms.

Any torsions that are defined are automatically selected. All selected torsions are considered to be variables in a search procedure. You may limit selected torsions to a subset using **Select Torsions** to open a selection palette. The opportunity to select a subset of torsion angles enhances the flexibility of search procedures.

Tools are available for defining, selecting, listing or saving torsion angles. Up to 2000 torsions can be defined. To enable easy referencing of various torsion angles, a naming convention is employed. The naming convention makes it easier to deal with torsion angles that belong to various families.

Torsion selections employ two different kinds of ASCII files for saving and retrieving torsion angle definitions:

- .tor files — ASCII files containing torsion angle definitions.
- .trn files — Torsion template files.

Four selections and options aid in searching conformations of cyclic structures: **Elastic Bond** and **Define Peptide Backbone Torsions** from the Torsions palette, **and Initialize Go Scheraga Cyclization** and **Find Go Scheraga Cyclization Type** from the Cyclization dialog box.

Elastic Bond properly defines torsion angles in cyclic structures to allow conformational search procedures to execute. One or more bonds in the ring becomes temporarily breakable. The break is hidden from the force field so only a stretched bond is seen. Subsequent energy minimization is used to restore the bond to its equilibrium length.

Define Peptide Backbone Torsions automatically defines and selects all backbone torsion angles of a peptide. It detects if the peptide is linear or cyclic. Cyclization detection is limited to cyclization through the back-

bone atoms. After backbone torsion angles are selected for cyclic peptides, the Go-Scheraga method is employed in conformational searching.

The **Initialize Go Scheraga Cyclization** option sets up the Go-Scheraga cyclization procedure. **Define Peptide Backbone Torsions** must be used to detect a cyclic structure before the **Initialize Go Scheraga Cyclization** option is employed. **Initialize Go Scheraga Cyclization** establishes the Go-Scheraga cyclization solution type.

In addition to torsions, other geometry properties such as interatomic distances, additional torsions, and general dihedral angle monitors can be defined either during a search or from within the Analysis application.

Interatomic Distances from the Conformational Search palette is selected to define specific interatomic distances in a displayed structure, A dialog box opens offering distance handling options. The CHARMm constraints facility can also be accessed from this dialog box.

Using the Geometry palette, you can define and monitor distance, bond, and dihedral parameters. These monitors can be carried into Analysis and used in the same way as other geometry measures. **Dihedral Monitors** allows you to define general dihedrals composed of any four atoms (connected or not).

Other definable properties include Radius of Gyration, Dipole Moment, Number of Hydrogen Bonds, and Fractional Free Volume. They are defined using **Define Property** in the Conformational Search palette. There properties can be calculated for each conformation during a search procedure. They also can be analyzed using the Analysis application.

References

1. Nobuhiro Go and Harold A. Scheraga. 1970. *Macromolecules* 3:178.

4. Understanding Search Methods

This chapter describes the various search methods available in the QUANTA Conformational Search application. It is a continuation of information on Conformational Search begun in Chapter 3, *Defining Geometric Properties.* Read this chapter to get information for planning your conformational search. Then continue with Chapter 5, *Performing a Search*, for specific procedural information and practice exercises.

Conformational search procedures explore conformational space by varying torsion angles in one or more molecular structures or by varying the relative displacements and orientations of several molecular structures. Therefore, search procedures are accessible only when one or more structures for which a CHARM energy can be calculated are displayed in the viewing areas, and after torsions are defined.

The Conformational Search application provides a variety of search procedures for exploring the conformational space of a molecular system. Search procedures may be broadly classified into two categories:

Systematic (deterministic) search procedures

- Grid Scan
- Custom Search
- Cyclic Modeling

Stochastic (probabilistic) search procedures

- Random Sampling
- Random Minimization
- Boltzmann Jump
- Hybrid Jump
- Custom RIS Search
- RIS Random Sampling
- Filter Search
- Peptide Flip

- Loop Modeling

An exhaustive systematic search of the entire conformational space is a very time consuming process for most flexible molecules. The size of the conformational space to be searched increases dramatically with the number of rotatable torsion angles. A thorough search of conformational space can be done in a reasonable length of computer time only for molecules with a small number of rotatable bonds.

There are two factors involved in the increased complexity of a search as the size of a molecule increases:

- The number of variable torsion angles generally increases. If M_i states have to be considered for the i-th torsion angle, then the total number of states for the molecule with n torsion angles is the product $M_1 \times M_2 \times ....\times M_{n-1} \times M_n$. With increased number of torsion angles, the total number of conformational states to be considered increases drastically.
- The resolution with which each torsion angle needs to be scanned also generally increases. For larger molecules, a variation of a torsion angle produces larger Cartesian displacements of many atoms. A larger number of states (M_i) have to be considered for the i^{th} bond as the molecular size is increased.

Both these factors compound to enhance the difficulty of locating minimum energy conformations of a large molecule. A systematic search can be carried out in reasonable length of time only for very small molecules with four or fewer variable torsion angles. In the case of larger systems, either a systematic search is more local, in the sense that the search is carried out only in part of the conformational space, or a stochastic search is used to sample the conformational space.

Using a Systematic Search Method

There are two ways to perform a systematic search, Grid Scan and Custom Search. In a Grid Scan search, each specified torsion angle is varied over a grid of equally spaced values. If more than one torsion angle is involved, the variation of the torsion angles are nested. If there are two angles α and β, for a given value of α, angle β assumes a grid of values. If β is the faster torsion angle, the β loop is inside the α loop (see case 1). Although the application is capable of handling up to 10 grid torsions, it

is impractical in most cases to employ grid scan for more than four torsion angles.

In a Custom Search, torsion angles are assigned specific values. These values do not need to be equally spaced. This is an advantage in those cases where favorable states of a torsion angle are known from previous modeling studies and the intent is to restrict the systematic search to these values. A further advantage of Custom Search is that it can handle, if so desired, simultaneous changes in several torsion angles. As in Grid Scan, these changes may also be nested.

Using a Stochastic Search Method

By definition, stochastic procedures involve random perturbations to a conformation as a method of moving in the conformational space. The nature of the random moves and the criteria employed for accepting the results of the random moves vary from one procedure to another.

In Boltzmann Jump, the torsion angles of a molecule are randomly altered within a specified angular window. In the Peptide Flip procedure, a peptide unit is rotated about the C^{α}_{i} - C^{α}_{i+1} line. The angle of rotation can be a random value, and the peptide units to be rotated can be chosen randomly from the polypeptide sequence. When there is more than one molecular fragment in the system, the random moves may also involve random reorientation of one fragment with respect to another.

In some stochastic procedures, after each random move a selection criterion is employed to either select or reject the move. The most commonly employed selection criterion is the Metropolis criterion (see citation 1 in the References section):

- If the move results in a decrease of energy, the move (perturbed conformation) is selected.
- If energy increases, the value of $f = e\ (-\Delta E/RT)$ is computed (where R is the Boltzmann gas constant and T is the temperature in K), and a random number from 0 to 1 is generated. The move is selected if the random number generated is less than f.

At higher temperatures, larger upward jumps in energy are likely to be accepted. If the temperature is taken to be zero degrees, no upward jumps in energy are allowed. This special case is employed by the procedure Random Minimization.

Performing a Conformational Search

The general procedure for performing a conformational search includes the following steps:

1. Define torsions and other geometric quantities.
2. Specify a torsion subset (optional).
3. Select the tag generation method (optional).
4. Choose a search procedure.
5. Specify search parameters.
6. Initiate the search.
7. Provide a filename, supply the tag prefix and number, if prompted.

With these steps completed, the search executes. After the procedure is complete, you have several choices for next steps. You can select:

- **Do Search** to repeat the procedure
- **Setup Search** to perform another search procedure
- **Analysis** to analyze conformations generated by the search procedure
- **Exit Conformational Search** to return to the Modeling palette.

Conformations generated during a search procedure are saved in binary search files, (*filename*.csr). Search files contain coordinate data, identifying tags, and optionally, the potential energy for each conformation generated by a search procedure.

The Conformational Search palette, displayed when you select **Conformational Search** from the **Applications** menu, includes selections for defining torsions and other geometric properties, selecting a search method, specifying search parameters, and initiating search procedures. The Conformational Search palette also allows you to transfer to the Analysis application without exiting from Conformational Search. Table 14 lists and briefly describes the selections in this palette.

Setup Search in the Conformational Search palette provides access to search procedures and search procedure parameters. When **Setup Search** is selected, the palette changes by making all the search procedures selectable.

Table 14. Conformational Search Palette

Selection	Description
Torsions...	Displays the Torsions palette that contains selections for defining and selecting torsions.
Interatomic Distances...	Provides options for working with interatomic distances including **Pick Distances, Read Distances, Save Distances,** and **List Distances.**
Dummy Atoms...	Provides options for working with dummy atoms including **Define Dummy Atoms, Read Dummy Atoms,** and **Save Dummy Atoms**.
Define Properties	Provides options for working with properties including **Radius of Gyration, Dipole Moment,** and **Fractional Free Volume**.
Cyclization...	Provides options for working with cyclic structures including **Initialize Go Scheraga Cyclization** and **Find Go Scheraga Cyclization Type**.
Rigid Body Options...	Provides options for carrying out manipulations on rigid structures during search procedures. This option makes it possible to execute a search with more than one molecular fragment
Setup Search...	Activates all search setup selections in the palette and initiates the setup of the selected search procedure.
Do Search	Executes the selected search procedure.
Grid Scan	Generates conformations systematically by varying torsions over a grid of equally spaced values.
Random Sampling	Generates conformations by randomly changing torsion angle variables within a specified torsion window.
Filter Search	Applies filters to randomly generate conformations, eliminating those conformations that do not meet specified geometric or energetic criterion.
Random Minimization	Locates a minimum energy structure by accepting only those random perturbations that result in lower energy.
Boltzmann Jump	Uses the Metropolis algorithm to explore conformational space for energy minima. Random perturbation is performed in torsional space while quenching is performed in Cartesian space.
Hybrid Jump	Searches conformational space by varying two sets of torsion variables. The first set is varied using the Boltzmann jump perturbation; the second set is varied using RIS sampling.
Custom Search	Reads a user-defined loop setup file (*filename.LSF*) that defines torsion variables for systematically searching conformational space.
Custom RIS	Reads a user-defined random setup file (*filename.RSF)* that defines states and statistical weights for each torsion family. New conformations are generated randomly.
RIS Sampling	Assigns torsion values randomly to energetically preferred states (180°, -60°, and 60°) or user-defined states, according to statistical weighting factors supplied for each state.

Table 14. Conformational Search Palette (Continued)

Selection	Description
Peptide Flip	Reorients peptide units randomly in a peptide using sclerotizations about inter-alpha carbon atom lines. Using such flips followed by energy minimization, searches conformational space of cyclic and linear peptides.
Cyclic Modeling	Provides an interactive method of making torsion angle changes in any cyclic structure using dihedral constraints and energy minimization.
Loop Modeling	Modifies peptide loops in polypeptide chains.
Show Search Setup	Reports the current status of the search parameters in the Textport.
Set Background Search...	Records a search procedure for use in non-graphic QUANTA.
Analysis	Exits the Conformational Search application and starts the Analysis application. If a search procedure has just been completed, the current search file is automatically used as the file under analysis, enabling analysis of conformations contained in that file.
Exit Conformational Search	Exits the Conformational Search application.

When a search procedure is selected, dialog boxes are displayed to set appropriate parameters for the procedure, then the palette changes to highlight the particular search procedure. All the remaining search procedures are grayed out and not selectable. **Do Search** becomes selectable.

When **Show Search Setup** is chosen, a list of parameters for the search procedures that have default parameter settings are displayed in the textport.

A search is executed by selecting **Do Search** from the Conformational Search palette. A File Librarian dialog box is displayed for you to specify an output search file where the conformations calculated during the search procedure are stored. All search files have the file extension .csr automatically appended to the base filename.

A search procedure can be interrupted by clicking any mouse button. A dialog box offers the option to continue or terminate the search.

Setting Up a Background Search

All Conformational Search procedures can be run using non-graphical QUANTA by typing the appropriate commands. **Set Background**

Search in the Conformational Search palette facilitates the setup and execution of a non-graphical search. The selection turns on the command record option that automatically records all steps taken to set up and run a search including defining torsions.

When **Do Search** is selected, the recorded commands are manipulated so that they can be run in a fresh directory. A new directory containing appropriate files is created. In some cases, it may be necessary to manually copy some files. In addition, if a constraints table is to be read and applied during the search, the command record must be edited to include commands to read the table and activate the constraints.

QUANTA is started in non-graphical mode and the recorded search protocol is executed. The current graphical QUANTA session can continue without interference while the non-graphical procedure runs.

Complex search procedures that require a lot of CHARMm processing can generate very large CHARMM.LOG files that may exceed available disc space. To address this issue, select **Initialization Options** from the **CHARMm** menu. In the **Print Level** field, enter an integer smaller than the default value 5 to generate less output for the log file. To suppress nearly all output, enter zero.

Alternatively, the log file can be suppressed altogether by selecting the option, **User-specified file**, under CHARMm log destination. Use the filename /dev/null.

Performing a Search on More than One Structure

All conformational search procedures sample conformational space by manipulating specified torsions. In systems comprised of more than one molecular fragment, manipulations of internal coordinates may not be sufficient to explore conformational space.

The selection **Rigid Body Manipulation** in the Conformational Search palette rotates a structure's coordinates about a randomly oriented vector placed at the center of mass. The extent of rotation is determined by random selection within the specified angular window. After rotation, each fragment is translated along a direction chosen at random by an amount specified by the translation window.

One or more structures may be held rigid during the search. All fragments to be held rigid are loaded into the same MSF. That MSF is designated as the first MSF encountered by the system and all fragments in it are fixed.

Remaining fragments held in other MSFs are manipulated during the search procedure.

Using Tags

Tags are unique names that identify conformations. They are assigned during a search procedure just prior to initiating the search. The general format of a tag is:

```
prefix[code]:number
```

where prefix is a character string, code designates the search procedure, and number is the rank number (the position in the search file at the time the structure was created by a search procedure). Generally, many conformations are saved into a search file by a single search procedure. The tag format enables the generation of unique tags for each structure saved into the search file.

When a conformation is displayed in either the Conformational Search or Analysis application, the assigned tag is displayed in the upper left corner of the viewing area. Tags also provide a mechanism for identifying the search techniques that generate a set of conformations

When you select **Do Search** from the Conformational Search palette, a Define Output Search File dialog box is opened with options for defining tag specifications. The application automatically generates a tag containing a prefix, a code, and a starting number for the suffix. The default tag prefix is determined by a set of rules based on the origin of the starting structure at the time the search procedure is started.

The rules for generating the tag prefix are:

- When the starting structure is from a search file, the tag of the starting structure becomes the default tag prefix for the search procedure.
- When the starting structure is from an MSF, the tag prefix consists of the MSF filename (*filename*.msf).
- When the starting structure is from a dynamics trajectory, the tag prefix is the filename of the dynamics trajectory (*filename*.DCD).

A two-letter search key is inserted in the tag if the option **Insert Search Key into Tag** is selected in the dialog box. Search keys are two-character abbreviations of a search procedure enclosed in brackets. Table 15 lists the keys and the procedures that they identify.

Table 15. Search Keys

Key	Procedure That Generated File
[gs]	Grid Scan Search
[rs]	Random Sampling Search
[rm]	Random Minimization Search
[ri]	RIS Sampling Search
[fs]	Filtered Search
[bj]	Boltzmann Jump Search
[hj]	Hybrid Jump Search
[cs]	Custom Search
[cr]	Custom RIS Search
[hl]	Helix Modeling (generated by the Helix Modeling application)
[cy]	Cyclic Modeling
[pf]	Peptide Flip
[ms]	Torsional Flexible Fitting (generated by Molecular Similarity application)
[ag]	Amorphous Builder application
[??]	Any other unspecified procedures

As an example of automatic tag generation, consider a search file generated by the Random Sampling Search method. The fifth conformation in a search file generated by this search procedure will automatically have the tag: *filename*.msf*[*rs*]*:5. If this conformation is then used for a Grid Scan search, the first conformation generated by the Grid Scan search is assigned the tag *filename*.msf*[*rs*]*:5*[*gs*]:*1.

Understanding the Grid Scan Search

The **Grid Scan** selection in the Conformational Search palette initiates a grid scan search that generates conformations systematically by varying specified torsion angles over a grid of equally spaced values. The number of torsion angles that can be employed in a grid search must be less than or equal to 10. Not more than four torsions are recommended to keep search time reasonable. If more than 10 torsions are defined, a subset of 10 or less must be specified with the **Select Torsions** tool.

A dialog box is displayed for entering the initial value, a final value, and a torsion increment for each torsion variable. In this box, the numbers entered can be absolute values or values relative to the torsion angles of the starting reference conformation. For example, consider a torsion angle with the initial value of 160^{o}. If the values -30^{o}, $30°$, 10^{o} are set in the Grid Scan Torsion Angle dialog box, and if the relative values are specified in the dialog box, the angle will vary from 130^{o} to 190^{o} in steps of 10^{o}. If absolute values are specified in the dialog box, the angle varies from - 30^{o} to 30^{o} in steps of 10^{o}.

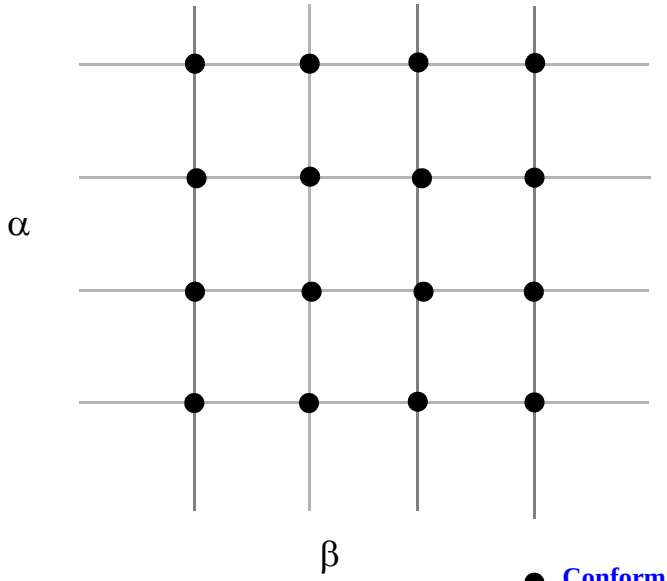
A second dialog box is displayed at the start of the grid scan procedure to specify the grid scan processing options. Any combination of these options is selectable. The options are used to specify how the conformations generated on the grid are to be processed. Table 15 lists and briefly describes the options.

Table 16. Grid Scan Setup Dialog Box

Option	Description
Calculate CHARMm energy for each structure	Calculates the potential energy of each conformer.
CHARMm minimization for each structure	Minimizes each structure before generating another conformation.
Constrain Torsions during Minimization	Constrains the grid torsion to the grid point using a penalty energy function. All other torsion angles are allowed to relax. The precision with which the conformation is fixed at the grid point depends on how strained the initial conformation is and how far energy minimization is carried out. The extent of energy minimization (the number of steps and the convergence criteria) depends on minimization options setup using the **CHARMm** menu.
Keep Minimized Conformations (do no reset)	Uses a minimized conformation to apply subsequent modifications. Adjustments are worked into the structure due to energy minimizations. If this option is not selected, all conformations on the grid are obtained by torsional manipulations of the same initial starting structure.
Display Each Structure	Displays each structure as search proceeds.

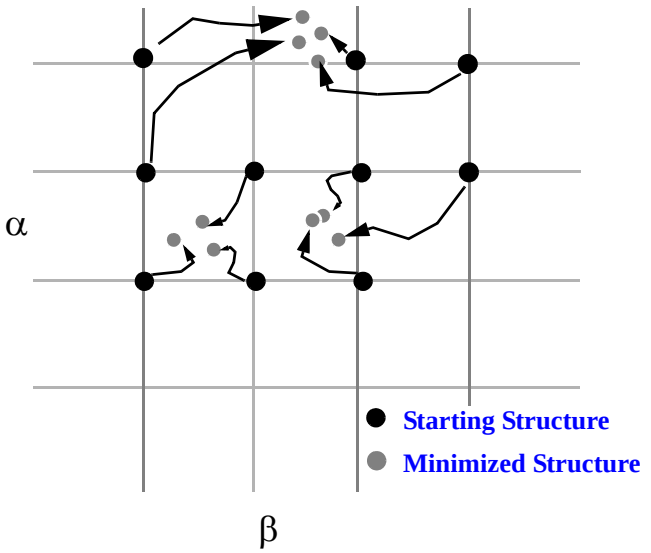
The simplest option is just to generate all the conformations and save them into the output .csr file. The CHARMm energy of each generated conformation can also be calculated by turning on the appropriate toggle in the options dialog box. There may be cases where an energy minimi-

zation on each conformation is desired. For example, consider a two dimensional grid scan (with two grid torsions α and β) as shown below:



The grid scan explores low and high energy regions. If an energy minimization is not performed at each grid point, the resulting energy variation with α and β is a quick way to identify low and high energy regions. But the conformation will not be a minimum energy structure. Selection of the energy minimization option gives a minimum energy structure but increases the computing time.

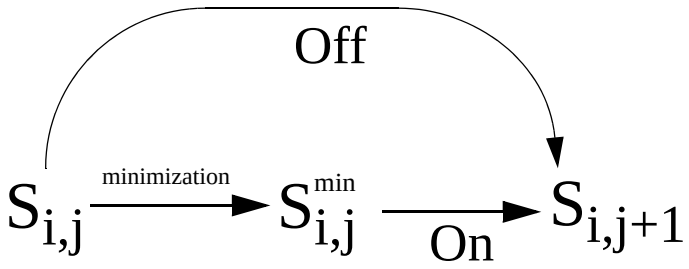
The CHARMm energy minimization option is an unconstrained Cartesian minimization process in which all coordinates are adjusted to obtain a minimum energy conformation. During minimization, the grid torsion angles (grid torsion angles α and β in this example) will tend to wander away from the grid point. As shown below, minimizations from some set of grid points may converge towards one region while other grid points may move towards other regions.



Depending on the grid resolution and the size of the grid, this may be a good way to explore low energy regions of this conformational space. A contour plot (see **Analysis** in the **Applications** menu) obtained from this search file exhibits a distorted contour shape because the drift of the conformation away from the grid points due to energy minimization is ignored by the contouring scheme which computes a contour of a property varying over a set of grid points.

For this reason, it is often necessary to constrain the grid torsion angles (α and β in the diagram) during the energy minimization using the **Constrain Grid Torsions During Minimization** option in the dialog box. If this option is selected, during the CHARMm energy minimization, the grid torsion is constrained to the grid point using a penalty energy function. All other torsion angles are allowed to relax in this process. The precision with which the conformation is fixed at the grid point depends on how strained the initial conformation is and how far energy minimization has been carried out. The extent of energy minimization (the number of steps and the convergence criteria) depends on minimization options setup using the **CHARMm** menu.

With an energy minimization calculation available for each grid conformation comes another interesting question: how does the program go from one grid point (i,j) to the next grid point (i,j+1) as part of the scan? Consider the scheme shown below:

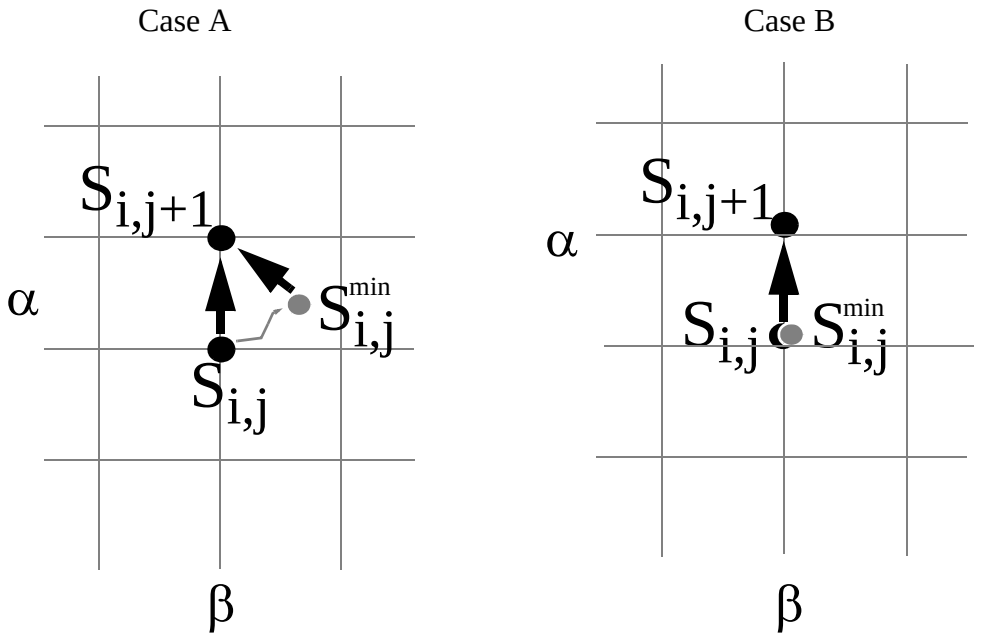


A conformation, $S_{i,j}$, is generated at the grid point (i,j). If an energy minimization is performed (with or without torsional constraints), a new conformation $S_{i,j}^{min}$, is obtained. The minimized conformation is then saved into a .csr file. The (i,j+1) conformation can be generated, by suitable torsional changes, from either $S_{i,j}$ or $S_{i,j}^{min}$. Note that these two starting conformations may differ in bond lengths, bond angles, and in non-grid torsion angles even if the **Constrain Grid Torsions** option was employed.

The program provides a choice: to construct $S_{i,j+1}$ out of $S_{i,j}$ or $S_{i,j}^{min}$. This choice is made by either selecting or deselecting the option **Keep Minimized Conformation**. If this option is not selected, all conformations on the grid are obtained by torsional manipulations of the same initial starting structure. If this option is selected, the conformational changes produced by minimization are carried over to the next state, rather than starting with the original conformation of the structure.

In this case, in scanning through the grid points, adjustments are worked into the structure due to energy minimizations. The resulting structure is used as the reference template from which to generate the subsequent conformations by torsional manipulations.

The following figure illustrates these options:



Display each Structure is an option that enables each structure generated by the grid scan to be displayed as the search procedure progresses. When **Display each Structure** is not selected the procedure is somewhat faster since the display of the generated structures is suppressed.

Understanding the Custom Search

The Custom Search method performs a systematic search, varying an arbitrary number of torsion angles to sample a set of torsion angle values. The values assumed by a torsion angle are not required to be grid values at equal intervals.

Custom Search also permits a set of torsion angles to be varied simultaneously. The scope of the search (the torsion angles to be varied and the values to be assigned) is specified in a loop setup file. Prior to selecting **Custom Search,** a loop setup file is created to describe the scope of the search.

The loop setup file (*filename*.lsf) is a pre-existing user-defined ASCII file. The file consists of several blocks of data, each block specifying a loop in the search. Even though blocks are listed in sequential order, they specify a nested set of loops in the search. The first block is the outermost loop, and the last block is the innermost loop. Therefore, the torsions specified in the last block will be the fastest varying angles.

Each block can consist of several records (lines). The first record gives the number of torsion angles to vary simultaneously in the loop. The second record gives the angle numbers or names of these torsions. The third and subsequent records list the set of values for these torsion angles. The last set of values is followed by an end of block signal (a record containing the number 999.0). One column is devoted to each torsion angle.

For example, this block of a loop setup file:

Data	Description
1	total number of torsions
22	torsion number/torsion name
180.0	torsion value
-160.0	torsion value
-145.0	torsion value
55.0	torsion value
60.0	torsion value
65.0	torsion value
999.0	end of block

assigns six values to torsion angle number 22: -180.0°, -160.0°, -145.0°, 55.0°, 60.0°, and 65.0°.

In the next example, torsion names are used to assign four backbone conformations to residue number 3:

Data		Description
2		number of torsions
phi(3)	psi(3)	torsions number or name
-60.0	-50.0	torsion value
-65.0	70.0	torsion value
-70.0	120.0	torsion value
60.0	55.0	torsion value
999.0	999.0	end of block

This example assumes the torsion angles of the residue are defined using a torsion template file that includes entries for phi and psi, and the two torsion angles, phi (3) and psi (3), will vary simultaneously.

A maximum of 10 angles can be included in a given specification, and a maximum of 200 sets of values can be assigned in a block. A maximum of 20 specifications (blocks) can be supplied for a given Custom Search.

Understanding Cyclic Modeling

Cyclic Modeling is an interactive method that makes it possible to drive the torsion angles of a cyclic molecule. This procedure provides tools for changing the value of any specific torsion angle in the ring system. It then employs CHARMm energy minimization, with suitable dihedral constraints, to achieve low energy structures with the desired torsional values.

Steps involved in initiating Cyclic Modeling are:

1. Select **Conformational Search** in the **Applications** menu. Open the Torsions palette by selecting **Torsions** from the Conformational Search palette.
2. Select **Elastic Bond** from the Torsions palette and specify the bond to break by clicking on it. The bond will be highlighted momentarily. For

cyclic peptides, you can bypass the **Pick Torsions** and **Elastic Bonds** steps by selecting **Peptide Backbone Torsions** instead.

3. Define all torsions using **Pick Torsions** from the Torsions palette.
4. Select **Setup Search** and then **Cyclic Modeling**. The Cyclic Modeling palette is displayed. This palette contains tools to modify the conformation of the molecule. Table 17 lists selections and a brief description of each. Make selections from this palette, exit, and return to the Conformational Search palette.
5. Select **Do Search** from the Conformational Search palette and specify an output search filename. A new palette is displayed. The conformation of the molecule may be modified by selecting various tools from this palette.

Since cyclic modeling uses CHARMm energy minimization, set up CHARMm minimization options before running Cyclic Modeling. Also minimize the starting structure before you begin.

Table 17. Cyclic Modeling Palette

Selection	Description
Up	Scrolls up the list of torsion angles if there are more torsion angles than can be accommodated in the space provided in the palette. Toggles with **Down**.
Torsion Angles	Lists all defined torsion angles in the active structures displayed in the viewing area.
Down	Scrolls down the list of torsion angles if there are more torsion angles than can be accommodated in the space provided in the palette.
1.0	Changes torsion angle in one-degree increments.
2.0	Changes torsion angle in two-degree increments.
5.0	Changes torsion angle in five-degree increments.
10.0	Changes torsion angle in ten-degree increments.
Custom Value	Changes torsion angle according to a custom value.
Set Value	Specifies a custom value torsion angle increment.
Torsion Name Value	Changes the value of a specified torsion angle. The change in the value is a constant amount determined by the angular value highlighted and also by the use of **Add** or **Subtract**.
Add	Changes torsion angle by the increment highlighted. **Subtract** is inactive.
Subtract	Changes torsion angle by the decrement highlighted. **Add** is inactive
Cyclize Each Structure	Changes torsion angles and automatically generates an energy minimum to keep the molecule cyclic. When the selection is inactive, each change in torsion angle will not take effect until the next energy minimization.

Table 17. Cyclic Modeling Palette (Continued)

Selection	Description
Energy Minimize	Initiates an energy minimization on the current structure with changes, if any, in the torsion angles that have been specified since the last energy minimization.
Continuous Save to Search File	Automatically saves each new structure into the output search file.
Save to Search File	Saves the displayed structure into the output structure file.
Peptide Flip	Performs a rotation through a specified angle of a segment of specified range of consecutive peptide units about the line connecting the two alpha carbon atoms at the ends of the segment.
Save to MSF	Saves the current structure into an MSF file using the normal method currently in effect in QUANTA for saving structures into MSFs.
Exit Cyclic Modeling	Exits Cyclic Modeling and returns to the Conformational Search palette.

Understanding Random Sampling Search

The Random Sampling search method is a procedure applicable to molecules having a large number of variable torsion angles. Random Sampling randomly changes all defined torsion angles within a predefined angular window. The range of the torsion angle varies during the search procedure and generates new conformations. The search continues for a specified number of steps defined as the Number of Samples. CHARMm energy can be calculated for each randomly altered conformation. CHARMm energy minimization also can be performed. All perturbed conformations are saved to the output search file.

An angular window specification controls the variation in torsion angles for each conformation. If a large angular window value is used, large perturbations are produced. These generate high energy conformations. If a small angular window is used, perturbations are more likely to produce reasonable conformations. However, several cumulative perturbations may be required to significantly migrate from one of part of conformational space to a distant part.

When Random Sampling is selected, a dialog box is displayed to specify Random Sampling parameters. Table 18 lists and briefly describes the options.

Table 18. Random Sampling Setup Dialog Box

Option	Description
Number of Samples	Specifies the number of conformations to generate and write to the output search file.
Torsion Angle Window	Determines the angle each selected torsion can vary during the search.
Calculate CHARMm Energy for Each Structure	Calculates the CHARMm energy for each new conformation.
Minimize Each Structure	Performs a CHARMm minimization of each new conformation.
Cumulative Random Modifications	Specifies that the random modification is to be performed on the previously obtained conformation. Normally all random modifications are made on the same starting structure. However, if this option is set, the nth perturbed or minimized conformation becomes the starting conformation for perturbation number n+1.
Go-Scheraga Cyclization in Peptide Rings	Uses mathematical procedure to maintain closed peptide rings.
Display Each Structure	Displays each new conformation in the viewing area.

Understanding Random Minimization

Random Minimization performs a minimization based on a random search. No upward jumps in energy are permitted. Torsion angles are used as variables. When Random Minimization is selected from the Confor-

mational Search palette, a dialog box is displayed to set the search parameters. Table 19 lists the options and provides a brief description of each.

Table 19. Random Minimization Setup Dialog Box

Option	Description
Number of Trials	Specifies the number of conformations to generate and write to the output search file.
Torsion Angle Window	Determines the angle each selected torsion can vary during the search.
Maximum Number of Failures	Specifies the maximum number of conformations that do not fall within the specified energy parameters.
Go-Scheraga	Uses mathematical procedure to maintain closed rings.
Display Trial Structures	Displays each new conformation in the viewing area.
Display Successful Trials only	Displays only new conformations that meet the specified energy parameters.

When more than one fragment is displayed in the viewing area, the options **Rigid Body Translation Window**, **Rigid Body Angular Window**, and **Perform Rigid Body Manipulations** appear in the dialog box. **Perform Rigid Body Manipulations** translates and rotates the rigid bodies along a randomly oriented vector placed at the center of mass of each rigid body. The magnitude of the translation and rotation is within the prescribed windows. This option is available only when more than one fragment is displayed in the viewing area.

Conformations displayed in the viewing area are randomly altered within the Torsion Angle Window. If a new conformation is of lower energy than the specification, the next perturbation is performed on that conformation. If the new conformation is of higher energy, it is rejected as a failure, and the previous conformation is retained. The procedure is terminated if a specified number (**Maximum Number of Failures**) of consecutive conformations results in high energy conformations.

Understanding the Boltzmann Jump Search

The Boltzmann Jump search method explores conformational space through random perturbation of torsion angles. Boltzmann Jump differs

from Random Sampling in that a Metropolis selection criterion is employed to accept or reject perturbed conformations. See citations 1 and 2 in the References section for information on the Metropolis criterion.

The Boltzmann Jump search is a useful procedure for getting from point A to point B in conformational space using a low energy pathway.

Unlike Random Minimization, in the Boltzmann Jump method, upward jumps in energy are permitted. If an upward jump occurs, a stochastic decision is made to select or reject the new structure.

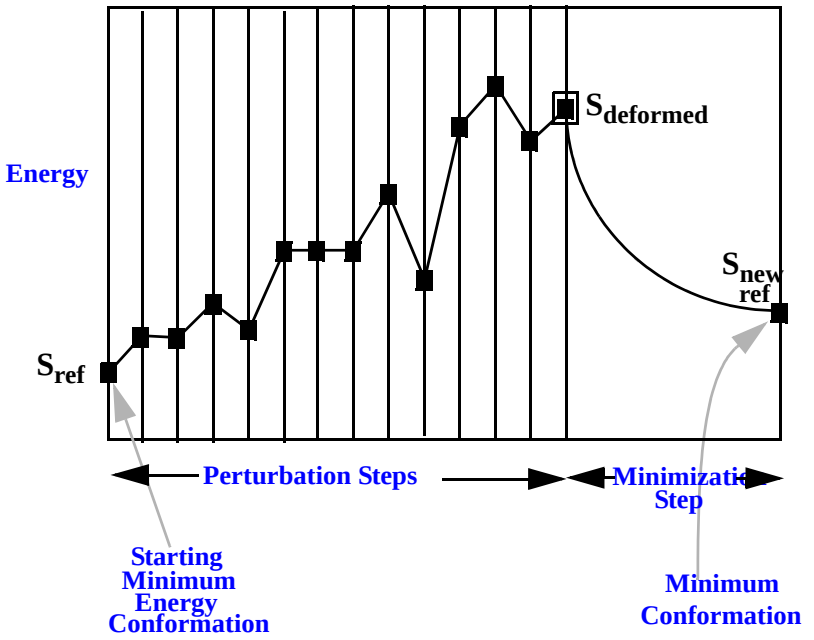
The probability of selecting the new conformation is equal to the Boltzmann factor defined as $e^{(-\Delta E/RT)}$. The chances of making an upward jump in energy are greater at a higher temperature (T) or at a smaller energy difference (ΔE).

Starting with an energy-minimized conformation, a random perturbation is carried out within the specified torsion angle window. If the resulting conformation is of lower energy, that conformation is selected. If the perturbed conformation is of higher energy, a stochastic decision is made to select or reject the new conformation.

Random perturbation, followed by a conditional selection of the perturbed conformation, is continued until the root mean square difference (rmsd) in torsion angles between the current conformation and the starting minimum conformation exceeds the specified maximum rmsd. This causes the thermal perturbation sequence to terminate, and a CHARMm energy minimization is performed on the conformation. This procedure is repeated a specified number of times to generate a collection of low energy conformations for the molecule.

A sequence of random perturbation followed by an energy minimization constitute a Boltzmann Jump cycle. An example of an energy profile during a cycle is illustrated in the following figure:

Energy Trace During One Cycle of Boltzmann Jump Sequences



When **Boltzmann Jump** is selected from the Conformational Search palette, a dialog box is displayed to select parameters for the search procedure. Table 20 lists and briefly describes the options.

Choosing Boltzmann Jump Parameters

There are three parameters that need to be set for a successful execution of the Boltzmann Jump procedure:

- Torsion angle window
- Temperature
- Rms limit

Table 20. Boltzmann Jump Setup Dialog Box

Option	Description
Number of Samples	The number of conformations to generate and write to the output search file.
Temperature (Kelvin)	The value used in the formula, $e^{(-\Delta E/RT)}$, that determines if a conformation is accepted or rejected.
Set Torsion Window	Determines the maximum angle each selected torsion can vary during each perturbation.
Maximum RMS Difference	Determines the conditional selection criteria for termination of a thermal perturbation sequence.
Torsion Space	Specifies the value (degrees in torsion space) used as the cutoff for RMS calculations. Reaching this value causes the thermal perturbation sequence to terminate and a CHARMm energy minimization to be performed.
Cartesian Space	Specifies the value (degrees in Cartesian space) used as the cutoff for RMS calculations. Reaching this value causes the thermal perturbation sequence to terminate and a CHARMm energy minimization to be performed.
Display Each Structure	Displays each new conformation in the viewing area.
Save Minimized Structures Only	Saves only minimized structures as a result of the search. If this option is not selected, all structures accepted during the jump sequence are saved.
Go-Scheraga Cyclization in Peptide Rings	Uses mathematical procedure to maintain closed peptide rings.

The magnitude of the torsion angle window determines the magnitude of the conformational change produced by one perturbation. As this magnitude increases, the chances of producing higher energy conformations increases. At a given temperature, the rate of conformation rejection will increase. If the angular window is decreased, the rate acceptance of conformation by the Metropolis criterion will increase. If too small a value for the window is employed, a large number of perturbations may be required to develop an RMS difference greater then the specified limit.

For a given temperature, it is recommended that trial calculations be performed by gradually increasing the angular window. The highest window value that results in a reasonable number of successful jumps is the optimal value for the window.

The rms limit specified should neither be very small or very large. If it is too small, the perturbation sequence is too short, energy minimization is initiated too soon, and the minimization will most likely bring the confor-

mation back to the starting conformation. If the rms limit is set at too high a value, it may take too many perturbations for the conformation to move very far away from the reference conformation.

Conformation may not change at all if a conformation is unable to develop the specified rms difference. This situation can happen if there is an appreciable energy barrier separating the current class of conformations and the conformations that are farther away. In such a case, a larger rms difference can be obtained only with a higher temperature and possibly a larger angular window. A low value for the rms limit is recommended during trial calculations, followed by iterative increases in the rms limit if the minimization is reached too soon, that is, before a dozen perturbations are performed.

Understanding the Hybrid Jump Search

The Hybrid Jump search method is a variation of the Boltzmann Jump. The procedure searches conformational space by varying two sets of torsion variables. The first set, Selection A, is modified using the Boltzmann Jump random perturbation method. The second set, Selection B, uses RIS Sampling.

When **Hybrid Jump** is selected, the palette changes to accommodate torsion selections for the first set of torsion angles. After you select the first set, the palette changes to accommodate torsion selections for the second set of torsion angles. When the torsion sets are selected, a dialog box displays the parameters for the Hybrid Jump search procedure. Table 21 lists the options and provides a brief description of each.

Table 21. Hybrid Jump Setup Dialog Box

Option	Description
Number of Samples	Specifies the number of samples in the run. Default is 3.
Torsion Window (random)	Specifies a torsion window value. Default is 15.
Temperature (Kelvin)	Specifies the run temperature. Default is 5000 Kelvin.
RMSD Trigger (degrees)	Specifies the RMSD trigger for a search. Default is 5.00.
Options for Torsions Set using RIS Sampling (heading)	
Sample State t,g+, g-	Specifies assignment to a sample state *trans*, *gauche* $^{+}$ or *gauche* -. This is the default selection

Table 21. Hybrid Jump Setup Dialog Box

Option	Description
Exclude g+/g- or g-/g+ sequences	Excludes the specified sequences. This is a default selection.
User Defined Three-State Sampling as below:	Specifies user-defined sampling with the states and weights defined in the next data fields.
States	Specifies user-defined states. Defaults are 60.00, -60.00, and 180.00.
Weights	Specifies user-defined statistical weights. Defaults are 0.25, 0.25, and 0.50.
Display Each Structure	Displays each conformation as it is generated.
Save Minimized Structures Only	Saves only conformations that have been minimized.
Go-Scheraga Cyclization in Peptide Rings	Uses mathematical procedure to maintain closed peptide rings.

Do Search in the Conformational Search palette starts the search. The first set of torsion angles (Selection A) is randomly perturbed within the Torsion Angle Window. The second set (Selection B) is assigned to the rotational isomeric low-energy states or user-defined states. This is done for a specified number of conformations (**Number of Samples**).

Understanding the Custom RIS Search

The Custom RIS Search method randomly samples torsion angles of a given torsion family from a list of possible states with corresponding statistical weights. The states and the weighting factors must be supplied in a pre-existing, user-defined rotational state file (*filename*.rsf).

The random search setup file can have up to 20 blocks of data, one block for each torsion family. Each block consists of several records. The first record of each block contains the torsion family name. The second and subsequent records contain specific torsion values and associated statistical weights the family can sample. Up to 20 such records can be supplied, allowing a 20-state model for each family of torsions.

The first record in the .rsf file must contain a 0 in the first space. This record is necessary for other applications using this file though it is

ignored by Conformational Search. The last record for each family contains the entries 999.0 and 0.0, to mark the end of each block.

In the following example, five different states are assigned for the families phi and psi, and two states are assigned for the family omega. The statistical weights are automatically normalized to 1.0.

Data		Description
0		
phi		torsion family name
50.0	0.1	torsion value, statistical weight
70.0	0.3	torsion value, statistical weight
-60.0	1.0	torsion value, statistical weight
-145.0	2.0	torsion value, statistical weight
-180.0	0.7	torsion value, statistical weight
999.0	0.0	end of block
psi		torsion family name
30.0	0.3	torsion value, statistical weight
-20.0	1.0	torsion value, statistical weight
80.0	2.0	torsion value, statistical weight
90.0	9.2	torsion value, statistical weight
140.0	0.1	torsion value, statistical weight
999.0	0.0	end of block
omega		torsion family name
180.0	0.5	torsion value, statistical weight
0.0	0.5	torsion value, statistical weight
999.0	0.0	end of block
END		

To execute a Custom RIS Search, torsion families must be defined first by reading the appropriate torsion template file (*filename*.trn). **Do Search** starts the search procedure.

Understanding RIS Random Sampling

The RIS Random Sampling search method uses a rotational isomeric state model to limit torsion values to the energetically preferred trans, gauche+, and gauche- states. These states correspond to 180^{o}, -60^{o}, and 60^{o}, respectively.

When RIS Random Sampling is chosen from the Conformational Search palette, a dialog box is displayed to specify the parameters for the search procedure. Table 22 lists the options and provides a brief description of each.

Statistical weights entered in the dialog box are normalized to 1.0, based on their sum. For example, if the values are 10, 4, and 2, the statistical weights will be 0.625, 0.25, and 0.125, respectively. At each perturbation, each torsion angle is reassigned to the trans, gauche+, or gauche- state with the probability of assignment determined by the statistical weights.

Table 22. RIS Sampling Setup Dialog Box

Option	Description
Number of Samples	Specifies the number of conformations to generate and write to the output search file. Default is 10.
Torsion States	Specifies assignment of torsions states. Default is *trans*, *gauche+*, and *gauche -*.
User-defined Three-state Sampling	Specifies user-defined sampling with the states and weights defined in the next set of data fields.
States	Specifies user-defined states. Default values are 60, -60, and 180.
Weights	Specifies user-defined statistical weights. Default values are 0.250, 0.250, 0.500.
Calculate CHARMm energy for each structure	Calculates the potential energy of each conformer as it is generated.
CHARMm minimization for each structure	Minimizes the energy for each conformation as it generated.
Display each structure	Displays each conformation as it is generated.

Understanding the Filter Search

The Filter Search method is a variation of the Random Sampling and the RIS Random Sampling procedures. After new conformations are generated, property filters are applied to reduce the number of conformations chosen for further processing. A filter is specified in terms of a geometric property or in terms of an energy value. If the filtering conditions are too stringent, an empty output file may be created. An empty output file is rejected by the Analysis application.

Filter Search offers two methods for generating conformations: a random sampling technique and random choice of a rotational isomeric state (RIS). A dialog box is displayed to choose the generation method.

After the generation method is selected, the Filter Search palette is displayed with additional selections to process conformations, define filters, and save new conformations. Table 23 lists and briefly describes the palette selections.

Table 23. Filter Search Palette

Selection	Description
Energy	Calculates energy for each generated conformation.
Minimize	Minimizes energy for the conformations that are generated by the search.
Dynamics Burst	Performs a quick dynamics calculation on generated conformations that fall within specified parameters.
Jump Sequence	Specifies the method and sequence for generating conformations.
Define Filter	Displays the Filter palette listing property type selections to define the filter. Each filter can include up to 10 different properties.
Save/Finish	Saves filter search selections and exits the Filter Search palette.

Filters impose criteria on new conformations. The conformations that meet the criteria proceed to the next step in the process. Filters can apply to torsions, distances (including distances to dummy atoms), energy, radius of gyration, and dipole moment. When **Define Filter** is selected from the Filter Search palette, the Filter palette is displayed listing property type selections to define the filter. Each filter can include up to 10 different properties.

Properties are combined by selecting an appropriate Boolean operator from the Filter Search palette. The operators **.AND.** and **.OR**. are provided for making combinations. These operators appear on the palette after the first property is defined.

Each time a new property is selected, it is combined with the previously defined property by the highlighted operator. For example, let A, B, C, and D be a set of interatomic distances defined using the **Geometry** palette. If the **.OR**. tool is highlighted, selection of the **Distance** property, followed by a distance name specification for each distance, constructs the following filter:

(((A or B) or C) or D)

After a filter is defined, two additional selections appear in the palette: **.AND. Filter** and **.OR. Filter**. These selections combine filters. For example, two filters are combined by first defining the first filter, selecting **.AND. Filter** or **.OR. Filter**, then defining the second filter. For example,

Filter#1 or Filter#2 == (A or B) and (C or D)

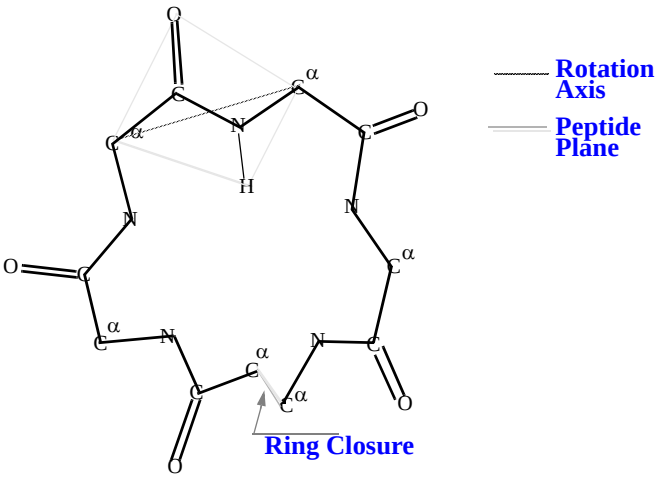
Understanding the Peptide Flip Search

The Peptide Flip search method locates low-energy conformations for cyclic peptides. It is a special random sampling procedure where peptide units are permitted to rotate about pseudobonds connecting alpha carbon atoms. Cyclization algorithms built into the search procedure ensure that a cyclic structure is preserved during torsional manipulation.

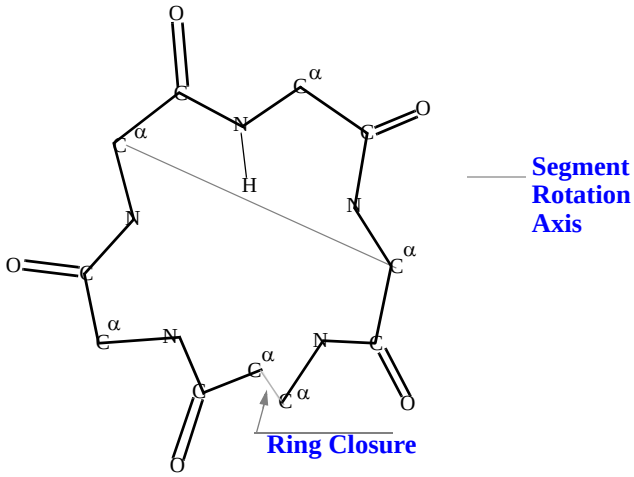
In a cyclic system, flexibility of the molecule is often characterized by correlated changes in torsion angles. This is particularly the case where two single bonds are roughly colinear and a crankshaft motion of the intervening section can occur. Such a motion results in a change in the torsion about the first bond and a change of roughly equal magnitude but opposite sign around the other bond.

Such a situation exists approximately for a planar trans peptide unit where a tilt of the peptide plane about the line joining its terminal alpha carbon atoms corresponds to a change in $\mathbf{psi_i}$. A change of roughly the same magnitude but opposite in sign also occurs in $\mathbf{phi_{i+1}}$. As long as the magnitude of these changes is not too large, they produce a local deformation that can be used to rapidly search for better energy minima.

The Peptide Flip procedure utilizes two types of pseudorotations: peptide flips and segment rotations. A peptide flip consists of the rotation of a single peptide unit about the line joining the alpha carbons at both ends of the peptide bond. The following figure locates the rotation axis for a peptide flip:



A segment rotation is a rotation of a sequence of connected peptide units, as a whole, about the line joining the two alpha carbon atoms at the ends of the segment. The following figure locates the axis of rotation for a segment rotation:



Two features of these rotations should be noted:

- The conformation of the rotating group is preserved in the process.
- The only changes resulting from such a rotation are in the two skeletal bond angles at the alpha carbon atoms, and the four torsion angles at the alpha carbon atoms. Such rotations lead to localized changes in conformation.

The Peptide Flip and Segment Rotation procedures affect the conformation of the cyclic system in different ways and therefore, the parameters used for them are different. In most cases peptide flips of 180 degrees produce satisfactory results. On the other hand segment rotations have to be small, preferably between 30 and 60 degrees.

While peptide flips generally do not dramatically alter the alpha carbon atom framework of the peptide after the energy minimization, segment rotation does change the shape of the molecule. Peptide flips do dramatically alter the backbone torsion angles and the hydrogen bonding patterns in the system.

Segment rotation can be viewed as starting a new shape of the molecule, while peptide flips explore the variation of the overall shape. It is for this reason the algorithm employs a series of segment rotations, with each segment rotation being followed by several peptide flips. One starts a new shape for the cyclic system and the other develops energetically favorable peptide plane orientations consistent with that shape.

Results of the Peptide Flip search also depend on:

- The number of segment rotations per search
- The number of peptide flips performed within each segment rotation
- The number of random peptide flip rotations performed before the lowest energy structure is selected, minimized, and saved

Peptide flips can be performed on more than one peptide unit. When one or more peptide units are rotated, the peptides that undergo such rotations are selected randomly from the total number of rotatable peptide units. Peptide flips are not performed on a prolyl peptide unit.

Segment rotations can have an angular value that is either fixed or a window for random variation. The length of the segment also must be specified using the number of connected residues that make up the segment to be rotated.

Parameters required for setting up a Peptide Flip procedure are specified in a dialog box. Table 24 lists and briefly describes the options.

Table 24. Peptide Flip Options Dialog Box

Option	Description
Perform Segment Rotation	Performs a segment rotation as part of the search. If this option is not selected, only peptide flips are performed, randomly selecting any peptide in the peptide backbone.
Number of Times Rotations are Repeated	Defines the number of times a segment rotation is performed in one peptide flip search procedure.
Length of the Segment	Defines the number of connected residues that compose the segment to be rotated.
Use Random Segment Length	Uses a segment of random size in the search procedure.

Table 24. Peptide Flip Options Dialog Box (Continued)

Option	Description
Rotation Angle/Window (degrees)	Specifies the angle for segment rotations. **Constant Angle** — rotations occur in increments of the specified value. **Random Rotation** — rotations occur anywhere within a range of the specified number.
Number of Energy Minimum Conformations per Segment Rotation	Defines the number of peptide flip sets that are performed after each segment rotation. Each set of peptide flips consists of a series of peptide flip trials followed by an energy minimization.
Number of Peptide Flip Trials Before Energy Minimization	Defines the number of peptide flips that are performed before the lowest energy structure is selected and submitted to CHARMm for minimization.
Peptide Flip Angle/ Window (degrees)	Specifies the angle for peptide flip rotations. Constant Angle — rotations occur in increments of the specified value. Random Rotation — rotations occur anywhere within a range of the specified number.
Number of Peptide Units Involved in a Flip	Allows rotation of more than one peptide unit. Peptide units are selected randomly from the total number of rotatable peptide units.

For a cyclic peptide with *n* residues, use a procedure similar to the following set of steps. In this description, each parameter is identified by the name used for it in the dialog box.

1. Choose a segment of length L (**Length of the Segment**) randomly from *n* residues. Alternatively, choose a segment of a random size by turning the **Use Random Segment Length** toggle on in the dialog box.
2. Rotate the chosen segment either through a constant angle or through a random angle within a window of -1/2a to 1/2a. Select **Constant Angle** for the first option and **Angular Window for Random Rotations** for the second case.
3. Energy minimize the conformation.
4. Perform *M* (**Number of Peptide Flip Trials before Energy Minimization**) trials of peptide flips. Each trial of the peptide flips consists of the following:

- Randomly choose *m* **(Number of Peptide Units**) peptide units. These are rotated about their own inter-C^{α} line through either a constant angle b (Peptide Flip Angle/Window) or through a random angle within a window 180-1/2b to 180+1/2b.
- Calculate the CHARMm energy of the resulting structure. Once again the two radio buttons in the dialog box, **Constant Angle** and **Angular Window for Random Rotations**, may be employed to apply either case.

5. Recall the lowest energy structure from the M trials done in Step 4. Energy minimize this structure in CHARMm and save the structure. A single execution of Steps 4 and 5 results in one minimized structure being saved into the output structure file.
6. Repeat the Steps 4 and 5 N times (**Number of Energy Minimum Conformations per Segment Rotation**), so that a total of N structures are saved into the search file.
7. Repeat Steps 1 to 6 a total of K times (**Number of Times Rotations are repeated**). At the end of this procedure, a total of N×K energy minimized conformations are saved into the output search file.

Understanding Loop Modeling

Loop Modeling is used to generate low energy conformations in loop regions of a polypeptide or protein. Only the loop portion of the molecule is involved in generating new conformations. The rest of the molecule is held rigid during the procedure. Torsion angles within the loop region are adjusted to ensure acceptable joining of the loop region with the rest of the molecule.

You identify the loop region you want to model by identifying the residue numbers at either end of the loop. An anchor residue is added by the program at each end. These residues belong to the fixed region of the protein.

Torsion angles within the loop are randomly altered. Each torsion angle chi is altered such that each new value is within the chi -0.5 * window and chi +0.5 * window. The window is an angular parameter that is specified in the setup dialog box.

When all torsion angles of the loop are modified, the loop adopts a conformation with anchor residues displaced from their original positions.

The loop then goes through rigid and flexible fit procedures and energy minimization. Non-loop portions of the molecule are kept fixed during minimization. Conformation of the protein is saved to an *.csr* output file. This modeling procedure may be repeated a specified number of times.

The starting and ending residues of the loop are specified along with the torsion windows for the ends and the middle of the loop. Matches may be displayed during the procedure and the loop can be minimized at the end of the design phase.

When Loop Modeling is chosen from the Conformational Search palette, a dialog box is displayed to specify the parameters of the search procedure. Table 24 lists and briefly describes the options.

Table 25. Loop Modeling Setup Dialog Box

Option	Description
Number of Loop Conformations	Determines the number of conformations to be generated. Default is 1.
Start and End residue	Lists the numbers of the residues at either end of the loop region in the protein.
Torsion window at ends	Specifies the torsion window to be applied to torsion angles at the ends of the loop. Generally, changes in the torsion angles at the ends of the loop are larger than in the middle, so a smaller window can be specified to give satisfactory results. Default is 60.0.
Torsion window at middle	Specifies the torsion window to be applied to torsion angles in the middle of the loop. Generally, changes in the torsion angles in the middle of the loop are smaller than at the ends, so a larger window can be specified to give satisfactory results. Default is 120.0.
Maximum number of iterations in simplex	Specifies the number of iterations for flexible fitting, simplex minimization of the RMS. Default value is 200. Generally values between 200 and 400 are appropriate.
Tolerance of simplex	Specifies the tolerance value for the simplex minimization. Default is 0.00.
Display matches during design	Shows displacement of anchor residues from their original positions as the procedure progresses.
Energy minimization after design	Executes an energy minimization procedure at the end of the modeling procedure. Strongly recommended.

Additional Processing of Conformations

Conformations can be processed to update pre-existing search or dynamics files without running a search procedure. For example, when a structure does not converge to a real minimum, additional cycles of energy minimization can be carried out.

Summary

This chapter describes a variety of conformational search methods available in QUANTA. These methods explore conformational space by varying torsion angles in one or more molecular structures or by varying the relative displacements and orientations of several molecular structures. The search procedures may be broadly classified into two categories:

1. Systematic (deterministic) search procedures

- Grid Scan
- Custom Search
- Cyclic Modeling

2. Stochastic (probabilistic) search procedures

- Random Sampling
- Random Minimization
- Boltzmann Jump
- Hybrid Boltzmann Jump
- Custom RIS Search
- RIS Random Sampling
- Filter Search
- Peptide Flip
- Loop Modeling

An exhaustive systematic search of the entire conformational space is a very time consuming process for most flexible molecules. The size of the

conformational space to be searched increases dramatically with the number of rotatable torsion angles. In addition, as molecular size increases, the resolution with which each torsion angle needs to be scanned generally also increases. Both these factors compound to enhance the difficulty of locating minimum energy conformations of a large molecule.

A systematic search can be carried out in reasonable length of time only for very small molecules with four or fewer variable torsion angles. In the case of larger systems, either a systematic search is more local, in the sense that the search is carried out only in part of the conformational space, or a stochastic search is used to sample the conformational space.

The general procedure for performing a conformational search includes the following steps:

1. Define torsions and other geometric quantities.
2. Specify a torsion subset (optional).
3. Select the tag generation method (optional).
4. Choose a search procedure.
5. Specify search parameters.
6. Initiate the search by selecting **Do Search**.
7. Provide a filename and supply the tag prefix and number, if prompted.

Begin a Conformational Search by selecting **Conformational Search** from the **Applications** menu and displaying the Conformational Search palette. This palette includes selections for defining torsions and other geometric properties, selecting a search method, specifying search parameters, and initiating search procedures. Each search is initiated after torsion definition is completed.

References

1. S. Kirkpatrick, C. D. Gelate Jr., and M. P. Vickie. 1983. *Science* 220: 671.
2. W. H. Press, B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling**.** 1986. *Numerical Recipes*. Cambridge University Press.

5. Performing a Search

This chapter describes the basics of performing simple searches within the Conformational Search application. It is the third chapter to describe conformational search procedures. Read this chapter for specific information on setting up and running a conformational search. The chapter includes practice exercises for the following search methods:

- Grid Scan
- Random Sampling
- Boltzmann Jump
- Peptide Flip

The Conformational Search application enables the exploration of a molecular structure's conformational space. Torsion angles are modified to generate various conformations and each conformation is processed according to user-defined criteria. When two or more structures are under study, it is also possible to explore spatial orientations of one structure with respect to the other. There are three basic steps for defining a search procedure:

1. Identify geometric properties
2. Select a search procedure
3. Define the scope of the search

Files containing the conformations generated during a search procedure are assigned user-defined names. These files contain: coordinate data, identification tags for each conformation, and a potential energy for each conformation generated by the search procedure (optional).

Selecting **Conformational Search** in the **Application** menu calls the Conformational Search palette. This palette provides a set of tools to execute the steps listed above.

Selections from the QUANTA Geometry palette are also accessible from within Conformational Search. Other palettes provide logical grouping of selections based on the functions they perform.

It is possible to move directly from the Conformational Search palette to the Analysis application. Transferring to Analysis from Conformational Search removes the Conformational Search palette. Subsequent exiting from Analysis returns to Conformational Search and restores the appropriate palette.

Many palette tools are dimmed when the Conformational Search palette is first displayed. Dimmed tools represent unavailable choices based on the currently active tool or the current point within the procedural definition. Dimmed tools cannot be selected, providing a safeguard against making inappropriate selections. Dimmed tools are restored to an active status (bright) when usage is permitted.

Performing a Grid Scan Search

The Grid Scan search explores conformational space by systematically varying torsion angles over a grid of equally spaced values to generate new conformations. Up to 10 torsion angles can be used in a given search. If more than 10 torsions are defined, a subset of 10 or fewer must be selected.

To perform a grid scan:

- Define (and, if necessary, select), the torsions in the displayed structure(s).
- Define the scope of the search by specifying the search method and associated criteria.

Separate palettes are provided for each step.

Sample Procedure — Grid Scan Search

The following exercise completes the three search steps for search1.msf. The structure was generated in Chapter 2 of *QUANTA Generating and Displaying Molecules.*

Steps 1–5 of this exercise repeat steps taken to define torsions in an exercise in Chapter 3 of this book. If you have completed these steps already, go on to Step 6. After search setup procedures are established, the grid scan search for search1.msf takes 5-10 minutes to complete.

1. **Open search1.msf.**

   Display the **File** menu. Select the **Open** function. A File Librarian dialog box is displayed.

   Select search1.msf from the scrolling list.

   Select the **Open** button. The structure *search1* is displayed in the viewing area.

2. **Start Conformational Search.**

   Display the **Applications** menu. Select **Conformational Search**. The Conformational Search palette replaces the Modeling palette and the Geometry palette remains displayed.

   The textport reports the following information about the displayed structure, search1.msf:

   ```
   Number of Fragments = 1
   Number of Ring Bonds= 6
   No of rotatable bonds= 6
   ```

3. **Display atom labels for all atoms. Display the Draw menu. Select Label Atoms.**

   From the pull-right menu that opens, select **Selection Tools**. The Label Atoms and Label Components and Utilities palettes are displayed.

   From the Label Components and Utilities palette, select **Atom Name, Residue ID,** and **Show Labels.**

   From the Label Atoms palette, select **Include** and **All Atoms.** A label containing the atom name is displayed for all atoms. These labels permits identification of the atoms composing the available torsions.

   From the Label Atoms palette, select **Finish.** The Conformational Search palette is redisplayed

4. **Define torsions in the displayed structure.**

   From the Conformational Search palette, select **Torsions.** The Torsions palette is displayed.

From the Torsions palette, select **Define All Torsions.** The command line displays:

```
No of Torsions = 6 Families = 6
```

Select **List Torsions**. The following torsion definitions are reported in the textport:

```
1       1:tor1 [PHEN1] C1C2C14C15 -77.9 back *
2       2:tor2 [PHEN1] C3C4O9H131 179.5 back *
3       3:tor3 [PHEN1] C4C5O11H121 -147 back *
4       4:tor4 [PROP2] C2C14C15C161 -63 back *
5       5:tor5 [PROP2] C14C15C16N211 -60 back *
6       6:tor6 [PROP2] C15C16N21H221 179.9 back*
```

Actual atom names may differ, depending on the sequence in which bonds and atoms were placed when the structure was built.

5. Specify a torsion subset.

From the Torsions palette, select **Select Torsions**. The **Torsion** Selection palette is displayed. Selected torsion bonds in the displayed structure are blue, and all other bonds are red.

The message line reads:

```
Torsion Selection: INCLUDE on 6 Torsns SLCTD out of 6.
```

From the Torsion Selection palette, select **Include** and **Select a Subset of Torsions.** A dialog box is displayed offering options for the selection of one torsion from all torsions currently available.

Select the options:

Include
and **Exclude all others**
4 tor4(2) C2-C14-C15-C16

Select the **OK** button. The dialog box is cleared from the screen. The torsion named tor4(2) comprised of the bond between the carbons alpha and beta to the para ring carbon, is selected. This bond remains displayed in blue. All others bonds are red.

The message line reads:

```
Torsion Selection: INCLUDE on 1 Torsns SLCTD out of 6
```

From the Torsion Selection palette, select **Finish.** The palette is no longer displayed. The search1 structure is redisplayed in the original colors. The message line reads:

```
1 of the 6 torsions selected
```

From the Torsions palette, select **Exit Torsions.** The palette is removed from the screen. Defining torsions for the search procedure is completed.

6. Define the scope of the search

From the Conformational Search palette, select **Setup Search.** All of the search procedure selections become active.

Select **Grid Scan.** A dialog box is displayed permitting the entry of values that define the rotational range (initial and final torsion values) and the torsion angle increment within this range.

You can also specify torsion values as absolute or relative values.

Select the option:

Torsion Values are ABSOLUTE

Enter the values:

From: 0.000
To: 330.000
Step Size: 30.000

Select the **OK** button. A dialog box is displayed, offering options for specifying how each conformation is processed.

Select the options:

CHARMm Minimization for each structure
Constrain Grid Torsion during Minimization
Display Structure

The second option in the list above constrains the torsion angles which the previous dialog box specified. This ensures they are not altered by the subsequent energy minimization in CHARMm. The program employs dihedral constraints in CHARMm to retain grid values for these torsions.

Select the **OK** button. **Grid Scan** remains checked and highlighted, indicating it is the selected search method. All other procedure selections are dimmed. **Do Search** is activated, indicating the appropriate conditions now exist for starting a search.

7. Setup minimization for conformations.

Since each conformation is to be minimized, establish CHARMm minimization specifications.

Display the **CHARMm** menu. Select **Minimization Options**. A dialog box containing the minimization setup options is displayed.

Select the option:

Steepest Descents

Enter the values:

Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000

Select the **OK** button. The dialog box is cleared from the screen. The new minimization conditions are established.

8. Start the grid scan search.

From the Conformational Search palette, select **Do Search.** A File Librarian dialog box is displayed.

Enter the name grid1 for the file to contain search results. The application automatically adds the extension *.csr.*

Accept the default values:

Tag prefix: search1.msf
Starting Tag Number: 1

Select the **New** button. The search procedure is started. As the search proceeds, each conformation is displayed in the viewing area and minimized before proceeding to the next conformation. Tags are displayed in the upper-left corner of the viewing area, identifying which conformation is currently displayed.

This search procedure takes 5-10 min. When the search is completed, the last conformation is displayed in the viewing area. The message line reads:

```
GRID SEARCH completed.
```

Performing a Second Grid Scan Search

A second grid scan search, employing two torsion angles in the search1 structure is performed in the same manner as the first. The results, however, are much different when two torsions are used. After search setup procedures are established, the Grid **Scan** search takes 30 to 35 minutes.

1. Define torsions in the displayed structure.

Display the Conformational Search palette. Select **Torsions**. The Torsions palette is displayed.

Select **Define all Torsion. Use Default Names** is already selected. A dialog box is displayed, providing options to add or replace the current torsion definition.

Select the option:

Define New Torsion Angles

Select the **OK** button. The message line reads:

```
No of Torsions= 6 Families=6
```

The following torsion definitions are reported in the textport:

```
1    1tor1   C1      C2      C14     C15     1
2    2tor2   C3      C4      O9      H13     1
3    3tor3   C4      C5      O11     H12     1
4    4tor4   C2      C14     C15     C16     1
5    5tor5   C14     C15     C16     N21     1
6    6tor6   C15     C16     N21     H22     1
```

The torsions may have different designations in your system depending how the structure was generated.

2. Select two torsions for the search.

Display the Torsions palette. Select **Select Torsions.** The Torsion Selection palette supplements the display of the Torsions**,** Conformational Search, and Geometry palettes. Selected torsion bonds in the displayed structure are colored blue. All other bonds are colored red.

The message line reads:

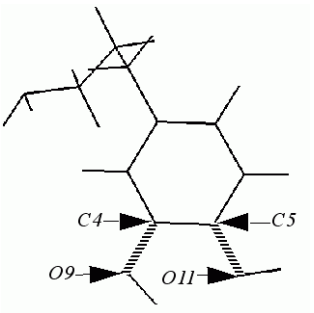
```
Torsion Selection: INCLUDE on 6 Torsns SLCTD out of 6
```

From the Torsion Selection palette, select **Select a Subset of Torsions**. A dialog box is displayed, permitting the selection of specific torsions from all torsions currently available.

Select the options:

Include
and **Exclude all others**
2 tor2(1) C3--C4--O9--H13
3 tor3(1) C4--C5--O11--H12

Select the **OK** button. The dialog box is cleared from the screen. The torsions named tor2(1) and tor3(1) are selected. The bonds remain displayed in blue (between C4 and O9, and C5 and O11). All other bonds are displayed in red.



The message line reads:

```
Torsion Selection: INCLUDE on 2 Torsns SLCTD out of 6.
```

From the Torsion Selection palette, select **Finish**. The palette is removed from the screen. The structure is redisplayed in the original colors. The message line reads:

```
2 of the 6 torsions selected.
```

From the Torsions palette, select **Exit Torsions.** The palette is removed from the screen. The definition of torsions for the search procedure is completed.

3. Select the Grid Scan search method and define specifications.

From the Conformational Search palette, select **Setup Search.** All of the search method tools become active.

Select **Grid Scan**. A dialog box is displayed, permitting the entry of an initial value, the final value, and the torsion angle increment for both torsions.

Select the option:

Absolute Value

Enter the values:

tor2(1) From: 0.00
To: 330
Step Size: 30.00
tor3(1) From:0.00
To: 330
Step Size:30.00

Select the **OK** button**.**

A dialog box is displayed, offering options to specify how each conformation is processed.

Select the options:

CHARMm Minimization for each structure
Constrain Grid Torsion during Minimization
Display Structure

Select the **OK** button.

The following information is reported in the textport:

```
There are 12 states for tor2(1)
There are 12 states for tor3(1)
Number of conformations will be: 144
```

Grid Scan remains checked and highlighted, indicating this is the defined search method. All other method selections are dimmed. **Do Search** is activated, indicating the appropriate conditions now exist for starting a search.

4. Setup minimization for conformations.

Display the **CHARMm** menu. Select **Minimization Options**. A dialog box containing the minimization setup options is displayed.

Select the option:

Steepest Descents

Enter the values:

Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000

Select the **OK** button. The dialog box is cleared from the screen. The new minimization specifications are established.

5. Start the Grid Scan search.

From the Conformational Search palette, select **Do Search.** A File Librarian dialog box is displayed. Enter the name grid2 for the file to contain search results.

Accept the default values:

Tag prefix: search1.msf
Starting Tag Number: 1

Select the **New** button. The search process is started. This search procedure takes about 30 minutes to complete. When the search is completed, the last conformation is displayed in the viewing area.

The message line reads:

```
GRID SEARCH Completed.
```

6. Exit Conformational Search.

From the Conformational Search palette, select **Exit Conformational Search**. The Conformational Search palette is removed and the Modeling palette is displayed.

7. Reject changes to the structure.

From the Modeling palette, select **Reject Changes**. Search1 returns to its original conformation.

Performing a Random Sampling Search

The Random Sampling procedure randomly changes all defined torsion angles in a structure using a predefined range. This range (angular window) specifies the maximum angle that torsions may vary during each perturbation in the search procedure and generates the user-defined number of conformations. Torsion angle modifications can be performed using a starting structure or a previously obtained conformation.

As the search progresses, CHARMm energy and/or CHARMm minimization can be calculated for each randomly altered conformation. When the search is complete, all conformations are saved to a search file and given a user-defined name.

Sample Procedure — Random Sampling Search

Complete the following exercise to become familiar with the steps in a Random Sampling search. After search setup procedures are established, the random sampling search for the search1 structure takes five to ten minutes to complete.

1. **Display the original conformation of the structure.**

   Open search1.msf. The original conformation of the structure replaces the conformation displayed after exiting conformational search in the previous exercise.

2. **Start Conformational Search.**

   Display the **Applications** menu. Select **Conformational Search**. The Search palette replaces the Modeling palette and supplements the display of the Geometry palette. The textport reports the following information about the displayed structure:

   ```
   Number of Fragments = 1
   Number of Ring Bonds = 6
   No of rotatable bonds = 6
   ```

3. **Define torsions in the displayed structure.**

From the Conformational Search palette, select **Torsions**. From the Torsions palette, select **Define All Torsions**. The message line reads:

```
No of Torsions= 6 Families=6
```

The following torsion definitions are reported in the textport:

```
1    1tor1   C1      C2      C14     C15     1
2    2tor2   C3      C4      O9      H13     1
3    3tor3   C4      C5      O11     H12     1
4    4tor4   C2      C14     C15     C16     1
5    5tor5   C14     C15     C16     N21     1
6    6tor6    C15    C16     N21     H22     1
```

The actual atoms that define your torsions may differ, depending on the sequence in which bonds and atoms were placed when the structure was built.

From the Torsions palette, select **Exit Torsions.** The Torsions palette is removed from the screen. The torsions for the search procedure are now defined.

4. Select a search method and define specifications.

From the Conformational Search palette, select **Setup Search**. All of the search procedure selections become active.

From the Conformational Search palette, select **Random Sampling.** A dialog box is displayed, permitting the entry of setup specifications for the search.

Enter the values:

Number of Samples: 25
Torsion Angle Window: 60

Select the options:

Minimize Each Structure
Cumulative Random Modifications
Display Each Structure

Select the **OK** button. **Random Sampling** in the Conformational Search palette remains checked and highlighted, indicating it is the defined search method. All other procedure selections are dimmed. **Do Search** is activated, indicating the appropriate conditions now exist for starting a search.

5. Setup minimization for conformations.

Display the **CHARMm** menu. Select **Minimization Options**. A dialog box containing the minimization setup options is displayed.

Select the option:

Adopted-Basis Newton Raphson

Enter the values:

Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000

Select the **OK** button. The dialog box is cleared from the screen. The new minimization specifications are established.

6. Start the random sampling search.

From the Conformational Search palette, select **Do Search.**

A File Librarian dialog box is displayed. Enter the name random for the file to contain search results. The extension .csr will automatically be added.

Accept the default values:

Tag prefix: search1.msf
Starting Tag Number: 1

Select the **New** button. The search procedure is started. As the search proceeds, each conformation is displayed in the viewing area and minimized before proceeding to the next conformation. Tags are displayed in the upper left corner of the viewing area (search_msf:1), identifying which conformation is currently displayed.

This search procedure takes 5–10 minutes. When the search is completed, the last conformation is displayed in the viewing area. The message line displays:

```
Random Sampling Completed
```

7. Exit Conformational Search.

From the Conformational Search palette, select **Exit Conformational Search.** The Conformational Search palette is removed from the screen and the Modeling palette is displayed.

Performing a Boltzmann Jump Search

The Boltzmann Jump search explores conformational space for energy minima, generating the user-defined number of conformations through random perturbation of torsion angles. Upward jumps in energy are permitted.

Starting with a minimized structure, a random perturbation is carried out within a predefined range, specifying how much torsion angles may vary for each conformation during the search. If the resulting conformation is of a lower energy, the conformation is selected. If the perturbed conformation is of a higher energy, a stochastic decision is made to select or reject the new conformation.

Even after a conformation is selected, random perturbation continues until the RMS differences in torsion angles between the selected conformation and the starting minimum energy conformation exceeds a user-defined value. This causes the perturbation sequence to terminate, and a CHARMm energy minimization to be performed on the conformation.

Search procedures can also be performed on cyclic structures. Cyclization algorithms work transparently during a search procedure to ensure that the cyclic structure is preserved during torsional manipulation.

When you select **Boltzmann Jump** from the Conformational Search palette, a dialog box is displayed, offering options to set up the specifications for the search.

Sample Procedure — Boltzmann Jump Search

The exercise that follows uses the cyclic structure CCK7 built in Chapter 4 of *QUANTA Generating and Displaying Molecules*. The file CCK7.msf should be displayed and active in the viewing area. After search setup procedures are established, the Boltzmann Jump search takes 30–35 minutes to complete.

1. Start Conformational Search.

From the **Applications** menu. Select **Conformational Search**. The Conformational Search palette replaces the Modeling palette and supplements the display of the Geometry palette. The textport reports the following information about the displayed structure (CCK7.msf):

```
Number of Fragments = 1
Number of Ring Bonds= 43
No of rotatable bonds= 15
```

2. Select the torsion template to define backbone torsions.

From the Conformational **Search** palette, select **Torsions.** The Torsions palette displaces the Conformational Search palette and supplements the display of the Geometry palette.

From the Torsions palette, select **Read Torsion Template.** A File Librarian dialog box is displayed.

Select the torsion template:

protein.trn

Select the **Open** button. The template is applied to the structure and a list of torsions is displayed in the textport.

3. Define elastic bonds for cyclization.

From the Torsions palette, select **Define Peptide Backbone Torsions.** An elastic bond is automatically defined.

From the Torsions palette, select **Exit Torsions.** The palette is removed from the screen.

4. Select Boltzmann Jump and define specifications.

From the Conformational Search palette, select **Setup Search**. All of the search procedure selections become active.

Select **Boltzmann Jump.** A dialog box is displayed, permitting the entry of specifications for the search.

Enter the values:

Number of Samples: 20
Torsion Angle Window: 10.00
Temperature (Kelvin): 2000.00

If the **Boltzmann Jump** success rate is too low, you can reduce the **Torsion Angle Window** and increase the temperature.

Select the option:

Torsion Space

Enter the value:

Torsion Space: 5.00

Select the options:

Display Each Structure
Save Minimized Structures Only

Select the **OK** button. The dialog box is cleared from the screen. **Boltzmann Jump** remains checked and highlighted in the Conformational Search palette. All other procedure tools are dimmed.

5. Setup minimization for conformations.

Display the **CHARMm** menu. Select **Minimization Options**. A dialog box containing the minimization setup options, is displayed.

Select the option:

Adopted-Basis Newton Raphson

Enter the values:

Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000

Select the **OK** button. The dialog box is cleared from the screen. The new minimization specifications are established.

6. Start the Boltzmann Jump search.

From the Conformational Search palette, select **Do Search.** A File Librarian dialog box is displayed.

Enter the name jump for the file to contain search results. Select the **Save** button. A dialog box is displayed, enabling the definition of tag specifications.

Enter the values:

Tag prefix: CCK7.msf
Starting Tag Number: 1

Select the **OK** button. The search procedure is started. This search procedure takes 30–35 minutes. When the search is completed, the last conformation may be displayed off an edge of the viewing area. Select **Reset View** from the Dial Emulator to bring the entire structure into view.

The message line reads:

```
Boltzmann Jump Sequence Completed.
```

7. Exit Conformational Search.

Select **Exit Conformational Search.** The Conformational Search palette is removed from the screen and the Modeling palette is displayed.

8. Reject changes made to CCK7.msf as a result of the search.

From the Modeling palette, select **Reject Changes**. The changes made to *C*CK7.msf are not saved in the MSF. The original coordinates (the coordinates saved after minimization) of CCK7.msf are redisplayed in the viewing area.

Performing a Search Using the Peptide Flip

The Peptide Flip search randomly locates low-energy conformations in cyclic pipettes by rotating peptide units about pseudo-bonds connecting alpha carbon atoms. Cyclization algorithms built into the search procedure work transparently to ensure a cyclic structure is preserved during torsional manipulation. This search procedure uses two types of rotations:

- Segment rotation
- Peptide flip

The segment rotation creates an initial conformation by rotating a sequence of connected peptides (a segment) about the pseudo-bond joining the alpha carbon atoms at each end of the peptide sequence. A user-defined number of peptide flips is then performed on a peptide unit contained within the defined segment.

A random conformation for the Peptide Flip is selected after a series of random rotations and the lowest energy structure resulting from this random sampling is submitted to CHARMm for minimization.

A Peptide Flip Search can be set up so several segment rotations can be performed. Each segment rotation can have its own series of nested peptide flips. Once the search is completed, all resulting conformations are stored in a search file (*.csr*).

Sample Procedure — Peptide Flip Search

The exercise that follows uses the cyclic structure CCK7 built in Chapter 4 of *QUANTA Generating and Displaying Molecules*. If you are progressing through this chapter, the structure CCK7 should be open and displayed in the viewing area. After search setup procedures are established, the Peptide Flip search takes 30–35 minutes to complete.

1. Start Conformational Search.

Display the **Applications** menu. Select **Conformational Search**. The Conformational Search palette replaces the Modeling palette and supplements the display of the Geometry palette. The textport reports the following information about the displayed structure:

```
Number of Fragments = 1
Number of Ring Bonds= 43
No of rotatable bonds= 15
```

2. Define the backbone torsions.

From the Conformational Search palette, select **Torsions.** The Torsions palette is displayed.

Select **Define Peptide Backbone Torsions**. The message line reads:

```
Peptide is CYCLIC. Number of skeletal atoms = 21 No of
torsions = 18 Families = 3.
```

From the Conformational Search palette, select **Exit Torsions.** The Torsions palette is removed from the screen. The definition of backbone torsions for the search procedure is completed.

3. Select Peptide Flip search and define specifications.

From the Conformational Search palette, select **Setup Search.** All the search method selections become active.

Select **Peptide Flip.** A dialog box is displayed, permitting setup of specifications of a search that performs only peptide flips (no segment rotation).

Select the option for the **Rotation Angle/Window**:

Constant Angle

Enter the values:

Number of Energy Minimum Conformations per Segment Rotation: 20
Number of Trials Flips Prior to Minimization: 5
Peptide Flip Angle/Window (degrees): 180.0
Number of Peptide Units involved in a flip: 2

Select the **OK** button. The dialog box is cleared from the screen. **Peptide Flip** remains checked and highlighted. All other method selections are dimmed. **Do Search** is activated.

4. Setup minimization for CCK7.msf.

Display the **CHARMm** menu. Select **Minimization Options**. A dialog box containing the minimization setup options is displayed.

Select the option:

Adopted-Basis Newton Raphson

Enter the values:

Number of Minimization Steps: 100
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.0100000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000

Select the **OK** button. The dialog box is cleared from the screen. The new minimization parameters are established.

5. Start the Peptide Flip search.

From the Conformational Search palette, select **Do Search.** A File Librarian dialog box is displayed.

Enter the name flip for the file to contain search results.

Accept the default values:

Tag prefix: CCK7.msf
Starting Tag Number: 1

Select the **New** button. The search procedure is started. This search procedure takes 30–35 minutes to complete. When the search is completed, the last conformation is displayed in the viewing area. The message line reads:

```
Peptide Flip Completed
```

6. Exit Conformational Search.

Select **Exit Conformational Search**. The Conformational Search palette is removed from the screen and the Modeling palette is displayed.

7. Reject changes to CCK7.msf as a result of the search.

From the **Modeling** palette, select **Reject Changes**. The changes made to CCK7.msf are not saved in the MSF. The coordinates saved after minimization of CCK7.msf are redisplayed in the viewing area.

Summary

The Conformational Search application enables the exploration of a molecular structure's conformational space. Torsion angles are modified to generate various conformations and each conformation is processed according to user-defined criteria. When two or more structures are under study, it is also possible to explore spatial orientations of one structure with respect to the other.

Selecting **Conformational Search** in the **Application** menu calls the Conformational Search palette. Selections from the QUANTA Geometry palette are also accessible from within Conformational Search. Other palettes provide logical grouping of selections based on the functions they perform.

This chapter includes practice exercises for the following search methods: Grid Scan, Random Sampling, Boltzmann Jump, and Peptide Flip.

The Grid Scan search explores conformational space by systematically varying torsion angles over a grid of equally spaced values to generate new conformations. Up to 10 torsion angles can be used in a given search. If more than 10 torsions are defined, a subset of 10 or fewer must be selected.

To perform a Grid Scan search:

- Define (and if necessary, select), the torsions in the displayed structure(s).
- Define the scope of the search by specifying the search method and associated criteria.

The Random Sampling procedure randomly changes all defined torsion angles in a structure using a predefined range. This range (angular window) specifies the maximum angle that torsions may vary during each perturbation in the search procedure and generates the user-defined number of conformations. Torsion angle modifications can be performed using a starting structure or a previously obtained conformation.

The Boltzmann Jump search explores conformational space for energy minima, generating the user-defined number of conformations through random perturbation of torsion angles. Upward jumps in energy are permitted.

Starting with a minimized structure, a random perturbation is carried out within a predefined range, specifying how much torsion angles may vary for each conformation during the search. If the resulting conformation is of a lower energy, the conformation is selected. If the perturbed conformation is of a higher energy, a stochastic decision is made to select or reject the new conformation.

Even after a conformation is selected, random perturbation continues until the rms differences in torsion angles between the selected conformation and the starting minimum energy conformation exceeds a user-

defined value. This causes the perturbation sequence to terminate, and a CHARMm energy minimization to be performed on the conformation.

Search procedures can also be performed on cyclic structures. Cyclization algorithms work transparently during a search procedure to ensure that the cyclic structure is preserved during torsional manipulation.

The Peptide Flip search randomly locates low energy conformations in cyclic peptides by rotating peptide units about pseudo-bonds connecting alpha carbon atoms. Cyclization algorithms, built into the search procedure work transparently to ensure a cyclic structure is preserved during torsional manipulation. This search procedure uses two types of rotations: segment rotation and peptide flip.

Segment rotation creates an initial conformation by rotating a sequence of connected peptides (a segment) about the pseudo-bond joining the alpha carbon atoms at each end of the peptide sequence. A number of peptide flips is then performed on a peptide unit contained within the defined segment.

A random conformation for the peptide flip is selected after a series of random rotations and the lowest energy structure, resulting from this random sampling, is submitted to CHARMm for minimization.

6. Analyzing Results

This chapter describes the Analysis application. This application provides tools to compute, access and analyze physical properties of structures generated by dynamics simulations or conformational search procedures. Read this chapter if you intend to analyze the results of search procedures or dynamics simulations.

The Conformational Search and Dynamics Simulation applications produce files that contain multiple conformations of molecular structures. Up to 6000 structures can be handled by the Analysis application, but performance is best when smaller datasets are used.

The Analysis application provides tools to read or compute physical properties of these structures. It sorts, selects, and compares the structures using available physical properties data. Trends, correlations, and extremes of the data are revealed through a variety of plotting options. Use other l tools are provided to view structures one at a time or to view them overlaid after a least squares fitting procedure is applied**.** You can also cluster structures into subsets that have common conformational features.

Enter the Analysis applications either directly from the **Applications** menu or from the Conformational Search palette.

When you start Analysis**,** conformations in the input file are read and a table of properties is created. Each conformation has an identifying tag that consists of a meaningful prefix and a number that designates its order at the time it was created in the set of conformations.

Initially, the only defined property is energy. In some cases even energy values may not exist. If you only want to browse through the set of conformations, you need no additional properties.

To sort structures or plot information about them, define additional physical properties such as torsions or distances. Torsions defined in Analysis need not be the same as those used in a search procedure. The **Torsions** selection on the Analysis palette uses the same facility as the one on the Conformational Search palette. For more information about the facility as

it is used in Conformational Search, see Chapter 3, *Defining Geometric Properties*.

To define a general dihedral angle, bond angle, or interatomic distance as a property to be used in your analysis, use the Geometry palette to set up geometry monitors before you enter the Analysis application.

When a set of physical properties is defined, you can use the plots facility and its powerful selection tools to look for structures that satisfy the criteria you specify. For example, you can display the variation in a property through a set of conformations by generating a trace that plots the value of the property against the conformation number. The structures that display a specified value can be selected directly from the plot. Using **Save Selected Structures**, you can save the selected structures into a new file, read the new file, and resume work on a reduced data set.

The power of the Analysis selections is enhanced when you use **Intersection** accessed in the Analysis palette. This tool allows selections to be based on more than one property. For example, to select structures that are low energy and possess at least two hydrogen bonds: first select the low energy structures and then, changing the property, select the structures that have at least two hydrogen bonds. The result, depending on the status of the **Intersection** selection, is a set of structures that have both low energy and at least two hydrogen bonds, or a set of structures that are either low energy or possess at least two hydrogen bonds.

You can generate a histogram to see the distribution of a property value. Scatter plots and correlation maps are used to show correlation between two properties. Specialized range maps and box range plots provide quick visual overviews of variations in geometric properties.

The range of conformational space spanned by a search procedure can be visually inspected using overlays. Differences in structures due to simple rotations and translations are removed by fitting each structure to an external reference or a common frame. Root mean square difference in Cartesian coordinate space, torsion space, or distance space can be used as the basis for comparing or clustering structures.

During a search procedure, coordinates are saved to a .csr file along with header lines to identify the system under study. The header includes the total number of active atoms, the number of active molecules, and a list of atom numbers and names for each active molecule. When a .csr file is read into Analysis, the total number of active and inactive atoms is compared with the total number of active atoms in the .csr file. If these do not match, the file is rejected. It they do match, further testing is done to see

if the molecule names recorded in the file agree with the current list of MSF names. If the names do not agree, a warning is issued before analysis can proceed.

If you want to work with more than one active MSF in Analysis, the order in which the MSFs are opened does not have to be the same as their order at the time the search file was created. Inactive MSFs are ignored even if their names are in the search file. Inactive MSF coordinates are not read from the search file, and the coordinates are left unaltered during analysis.

When Analysis is started from Conformational Search and an output search file is currently open, the file is closed and reopened as an analysis file. In this case, Analysis does not prompt for a filename. If an output file is not open, dialog boxes prompt for a file type and a filename.

Using the Analysis and Plots Palettes

The Analysis application employs two main palettes, the Analysis palette and the Plots palette. Other palettes are opened depending on selections made from these two palettes.

The Analysis palette contains tools to browse through structures in the analysis file, to invoke overlay tools, and to control the display of the Plots palette. The Analysis palette also contains selections to define torsion angles, interatomic distances, dummy atoms, and other properties such as the radius of gyration and dipole moment. The second group of selections are the same as those found in the Conformational Search palette. Table 26 lists and briefly describes the selections.

Sample Procedure — Using the Analysis Palette

Complete the following exercise to become familiar with the basics of using the Analysis palette. This exercise uses search1.msf and the grid1.crs file generated during the first Grid Scan conformational search exercise in Chapter 5.

1. Open search1.msf.

Table 26. Analysis Palette

Selection	Description
Torsions...	Displays the Torsions palette that contains selections for defining and selecting torsions. (Use Geometry monitors for general dihedrals.)
Interatomic Distances	Provides options for working with interatomic distances, including **Pick Distances, Read Distances, Save Distances,** and **List Distances**.
Dummy Atoms	Provides options for working with dummy atoms, including **Define Dummy Atoms, Read Dummy Atoms,** and **Save Dummy Atoms**.
Calculate Properties	Provides options for working with properties, including **Radius of Gyration, Dipole Moment,** and **Fractional Free Volume**.
Sort By Property	Sorts structures by ascending values of a selected property.
File Options/Filters	Provides options that include changing the file under analysis, displaying a synopsis of the file under analysis, writing coordinates of the displayed conformation to an MSF, converting a search file to a dynamics file, concatenating search or dynamics files, showing tags, redefining tags, processing conformations, saving sorted conformations, and building the RSF.
Get Structure	Displays the conformation identified by number. For example, the second conformation is displayed by entering the integer 2 in the resulting dialog box.
First Structure	Displays the first conformation in the file under analysis.
Next Structure	Displays the next conformation in the file under analysis. If the last conformation in the file is currently displayed, the first conformation is displayed when this selection is made.
Previous Structure	Displays the previous conformation in the file under analysis. If the first conformation in the file is currently displayed, the last conformation is displayed when this selection is made.
Last Structure	Displays the last conformation in the file under analysis.
Lowest Energy Structure	Displays the lowest energy conformation in the file under analysis. This selection is useful for extracting the lowest energy conformation. You can return to the Conformational Search application to initiate another search procedure, starting with the lowest energy conformation.
Use Selected Structures	Allows all the browsing selection, **Get Structure, First Structure, Last Structure, Next Structure, Previous Structure,** and **Auto Browse**, to display only selected structures. This selection becomes active only after structures are designated.
Use Sorted Order	Allows all browsing selections, **Get Structure, First Structure, Last Structure, Next Structure, Previous Structure,** and **Auto Browse,** to use the sorted order previously established by **Sort By Property**. This selection becomes active only after a sort is created.

Table 26. Analysis Palette (Continued)

Selection	Description
Auto Browse	Displays all structures automatically, one at a time. The structures displayed and the order in which they are displayed may be modified if **Use Selected Structures** and/or **Use Sorted Order** are selected.
Continue Auto Browse	Resumes automatic browsing if the action of **Auto Browse** is interrupted with a mouse click. The display of structures resumes from the point where it was interrupted.
Average Structure	Computes an average structure from all conformations in the file under analysis and displays the structure. Coordinates are obtained by computing the average x, y, and z cartesian coordinates for each atom over the entire set of conformations. An average structure may not always be a reasonable structure, especially when the file under analysis contains a wide range of conformations. The average structure also may have spurious bonds, depending on the interatomic distances found in the average structure.
Store Structure	Stores the displayed conformation in the storage buffer, overwriting any previously saved structure.
Recall Structure	Recalls the conformation last saved in the storage buffer using **Store Structure.**
Compare Torsion	Compares torsion angles of the displayed conformation with those of the conformation in the storage buffer. RMSD of torsion angles is reported in the Textport. Torsion angles that differ by more than 20° are also identified. This tool becomes active only when torsion angles are defined.
Overlay Structures...	Allows comparison of structures, clustering of structures into groups, least squares fitting of structures using optimal multiple superposition, and simultaneous display of all the structures.
Cluster Analysis	Provides tools to cluster structures into geometrically similar subsets.
Select Clusters	Selects all members of a cluster or the lowest energy member from a cluster.
Clear Selection	Clears any previous structure selection that may be in effect.
Complement Selection	Replaces currently selected structures with all currently unselected structures

Table 26. Analysis Palette (Continued)

Selection	Description
Plots	Toggles the display of the Plots palette.
Intersection	Adds subsequent selections to the selection list (union), or makes subsequent selections based on existing conformations in the selection list (intersection). When **Intersection** is on, all unselected structures are ignored when making further selections.
Show Cell Contents	Shows the contents of the periodic cell instead of a structure. This selection becomes active only when periodic boundary conditions are in effect.
Show Table	Shows potential energy table for all conformations.
Exit Analysis	Exits the Analysis application returning to the application from which Analysis was started.

Display the **File** menu and select **Open.** From the scrolling list in the File Librarian, select search1.msf.

Select the **Replac**e button, then select the **Open** button. The structure search1 is displayed in the viewing area.

2. Start the Analysis application.

From the **Applications** menu, select **Analysis**. A dialog box is displayed to permit selection of the type of input file to use for analysis. Select:

Search File (.csr)

Select the **OK** button. A File Librarian dialog box is displayed with a scrolling list of available search files.

Select the file grid1.csr, then select the **Open** button.

From the Analysis palette, select **Show Table**. A table listing the 12 grid scan conformations is displayed along with their corresponding potential energies. The Analysis and Plots palettes are also displayed. A tag associated with search1 is displayed in the upper-left corner of the viewing area, and a potential energy is displayed in the upper right corner.

3. Browse through all conformations in the search file.

From the Analysis palette, select **Auto Browse**. Each conformation is automatically displayed in the viewing area in sequential order. The automatic browsing procedure stops when the last conformation in the search file is displayed.

The **Auto Browse** procedure can be interrupted by clicking the left mouse button in the viewing area. The procedure is resumed by selecting **Continue Auto Browse** from the **Analysis** palette.

4. **Display the first conformation in the search file.**

From the Analysis palette select **First Structure.** The first conformation in the search file is displayed in the viewing area. The tag search1_msf:1 is displayed in the upper-left corner and the potential energy is displayed in the upper-right corner.

5. **Display the remaining conformations in the search file.**

From the Analysis palette, select **Next Structure.** The second conformation in the search file (search1_msf:2) is displayed in the viewing area.

Continue selecting **Next Structure**. Each conformation in the search file is displayed in sequence. Selecting **Next Structure** when the last conformation is displayed redisplays the first conformation.

6. **Display the last conformation in the search file.**

From the Analysis palette, select **Last Structure.** The last conformation in the search file (search1_msf:12) is displayed.

7. **Display other conformations in the search file.**

From the Analysis palette, select **Previous Structure.** The previous conformation in the search file (search1_msf:11) is displayed in the viewing area.

Continue selecting **Previous Structure**. Each conformation in the search file is displayed in reverse sequence. Selecting **Previous Structure** when the first conformation is displayed, displays the last conformation.

8. **Display the conformation with the lowest potential energy.**

From the Analysis palette, select **Lowest Energy Structure.** The conformation with the lowest energy (search1_msf:11) is displayed in the viewing area along with its associated energy. The actual conformation number may differ, depending on the geometry and resulting energies of the structure as it was built.

9. Sort conformations by potential energy.

From the Analysis palette, select **Sort By Property**. A dialog box is displayed, permitting the selection of a property.

Select the option:

Potential Energy

Select the **OK** button. The dialog box is cleared from the screen and **Use Sorted Order** is activated in the Analysis palette.

10. Display conformations by ascending potential energy values.

From the Analysis palette, select **Use Sorted Order.** The selection is checked and highlighted.

From the Analysis palette, select **First Structure.** The structure with the lowest energy (search1_msf:11) is displayed since this is now the first conformation in the sorted order. The actual conformation number may differ, depending on the geometry and resulting energies of the original structure.

From the Analysis palette, select **Next Structure.** The structure with the next-lowest energy (search1_msf:7) is displayed.

Select **Last Structure**. The structure with the highest energy (search1_msf:1) is displayed.

Select **Auto Browse.** The conformations are displayed in order of ascending energy values. The display stops when the highest energy conformation is displayed.

Select **Use Sorted Order** again to turn it off.

11. Display a trace of potential energy.

From the Plots palette, select **Trace.** A dialog box is displayed, permitting the selection of the property to plot.

Select the option:

Potential Energy

Select the **OK** button. The **Trace** plot is displayed along with the Trace palette. The x axis represents the conformation number; the Y axis represents the corresponding potential energy value.

To remove the plot from the screen, open the **Trace File** menu and select **Quit**. A dialog box is displayed offering options for saving the plot.

Select the **No** button. The plot is removed from the screen.

12. Exit Analysis.

From the Analysis palette, select **Exit Analysis.** The Analysis and Plots palettes are removed from the screen. The highest energy conformation of the search file remains displayed in the viewing area.

The Plots palette provides tools to generate plots of various types from the data supplied in the analysis file.

Within the Analysis application, you can generate the following type of plots:

- Property Traces
- Histograms
- Box Range Maps
- Range Maps
- Scatter Plots
- Backbone Plots
- Contour Plots
- Pair Distribution Histograms

After you have specified the properties to be plotted, the plot appears in a new window with its own menu selections to change plot dimensions, inquire about specific conformations, make conformation selections, and create hard copy output.

Table 27 lists and briefly describes the selections.

Table 27. Plots Palette

Selection	Description
Trace...	Displays a tracing of a specified property through conformational space.
Residue Plots...	Displays a tracing of a specified property as a function of a conformation number.
Histogram...	Displays a histogram of a selected property.
Correlation Map...	Using a contour plot for all conformations, displays the partition function (the sum over Boltzmann factors) of any two properties.
Scatter Plot...	Displays a scatter plot of any two properties for all the conformations.
Range Map...	Displays the variation of torsion angles or interatomic distances in a collection of conformations. Individual tick marks represent each conformation that has a specific property value.
Box Range Map...	Displays the variation of torsion angles or interatomic distances in a collection of conformations. Boxes represent the range of values spanned by conformations.
Backbone Plot	Displays a scatter plot of phi-psi values for all conformations in a peptide**.**
Contour Plot...	Displays a contour plot of a specified property for a pair of torsion angles.
Pair Distribution Function...	Displays the distribution of all pair-wise distances in a given set of conformations.
Site Plot...	Displays the variation of inter-residue distance as a function of separation for any user-specified conformation in the file under analysis. The first atom in each residue is used as the reference atom for distance calculations.
Exit Plots	Removes the Plots palette, any of the displayed plots, and their palettes from the screen.

For example, contour plots are generated when **Contour Plot** is selected from the Plots palette. By selecting **Contour Plot**, the results of a Grid Scan search can be displayed in a contour plot comparing a property of two torsion angles. In the plot, the angle of one torsion is represented on the x axis of the plot, the angle of the second torsion is represented on the y axis of the plot. A legend to the right of the plot assists in color interpretation by displaying numbers representing the range of the selected property in the same colors as the corresponding contours in the plot.

Contour levels can be displayed in one of three ways:

- With a constant interval where the starting level, level difference, and number of levels is specified
- With levels on a logarithmic scale

- With custom specifications for levels

A maximum of 50 property levels can be used.

The Contour Plot palette provides tools for manipulating data to display in the plot. Table 28 lists and briefly describes the palette selections.

Table 28. Contour Plot Palette

Selection	Description
Select Contour Property	Displays a dialog box, allowing the plotted property to be changed.
Select X, Y Axis Torsions	Displays a dialog box, allowing the two torsions used as X and Y axis variables to be changed.
For Other Torsions	Provides a label for **Display Lowest Value, Display Highest Value,** and **Display Z-Sections**. No action is performed by selecting this label.
Display Lowest Value	Displays a plot of the lowest contour property where, as a result of the search, the other torsions are adjusted for each grid point. The reference property is selected or changed using **Select Contour Property.**
Display Highest Value	Displays a plot of the highest contour property where, as a result of the search, the other torsions are adjusted for each grid point. The reference property is selected or changed using **Select Contour Property**.
Display Z-Sections	Displays a plot containing a 2D section of multidimensional data as a result of the search. The non-XY axes torsions are fixed at values shown in the palette. The Z-axis assignment is modified by selecting one of the torsions from the list in the palette.
Next Z-Section	Changes the value of the Z-axis torsion to the next value and obtains the contours for that section. This is applicable only if **Display Z-Sections** is already selected.
Prev Z-Section	Changes the value of the Z-axis torsion to the previous value and obtains the contours for that section. This is applicable only if **Display Z-Sections** is already selected.
Other Options	Redefines the contour property to be used.
Set Contour Levels	Displays a dialog box, allowing a change in specifications that control contour levels, the number of levels, and the value of each level. Specifications are only applied if **Fixed Level** is not selected.
Automatic Levels	Adjusts contour levels to obtain 10 equally spaced levels between the lowest and the highest values of the property being contoured. When this tool is selected, contours are automatically readjusted after manipulation, using the lowest and highest values in the current 2D section.

Table 28. Contour Plot Palette (Continued)

Selection	Description
Fixed Levels	Uses the current values for contour levels. Subsequent manipulation of contours has no effect until this selection is turned off.
Display Grid Points	Toggles the display of grid points over the contour plot.
Select Subset of Structures	Defines a subset of structures involved in the current 2D contours by selecting grid points. Each grid point represents a unique structure in the search file.
Redraw	Toggles the automatic redrawing of plots. When selected, the contour plot is redrawn according to the current settings whenever a change is made to the plot through the use of other palette tools.
Write Plot File	Writes the currently displayed plot to an output device to create a hard copy.
Exit Plots	Clears the Contour Plot palette from the screen.

Sample Procedure — Using the Plots Palette

Complete the following exercise to become familiar with the basics of using the Plots palette and, specifically, the **Contour Plot** selection. This exercise uses search1.msf and the grid2.csr file that was generated in the second Grid Scan search exercise in Chapter 5.

1. Open search1.msf.

Display the **File** menu and select **Open.** From the scrolling list in the File Librarian, select search1.msf.

Select the **Replac**e button, then select the **Open** button. The structure search1.msf is displayed in the viewing area.

2. Start the Analysis application.

From the **Applications** menu, select **Analysis**. A dialog box is displayed permitting the selection of the type of input file to use for analysis. Select:

Search File (.csr)

Select the **OK** button. A File Librarian dialog box is displayed with a scrolling list of available search files.

Select the file grid2.csr, then select the **Open** button.

From the Analysis palette, select **Show Table**.

A potential energy table containing all 144 conformations (scrolling table) is displayed. The Analysis and Plots palettes replace the Modeling palette and supplement the display of the Geometry palette. A tag associated with search1 is displayed in the upper-left corner of the viewing area and a potential energy is displayed in the upper-right corner.

3. Display a contour plot.

From the Plots palette, select **Contour Plot.** A dialog box is displayed, permitting the selection of the property to plot.

Select the option:

Potential Energy

Select the **OK** button. A dialog box is displayed, allowing the choice of the torsion angles to plot.

Select the **Torsion Angle for the X Axis** option:
2:c3[1]-C4[1]-O9[1]-H13[1]

Select the **Torsion Angle for the Y Axis** option:
3:c4[1]-C5[1]-O11[1]-H12[1]

Select the **Apply** button. The Contour Plot palette and the plot are displayed. The contour levels indicate the potential energy for each conformation.

4. Smooth color to reflect energy values of contour levels.

Move the cursor into the command line of the Molecule window behind the plot window.

Type the command:

```
>  set color smooth
```

This is the command equivalent to selecting **Blue to Red Smooth Range** from **Color Definitions** in the **Preferences** menu. Changes may not show until **Redraw** is selected in the Contour Plot palette.

5. Display grid points on the plot.

From the Contour Plot palette, select **Display Grid Points.** The tool is checked and highlighted.

From the Contour Plot palette, select **Redraw.** The plot is redisplayed with grid points. The colors of the contour levels are changed so the low energy conformation levels are represented by blue and the high energy conformation levels are represented by red.

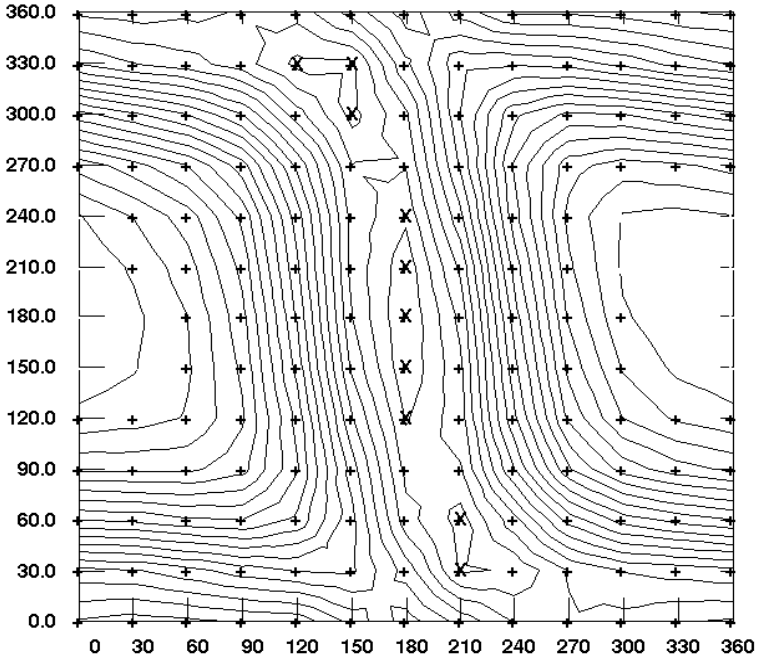
Redraw remains checked and highlighted. The plot is redrawn each time a change is made.

6. Select conformations with the lowest potential energy values.

From the Contour Plot palette, select **Select Subset of Structures**. The message line reads:

```
No structures selected
```

Move the mouse so the cursor is over a grid point located in the lowest energy contour. Click the left mouse button. The grid point is marked with an X, indicating the conformation has been selected.



Grid Points Selected (X) in Lowest Energy Area

Continue selecting grid points (conformations) located in or near the lowest energy contour level. When all conformations are selected, move the mouse until the cursor is outside the plot but still inside the plot window. Click the left mouse button. The selection process is concluded. The message line indicates the number of structures that have been selected.

From the Contour Plot palette, select **Exit Plots.** The plot and the Contour Plot palette are removed from the screen.

7. Save selected structures.

From the Analysis palette, select **File Options/Filters.** A dialog box is displayed offering several options for processing files.

Select the option:

Save Selected Conformations

Select the **OK** button. A dialog box is displayed, offering options for saving the conformation.

Select the option:

.csr File

Select the **OK** button. A File Librarian dialog box is displayed. Enter the name grid2_low_energy for the file to contain the structure subset.

Select the **Save** button. The conformations are saved as the new file grid2_low_energy.csr.

8. Display the structures contained in the new file.

From the Analysis palette, select **Use Selected Structures.** The selection is checked and highlighted.

Select **First Structure.** The first structure of the selected subset of structures is displayed.

Several times select **Next Structure.** Each conformation in the selected set of structures is displayed.

9. Exit the Analysis application.

From the Analysis palette, select **Exit Analysis**. The Analysis and Plot palettes are removed from the screen. The Modeling palette is redisplayed.

The search1 structure is displayed in the viewing area. The associated tag and energy are no longer displayed.

10. Reset the atom colors.

With the mouse in the Molecule window, type the command:

```
>  Set color atom
```

The structure is redisplayed in the original atom colors. The coordinates are *not* saved in a .msf file.

Additional Processing of Conformations

Conformations can be processed to update pre-existing search or dynamics files without running a search procedure. For example, when a structure does not converge to a real minimum, additional cycles of energy minimization can be carried out.

In the Analysis palette, the **File Options/Filters** selection provides access to further processing functions. In addition, this selection provides options for filing and tagging operations. When **File Options** is selected, a dialog box is displayed with a set of options. Table 29 lists and briefly describes the options.

Table 29. File Options/Filters Dialog Box

Option	Description
Change File	Closes the current input file, and allows another file to be selected for analysis. The file can be a binary search file, ASCII search file, or dynamics trajectory file. The specified file must correspond to the current MSF.
File Summary	Lists information about the current analysis file in the Textport. The information contains the input file name, lowest and highest energy structures, and the number of conformations currently selected.
Write MSF File	Creates a new MSF file for the conformation in the viewing area, after presenting file output options.
Convert a .csr file to .DCD format	Creates a dynamics trajectory file, corresponding to the specified search file. The search file (*filename*.csr) is not modified.
Concatenate Files	Displays a series of File Librarian dialog boxes to select an output file and input files to concatenate and write to the output file.
Redefine Tags	Displays a scrolling list dialog box to select a tag to rename. After the tag is selected, a dialog box is displayed to enter the new tag name.

Table 29. File Options/Filters Dialog Box (Continued)

Option	Description
Show Tags	Displays a scrolling list dialog box containing a sequential list of tags for all conformations in the current analysis file. When a tag is selected, the corresponding conformation is displayed in the viewing area.
Build RSF File	Constructs a rotational isomeric-state statistical weights file (*filename*.RSF) by analyzing the conformations in the input file for probable states of the various defined torsion angles using the conformational energies.
Save Sorted Conformations	Save the conformations in the input file into a new output file in a sorted order.
Process Conformations	Processes conformations by performing energy minimization and/or dynamics simulations for conformations contained in a previously generated search file (*filename*.csr).
Save Selected Conformations	Saves all the currently selected conformations into either a new search (.csr) file or a set of new MSFs. In the second case, the names of the new MSFs are automatically assigned by the program. The names are derived from the tags of each selected conformation.

Process Conformations is the selection to choose to read conformations from an existing search or dynamics trajectory file and perform calculations on each conformation in the file. This selection does not require any torsions to be defined.

When **Process Conformations** is selected, a File Librarian is displayed to choose an input structure file. Then another File Librarian is displayed to choose the output structure file. After the files are specified, a dialog box asks if a tag should be assigned to identify the set of conformations. A tag is useful to identify the procedure used to generate a particular set of conformations. The tag remains part of the conformation definition throughout the search and analysis procedures.

Finally, the calculations that process the conformation are selected from a dialog box. The calculations that can be applied to each structure include CHARMm energy, CHARMm energy minimizations, and CHARMm burst dynamics. In addition, a CHARMm command script can be applied.

When the **CHARMm Burst Dynamics for Each Structure** option is selected, a quick dynamics simulation is performed on each conformation if the energy of the conformation does not exceed a specified limit. This option is always calculated before an energy minimization is performed.

When this option is set, a dialog box is displayed to specify the temperature, number of steps, and energy upper limit to be employed in the calculation If the initial energy of the conformation exceeds the upper limit, the burst dynamics calculation is not performed.

When the conformations are processed, the CHARMm energy for each conformation is displayed in the upper right corner of the viewing area. The tag identifying each conformation, is displayed in the upper-left corner. To terminate calculation, click any mouse button. Processing is interrupted and a dialog box is displayed to specify if the calculations should be continued or terminated

Using Overlay Structures

When you select **Overlay Structures** from the Analysis palette, the Overlay Structures palette is displayed. This palette has selections that create and manipulate overlaid structures in the viewing area. When this palette is open, the Analysis, Plots, and Geometry palettes are not available.

Selections on this palette enable comparison of structures, clustering of the structures into groups, least squares fitting of structures using optimal multiple superposition and simultaneous display of all the structures. Table 30 lists and briefly describes the palette selections.

Sample Procedure — Using Overlay Structures

Complete the following exercise to become familiar with **Overlay Structures** functionality. This exercise uses the results of the Random Sampling search completed in Chapter 5 on the structure, search1. The Random Sampling search used all six torsions contained in search1.msf for its search procedure. Each torsion was sampled 25 times within a rotational range of 60°. The results of this search are stored in the search file random.csr.

1. Open search1.msf.

Display the **File** menu and select the **Open** function.

Select the file search1.msf from the scrolling list.

Table 30. Overlay Structures Palette

Selection	Description
Clear ID	Clears identification tags from a structure.
Select Atom Subset for Display...	Governs the atoms that are to be included in the molecular display. Default entry is All.
Select Atom Subset for Fit...	Provides options for selecting a subset of atoms to be used in fitting procedures. Does not have to be the same subset as that used for display.
Least Squares Fit to Reference	Performs a least squares fit procedure on each conformation using a reference conformation. Saves the new coordinates for all conformations into a new search file. Opens that file as the analysis file. The reference conformation employed is the one previously stored in the structure buffer using **Store Structure.** If no structure is stored in the buffer, the first conformation is taken to be the reference conformation.
Least Squares Fit to Common Frame	Performs a multiple least squares fit procedure for all structures in the search file, reorienting all structures to a common framework based on three or more user-defined atoms.
Structures Only Selected	Performs a multiple least squares fit procedure for selected structures in the search file, reorienting all selected structures to a common framework based on three or more user-defined atoms.
Display All	Displays all conformations in the search file.
Display Selected	Displays all selected conformations in the search file.
Single Step	Displays the next conformation.
Current Colors	Colors all atoms according to the current set of display colors.
Color by Energy	Assigns a color value to atoms (1 through 14) that matches the energy value.
Color by Clusters	Assigns a color value to an entire structure according to the cluster to which it belongs.
Generate Clusters	Classifies all conformations in the analysis file into families by comparing them on the basis of interatomic distances, torsion angles, or least squares optimal superposition.
Get Cluster	Selects a cluster for display.
All Clusters	Displays the nuclei of all the clusters.
Next Cluster	Displays all conformations of the next cluster in sequence.

Table 30. Overlay Structures Palette (Continued)

Selection	Description
Previous Cluster	Displays all conformations of the previous cluster in sequence.
Plot Overlaid Structures	Produces a plot file on the screen of the molecular display in one of the following formats: HP-GL, PostScript, or QUANTA plot file.
RMSD Table	Opens the Pairwise RMSD Calculations palette with selections for determining calculations and formatting of an RMSD table. The table contains all pairwise RMSD values for the selected conformation set.
Compare Files	Compares all conformations in the analysis file with all conformations in a specified search or dynamics file. The comparison is performed on the basis of defined torsion angles or interatomic distances. This selection is available only when torsions or interatomic distances are defined.
Exit Overlay Structures	Clears the Overlay Structures palette from the screen. Returns to the Analysis palette.

Select the **Replace** button and then the **Open** button. The structure search1 is displayed in the viewing area.

2. Start Analysis.

Display the **Applications** menu. Select the **Analysis** function. A dialog box is displayed permitting you to select the type of input file to analyze.

Select the option:

Search File (.csr)

Select the **OK** button. A File Librarian dialog box is displayed listing the available search files in the current directory.

Select the file random.csr from the scrolling list.

Select the **Open** button. The Analysis Property Table, listing all the random.csr conformations and their respective potential energies, is displayed in the viewing area in a separate window. The Analysis and Plots palettes replace the Modeling palette and supplement the display of the Geometry palette.

The search file random.csr is used as the file for analysis. The first conformation from this file is displayed in the viewing area and is tagged as search1.msf;1.

3. Select Atoms to define a common framework.

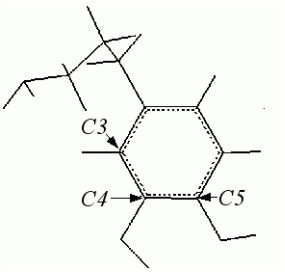
From the Analysis palette, select **Overlay Structures**. The Analysis, Plots, and Geometry palettes are removed from the screen. The Overlay Structures palette is displayed.

From the Overlay Structures palette, select **Select Atom Subset for Fit...** The Overlay Structures palette is removed and the Select Atoms and Selection Utilities palettes are displayed. These palettes are identical to the Active Atoms and Active Atoms Utilities palettes (described in *QUANTA Basic Operations*). From the Select Atoms palette, select **Pick Atom** and **Include.**

Pick the following atoms by clicking each:

atom C3 PHEN:1
atom C4 PHEN:1
atom C5 PHEN:1

Each atom is identified in the viewing area with a corresponding label and a colored dot.



From the Select Atom palette, select **Finish.** The selection palettes are removed and the Overlay Structures palette is redisplayed.

4. Display all conformations to common frame.

From the Overlay Structures palette, select **Least Squares Fit to Common Frame**. After a short interval, all conformations in random.csr are displayed with each structure showing the three selected atoms in the same location. All other atoms are placed accordingly.

Visual inspection of the overlaid structures show the areas of conformational space that have been sampled during the search. A new file

containing modified coordinates corresponding to the overlay, random_ovrly1.csr, is created and becomes the current analysis file.

This new .csr file contains the same molecular conformations as the previous file, but each molecule has been rotated and translated as a whole to obtain an optimum fit for the three atoms selected to define the common frame.

Select **Exit Overlay Structures.** The Overlay Structures palette is removed from the screen. The Analysis, Plots, and Geometry palettes are redisplayed. The first conformation (search1_msf:1) is displayed in the viewing area.

Creating 2D Plots and Making Selections

A variety of 2D plots are available with this application, supplying a variety of tools for visualizing data and for selecting subsets of structures based on property values. Plots are created in a separate window with their own pull-down menus, containing additional tools for plot manipulation. Different properties can be plotted and union and intersection tools allow conformation selection to span multiple properties.

Properties available for plotting are those originally present in the search or dynamics file and those defined using the torsions, interatomic distances, and calculate properties tools. Values for these properties may be assigned to the x axis and/or y axis of two-dimensional scatter plots.

Using a Potential Energy Trace to Create a Subset of Conformations

Conformations generated during the random sampling search include the base structure. To analyze only the conformations that result from the search, create a subset that excludes the base structure and includes all others.

Sample Procedure — Create a Conformation Subset

Complete the following exercise to create a subset of search1 conformations for analysis. Before proceeding, be sure that the Analysis applica-

tion is active, the file search1.msf is displayed in the viewing area, and random_ovrly1.csr is the current search file. If you have completed the previous section of this chapter, you are ready to continue with this exercise.

1. Display a potential energy trace.

From the Plots palette, select **Trace**. A dialog box is displayed, permitting the selection of a property to plot.

Select the option:

Potential Energy

Select the **OK** button. The trace plot is displayed. The x axis represents the number of conformations. The y axis represents potential energy values for the conformations. The plot shows a sharp decrease in energy from the first conformation to the second.

2. Define conformations to be generated in the random sampling search.

In the Plot window, display the **Trace Tools** menu. Select **Select X Range**.

Click on the point in the plot representing the second conformation in random_overly1.csr.

Click the point in the plot representing the last conformation in random_ovrly1.csr. Selected points are marked with a plus sign.

A message in the upper-left corner of the viewing area reads:

```
Number Selected = 25
```

The selection of the conformational subset is completed.

Display the Plot Window **File** menu and select the **Quit** function. The plot is removed from the screen.

3. Save the subset of conformations.

From the Analysis palette, select **File Options/Filters.** A dialog box providing options for manipulating files is displayed. Select the option:

Save Selected Conformations

Select the **OK** button. A new dialog box is displayed.

Select the option:

.csr File

Select the **OK** button. A File Librarian dialog box is displayed.

Enter the text:

random

in the data entry field. The existing file, random.csr, is overwritten.

4. Use the new version of random.csr.

From the Analysis palette, select **File Options/Filters.** A dialog box is displayed, offering several options.

Select the option:

Change File

Select the **OK** button. A dialog box is displayed, asking what type of file to use for analysis.

Select the option:

Search File (.csr)

Select the **OK** button. A File Librarian is displayed listing the available search files in the current directory.

Select the file random.csr from the scrolling list.

Select the **Open** button. The updated random.csr becomes the current search file. The tag in the upper-left corner of the viewing area shows search1_msf:2, indicating the second conformation, from the original file is now the first conformation in the search file.

5. Display a potential energy trace of the new file.

Display the Plots palette and select **Trace**... A dialog box is displayed offering an opportunity to select the property to plot.

Select the option:

Potential Energy

Select the **OK** button. The trace plot is displayed. Compare it with the previous plot.

Display the Plot Window **File** menu, select the **Quit** function. The Plot window is removed.

Creating a Range Map

Selecting **Range Map** from the Plots palette creates a plot for visually assessing the variation of torsion angles or interatomic distances in a collection of conformations. The x axis in the range map represents the torsion angle number or interatomic distance. The y axis represents the torsion angle value or interatomic distance. The plot consists of a set of horizontal tick marks where each mark represents a value of a property in each conformation. When the values are plotted, the range spanned by each torsion angle or interatomic distance is evident, and the density of tick marks help to visualize the number of conformations having a torsion angle or interatomic distance in a given range.

1. Select torsions for plotting.

From the Analysis palette, select **Torsions.** The Torsions palette supplements the display of the Analysis, Plots, and Geometry palettes.

From the Torsions palette, select **Define All Torsions.** The message line displays:

```
No of Torsions= 6 Families=6
```

The following torsion definitions are reported in the textport:

```
1       1tor1   C1      C2      C14     C15     1
2       2tor2   C3      C4      O9      H13     1
3       3tor3   C4      C5      O11     H12     1
4       4tor4   C2      C14     C15     C16     1
5       5tor5   C14     C15     C16     N21     1
6       6tor6   C15     C16     N21     H22     1
```

Select **Exit Torsions.** The Torsions palette is cleared from the screen. **Range Map** is activated (but not selected).

2. Display a range map.

From the Plots palette, select **Range Map**. A plot is displayed showing six torsions and the associated conformations that constitute the range of torsional values sampled in each minimized structure.

Using Scatter Plots

Scatter Plot creates a plot of any two properties for all conformations in the file under analysis. When **Scatter Plot** is selected, a dialog box is displayed, permitting the choice of the property to be assigned to the x axis. A second dialog box is then displayed, permitting the choice of the property to be assigned to the y axis.

The plot consists of a set of points. Each point represents a conformation with the corresponding property values given by the coordinates of that point. When the plot is displayed in the viewing area, the Scatter Plot palette is displayed. It is used to change the plot dimensions, make structure selections, and make a hard copy of the plot.

When you are observing cyclical characteristics of torsion rotations in 2D space plots, select the scale and range carefully. A conformation showing an angle of 1° and another conformation showing an angle of 360° are placed at extreme ends of a plot if a scale of 0° to 360° is chosen. In reality, there is only 1° difference between these two conformations.

Alternatively, data could be displayed showing a 360° rotational range using a -180° to 180° scale. The effects of repeated 360° rotations can be displayed as a scatter plot using a cumulative scale. In this situation, for example, torsion angle at 30° after one full rotation would be shown on the plot as 390°. Plotting search results using a different 360° rotational scale makes it possible to change the location of conformations in a plot and compare the same search results using a different format.

Table 31 lists and briefly describes the palette selections.

Sample procedure — Scatter Plot

Complete the following exercise to become familiar with generating and manipulating a scatter plot.

1. Display a scatter plot.

Table 31. Scatter Plot Palette

Selection	Description
Select Structure	Selects structures by picking corresponding data points in the plot. Click the <F1> key to exit this tool.
Select X Range	Selects all structures lying within the range of designated x values. This range is defined by picking data points in the plot.
Select Y Range	Selects all structures lying within the range of designated y values. This range is defined by picking data points in the plot.
Select X-Y Range	Selects all structures lying within a rectangular area of the plot. This rectangle is defined by picking data points in the plot.
Select Window	Selects all structures displayed in the plot window. When automatic X and Y scales are in effect, this tool is labeled **Select All** and selects all plotted structures. When zooming or manual scaling are in effect, this tool is labeled **Select Window** and only selects the structures currently visible in the plot window.
Change X-Y Property	Changes the property plotted on the X axis and/or Y axis without having to exit the Scatter Plot palette and reenter to define new specifications.
Next X Property	Displays the scatter plot with the next property for the x axis.
Prev X Property	Displays the scatter plot with the previous property for the x axis.
Next Y Property	Displays the scatter plot with the next property for the y axis.
Prev Y Property	Displays the scatter plot with the previous property for the y axis.
Use Energy Based Squares	Displays each datapoint in the scatter plot as a rectangle whose size is inversely related to the energy of the conformation (the larger the size of the rectangle, the lower the energy of the conformation). If this toggle is off, all data rectangles have the same size.
Color by Energy	Colors each data point (rectangle) by the energy of the conformation, using a smooth range of colors: blue corresponds to the lowest energy and red corresponds to the highest energy.
Color by Selection	Colors data rectangles using a two color system, one for selected conformations and the other for unselected conformations.
Show Trails	Toggles the display of either lines joining points in the scatter plot or plotting points only.
Cumulative Scale	Adjusts each torsion value by plus or minus 360 degrees, so that the modulus of the numerical difference between values of a given torsion angle for two adjacent conformations does not exceed 180 degrees.

Table 31. Scatter Plot Palette (Continued)

Selection	Description
180 Degree Scale	Adjusts the torsion values to lie within the range of -180° to +180°.
360 Degree Scale	Adjusts the torsion values to lie within the range of 0° to 360°.
Full Torsion Scale	Uses the full torsion scale either from -180° to 180° or from 0° to 360°, depending on current scale type. This tool has no effect if **Cumulative Scale** is used.
Set Scale	Changes the scale of the x axis and y axis. A dialog box is called that permits the entry of minimum and maximum values for each axis.
Reset Scale	Resets the scale to automatic scale value. This selection is equivalent to using the Auto X Scale/Auto Y Scale tools. **Reset Scale** does not alter the manual scales already defined by the most recent **Zoom, Set X Scale**, or **Set Y Scale**. If manual scales are defined, a rectangle is displayed showing the area covered by the manual scales, if it is contained within the plot.

From the Plots palette, select **Scatter Plot.** A dialog box is displayed to permit selection of the property for the x axis.

Select the option:

Torsion Angle

Select the **OK** button. A dialog box is displayed to permit you to select the torsion to display along the x axis.

Enter the value:

Enter>: tor2(1)

Select the **OK** button. A dialog box is displayed to permit you to select the property to be displayed on the y axis.

Select the option:

Torsion Angle

Select the **OK** button. A dialog box is displayed to permit you to select the torsion to display along the y axis.

Enter the value:

Enter>: tor3(1)

Select the **OK** button. The Scatter Plot is displayed.

2. Change the torsion value scale of the x and y axes.

From the Plot window, open the **Scatter Tools** menu**.**

Select **Set 360 deg Scale**. The scale of both axes are changed to 0 to 360. The location of the conformations in the plot change, based on the new scale.

Display the **File** menu and select **Quit.**

Using Correlation Maps

A scatter plot generally shows several regions sampled by a conformational search. Different regions may correspond to different energies and have different statistical weights. Statistical weights are not indicated in the scatter plot.

A correlation map adds a third dimension to a 2D plot by using contours to delineate regions of statistical weight based on the Boltzmann factor. The more dense the contours, the higher the statistical weight of a region. These regions can also be identified by color if the color definition is set to a smooth range. In this case, red represents regions of the highest statistical weight and blue represents regions of the lowest statistical weight. The empty spaces represent zero statistical weight.

Any two properties for all conformations can be plotted including:

- Kinetic energy
- Potential energy
- Total energy
- Torsion angles
- Interatomic distances
- Radius of gyration
- Dipole moment

When the plot is displayed in the viewing area, a palette is displayed offering selections to change the plotting properties of the x and y axes, alter the format used to label intervals on these axes, and generate of a hard copy of the plot.

Table 32 lists and briefly describes the palette selections.

Table 32. Correlation Map Palette

Selection	Description
Select Property Pair	Changes x/y axis properties without having to exit the Correlation Map palette and reenter to define new specifications.
Options	Changes the format of the x and y scale values, using a standard Fortran format notation.
Write Plot File	Writes currently displayed plot to an output device to create a hard copy.
Exit Correlation Map	Clears the Correlation Map palette from the screen.

Sample Procedure — Generating a Correlation Map

Complete the following exercise to become familiar with the procedure for generating a correlation map.

1. Display a correlation map.

Display the Plots palette and select **Correlation Map.** A dialog box is displayed to permit selection of the property to be displayed on the x axis.

Select the option:

Torsion Angle

Select the **OK** button.

Enter the value:

Enter>: tor5(1)

Select the **OK** button. A dialog box is displayed to permit selection of the torsion to display along the y axis.

Enter the value:

Enter>: tor6(1)

Select the **OK** button. The correlation map and the Correlation Map palette are displayed.

2. Smooth color to reflect statistical weights of contour levels.

Move the cursor into the QUANTA window. Enter the following in the command line:

```
>  set color smooth
```

This is the command equivalent of selecting **Blue to Red** in the **Color Definitions** pull-right menu of the **Preferences** menu. The colors of the contour levels are changed so that the low statistical weight conformation levels are represented by blue and the high statistical weight conformation levels are represented by red.

3. Change the property plotted in the map.

Display the Correlation Map palette and select **Select Property Pair**. A dialog box is displayed to permit selection of the property to be displayed on the x axis.

Select the option:

Potential Energy

Select the **OK** button. A dialog box is displayed to permit selection of the property to be displayed on the y axis.

Select the option:

Potential Energy

Select the **OK** button. The plot is redrawn, showing potential energy correlation between the torsions tor5(1) and tor6(1).

4. Reset structure colors.

Enter the following in the command line:

```
>  set color atom
```

This is the command equivalent to selecting **Reset All in the Color Definitions** pull-right menu of the **Preferences** menu. All structure colors are returned to their default colors.

5. Clear the correlation map from the screen.

Display the Correlation Map palette and select **Exit Correlation Map.** The map and the palette are removed from the screen.

6. **Exit the Analysis application.**

   From the Analysis palette, select **Exit Analysis.** The Analysis and Plots palettes are removed from the screen and the Modeling palette is redisplayed.

Comparing Conformations

It is possible to compare all structures in the file under analysis with structures in other search or dynamics files. The files selected for comparison do not need to be generated from the same MSF.

When data in both files pertains to the same structure, the comparison can be performed on the basis of either a set of defined distances, torsions, or least squares superposition. If the two files contain coordinates for different structures, the comparison may be done on analogous torsion angles. Distances cannot be compared.

In either case, each structure in the file under analysis is sequentially compared to each structure in the selected search or dynamics file. Pairwise root mean square differences (rmsd) between the structures in the file under analysis and the comparison file are computed and presented in an rmsd histogram upon completion of the calculation.

The x axis of this plot represents the rmsd. The Y axis represents the number of structure pairs with a given rmsd. From these results, it is possible to determine if the current analysis file contains any structures that are similar to any of those in the comparison file, or if the analysis file contains structures that are not found in the comparison file.

Whenever an rmsd between a given structure pair is below a specified threshold, the structure pairs are considered similar to each other. The classification of structure pairs as similar or dissimilar can be changed by supplying a different rmsd threshold value. Decreasing the threshold value, decreases the number of similar structure pairs. Increasing the threshold increases the number of similar structure pairs.

The threshold can be reset repeatedly and the selections can be reclassified. The selected structures, corresponding to the last comparison, can be saved and reviewed with any of the browsing selections or with plots.

The **Compare Files** palette contains selections for setting rmsd threshold values and structure selection. Table 33 lists and briefly describes the palette selections.

Table 33. Compare Files Palette

Selection	Description
Select Similar Structures	Selects all structures in the file under analysis that are similar (within the prescribed rmsd threshold) to any structure in the template file.
Select Unique Structures	Selects all structures in the file under analysis that differ from all the structures in the template file (unique structures).
Set Threshold	Defines the RMSD threshold used for comparison calculations.
Exit Compare Files	Exits the Compare Files palette.

Sample Procedure — Comparing Structures from Two Files

Complete the following exercise to become familiar with procedures for comparing conformations from two .csr or .DCD files.

1. Open CCK7.msf.

Display the **File** menu and select **Open**. A File Librarian dialog box is displayed.

Select CCK7.msf from the scrolling list of files.

Select the **Replace** button.

Select the **Open** button. The structure CCK7.msf is displayed in the viewing area.

2. Start Analysis.

Display the **Applications** menu and select **Analysis**. A dialog box is displayed to permit selection of the type of input file to analyze.

Select the option:

Search File (.csr)

Select the **OK** button. A File Librarian dialog box is displayed listing the available search files in the current directory.

Select flip.csr from the scrolling list. Select the **Open** button. The Analysis and Plots palettes replace the Modeling palette and supplement the Geometry palette. The search file flip.csr is used as the file for analysis.

3. Select torsions for plotting.

Display the Analysis palette and select **Torsions**. The Torsions palette supplements the display of the Analysis, Plots, and Geometry palettes.

From the Torsions palette, select **Define All Torsions.**

The message line reads:

```
No of Torsions = 15 Families =4
```

Definitions for these torsions are listed in the textport.

Select **Exit Torsions.** The Torsions palette is cleared from the screen.

4. Compare results of the Boltzmann Jump and Peptide Flip searches.

From the Analysis palette, select **Overlay Structures.** The Overlay Structures palette is displayed.

From the Overlay Structures palette, select **Compare Files.** A dialog box is displayed asking if the structures contained in the *.csr* files for comparison are chemically identical. If the same base MSF is used for comparison, the structure is considered chemically identical. If different MSFs are used, the structures are not chemically identical, and the File Librarian prompts for the name of the second MSF.

Select the **Yes** button. A dialog box is displayed, to choose the basis for comparison.

Select the option:

Torsion Angles option

Select the **OK** button. A dialog box is displayed to permit selection of the type of file template to compare with the current file (flip.csr).

Select the option:

Search File (.csr)

Select the **OK** button. A File Librarian dialog box is displayed.

Select jump.csr from the scrolling list.

Select the **Open** button. An rmsd histogram showing the pair-wise rms differences between the structures in flip.csr and jump.csr is displayed along with the Compare Files palette. By default, the rmsd threshold is set at:

$$rms_{min} + 0.3(rms_{max} - rms_{min})$$

where rms_{min} and rms_{max} are the lowest and highest rmsd values found in the histogram.

5. Define comparison specifications.

From the Compare Files palette, select **Select Similar Structures.** The following information is reported in the textport:

```
Number of Selected SIMILAR Structures= 9
```

Since a user-defined threshold value is not specified, the number of similar structures is based on the default threshold value.

Using the default threshold value, there are nine structures in flip.csr that are similar to structures in jump.csr. The rest of the structures in flip.csr are different from all the structures in jump.csr.

Select **Exit Compare Files.** The Compare Files palette and the histogram are removed from the screen.

Select **Exit Overlay Structures.** The Overlay Structures palette is removed from the screen. The first conformation is displayed in the viewing area.

6. Display similar structures in a scatter plot.

From the Plots palette, select **Scatter Plot.** A dialog box is displayed to permit selection of the property to be displayed on the x axis.

Select the option:

Torsion Angle

Select the **OK** button. A dialog box is displayed to permit selection of the torsion to display along the x axis.

Enter the value:

Enter: tor2(1)

Select the **OK** button. A dialog box is displayed to permit selection of the property to be displayed on the y axis.

Select the option:

Torsion Angle

Select the **OK** button. A dialog box is displayed to permit selection of the torsion to display along the y axis.

Enter the value:

Enter>: tor3(1)

Select the **OK** button. The plot is displayed along with the Scatter Plot palette. Plus signs on the plot indicate conformations from the Peptide Flip search that are similar to some conformations from the Boltzmann Jump search.

From the Scatter Plot palette, select **Quit**. The plot and the palette are removed from the screen. The Analysis and Plot palettes are redisplayed.

From the Analysis palette, select **Exit Analysis.** The Analysis and Plot palettes are removed from the screen and CCK7 is displayed in the viewing area.

Creating and Analyzing Clusters

The Analysis application provides the ability to classify all conformations in a .csr or .DCD file into families by calculating all pair-wise RMS differences among structures using torsion angles, interatomic distances, or least squares superposition as the basis for calculation. By selecting **Generate Clusters** in the Overlay Structures palette, you begin the process. When you make this selection, the Generate Clusters palette is displayed. Table 34 lists and briefly describes the palette selections.

To classify structures into conformational groups, you must establish an rmsd threshold as a similarity criterion. The following algorithm for classifying and grouping conformations is employed in QUANTA:

Table 34. Generate Clusters Palette

Selection	Description
Define Torsions	Opens the Torsion palette for defining torsions. Must be used if clustering is to be based on torsion angles.
Define Distances	Opens a dialog box that presents options for defining interatomic distances. Must be used if clustering is to be based on distances.
Clustering by Torsions	Performs clustering using torsion angles as the defining property.
Clustering by Distances	Performs clustering using interatomic distance as the defining property.
Clustering by Coordinates	Performs clustering using least squares superposition as the defining property.
Use Atom Subset...	Allows subset specification for clustering by coordinates
Compute RMS Differences	Calculates rmsd between all pairs of conformations that have been specified by other selections.
Show RMS Distribution	Displays a histogram plotting the distribution of rmsd.
Set RMS Clustering Threshold	Allows setting of a user-defined clustering threshold. When selection is made, a dialog box opens providing information on the current threshold, the range of values, and the number of clusters generated with this threshold. Values can be accepted and applied, or rejected.
RMS Difference Map	Displays an rmsd map where the difference between the i-th and the j-th conformation is indicated by a triangular plot with a continuous range of colors employed to indicate the rmsds.
Write RMS Differences	Writes calculated RMSD to a file (*filename*.rms).
Exit Generate Clusters	Exits the Generate Clusters facility and returns to Overlay Structures.

1. Choose a basis for comparing conformations by computing rmsd values in selected torsion angles or interatomic distances. Alternatively, compute rmsd values in Cartesian coordinates of all atoms or a subset of atoms using least squares superposition. If there are N conformations to be analyzed, there are N(N-1)/2 rmsd that can be computed.
2. Choose a threshold value of rms below which a conformational pair will be considered similar for the purpose of the algorithm. If the rmsd between a pair conformations exceeds the threshold, they are considered dissimilar.

3. From a list of all conformations, choose the lowest energy conformer and remove it from the list. This conformation is taken to be the nucleus of a cluster.
4. Examine the rmsds between this conformation and the remaining conformations in the list. If a conformation in the list has an rmsd with this nucleus that is less than the chosen threshold value, it is removed from the list and added to the first cluster. this is done until there are no more structures in the list with an RMS less than the threshold. At this point, a cluster is formed containing all the conformations similar to the lowest energy conformation.
5. Choose the lowest energy conformer from the remaining conformers. Start a new cluster with this structure as the nucleus.
6. Repeat Step 4 until the definition of the new cluster is complete.
7. Repeat Steps 5 and 6 until there are no more conformations in the list.

This sequence of steps describes the algorithm principles used in generating clusters. In practice, the list of generated clusters is not presented in sorted order. This means that cluster 1 is not necessarily the one that contains the lowest energy structure.

In the clustering procedure, the energy of the conformers plays a key role in the choice of the nucleus of each cluster. When the nucleus is chosen, the membership is decided purely on the basis of the rmsds with the nucleus. Note that two conformers with an rmsd between them exceeding the threshold may enter the same cluster as long their RMSDs with the nucleus is less than the threshold.

As the process proceeds, a range of possible threshold values is sampled. At the end of the process, a plot of the number of clusters versus the threshold values is displayed. The final number of clusters that are obtained from the procedure depends on the value of the threshold that you close by double clicking on the value in the plot. Alternatively, you may enter a threshold value in a dialog box by selecting **Set RMS Clustering Threshold** in the Generate Clusters palette.

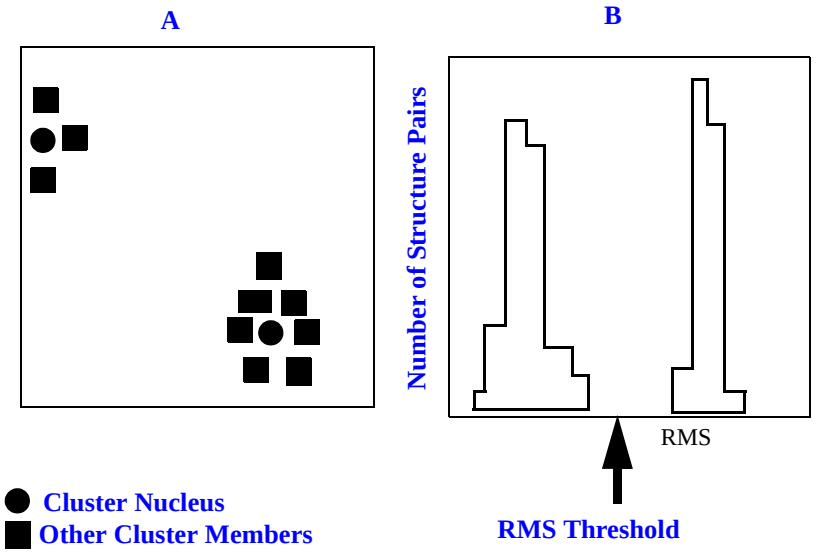
At the end of the process, results can be displayed in a rmsd histogram. This histogram plots the number of pairs of conformations with a given rmsd against the rmsd. it delineates the range of rmsds seen in the set of conformations.

Large peaks in this histogram correspond to rms values found among large number of conformational pairs. The distribution of conformational

pairs gives some idea of the range of variation in the rmsd. It helps you to choose a threshold value for the rmsd. The threshold value is needed for performing cluster analysis.

The histogram provides valuable clues regarding what may be too small or too large a value for the threshold. If a pronounced peak is seen in the histogram corresponding to an rms value of R_{max}, setting a threshold equal to R_{max} will not successfully separate most of the conformational pairs into different clusters. Very few clusters are generated, in this case. Setting a threshold at a smaller value than R_{max} gives rise to a reasonable number of clusters. The conformational pairs giving rise to rmsds of roughly R_{max} are partitioned into different clusters.

As an example, consider the simple case of a collection of conformations that fall into two clusters such as the ones schematically shown in diagram A. The rmsds between members of the same cluster are much smaller than the rmsds between members belonging to different clusters. Therefore, you might expect the rmsd histogram to have two peaks, one corresponding to intracluster rmsds and the other corresponding to intercluster rmsds. (See diagram B.) If you expected to get the two clusters seen in the drawing, then the threshold must be set somewhere between the two peaks, as indicated by the bold arrow.



Selecting Clusters

Select Clusters appears active in the Generate Clusters palette after structures are clustered. If this selection is made, a dialog box is displayed.

All the structures belonging to a specific cluster or the lowest energy structures from all clusters can be selected. The latter option selects all the cluster nuclei. When this is done, selected structures are viewed using browsing tools or with graph analysis tools.

Sample Procedure — Generating and Analyzing a Cluster

Complete the following exercise to become familiar with the procedures for generating and analyzing clusters. Before you begin, be sure that CCK7.msf is displayed in the viewing area.

1. Start Analysis.

Display the **Applications** menu and select **Analysis**. A dialog box is displayed to permits selection of the type of input file to analyze.

Select the option:

Search File (.csr)

Select the **OK** button. A File Librarian dialog box is displayed, listing the available search files in the current directory.

Select flip.csr from the scrolling list. Select the **Open** button. The Analysis and Plots palettes replace the Modeling palette and supplement the Geometry palette. The search file flip.csr is used as the file for analysis.

2. Define clustering specifications.

From the Analysis palette, select **Overlay Structures**. The Overlay Structures palette is displayed.

From the Overlay Structures palette, select **Generate Clusters.**

From the Generate Clusters palette, select **Define Torsions**. The Torsions palette is displayed.

From the Torsions palette, select **Define All Torsions**. Then select **Exit Torsions.** The Torsions palette is removed and the Generate Clusters palette is redisplayed.

From the Generate Clusters palette, select **Clustering by Torsions**. The textport lists threshold values and the number of clusters that will be produced for each value. When the calculation is complete, a histogram of the number of clusters versus the clustering threshold is displayed in a separate window. The message line reads:

```
Select a cluster threshold. Double click on graph.
```

3. Select a threshold value.

Double-click the threshold value you want to select in the x axis. The textport reports the trial clustering threshold value and the number of clusters that will be generated. If there are more than five clusters, repeat the process, selecting a lower threshold value.

From the Generate Clusters palette, select **Set RMS Clustering Threshold**. A dialog box is displayed that reports the current threshold value, the maximum and minimum rmsds, and the number of clusters that will be generated with these values. The information should coincide with the information in the textport for your last threshold value selection.

Select the **OK** button. The dialog box is removed.

Select **Exit Generate Clusters.** The histogram and the Generate Clusters palette are removed from the screen. The Overlay Structures palette is redisplayed.

4. Display generated clusters.

From the Overlay Structures palette, select **All Clusters.** The structures, representing the nuclei structures, are overlaid in the viewing area.

Select **Next Cluster**. The conformations composing the first cluster are overlaid in the viewing area. The number of structures selected is reported in the upper-left corner of the viewing area. A maximum of 150 structures can be shown simultaneously.

Again select **Next Cluster.** The conformations composing the second cluster are overlaid in the viewing area.

Select **Next Cluster** several more times. Clusters continue to be displayed in ascending order, based on the order in which they were defined.

Select **Exit Overlay Structures**. The palette is removed from the screen.

5. **Exit the Analysis application.**

   From the Analysis palette, select **Exit Analysis.** The Analysis and Plots palettes are removed from the screen and the Modeling palette is redisplayed.

Analyzing Trajectory Files

The Dynamics Animation application simulates the natural motion of a molecular structure, producing a set of coordinates and velocities that describe atomic motions of that structure through time. Using CHARMm dynamics, calculations are performed for heating, equilibration, and simulation stages of dynamics. The results of these calculations are saved in trajectory files. A separate trajectory file is created for each stage.

In the heating stage of a dynamics run, as kinetic energy is added to the structure, CHARMm potential energy and total energy increases proportionately. This can be illustrated in Analysis on a plot. In the plot, the x axis indicates the number of conformations while the y axis cycles through representations of temperature, potential energy, kinetic energy, and total energy.

In the second stage of a dynamics run, equilibration is achieved by allowing the structure to evolve spontaneously for a period of time by integrating the equations of motion until the average temperature and structure remain stable. Equilibration is facilitated by periodically scaling the velocities appropriate to the desired temperature. Generally, the procedure is continued until various statistical properties of the structure become independent of time.

Potential energies decrease in value during equilibration, as do the total energy values when more stable conformations are found. Kinetic energy and temperature both fluctuate as a result of velocities being scaled to the desired temperature. Fluctuations are illustrated by creating plots, cycling

through representations of potential energy, kinetic energy, total energy, and temperature.

Simulation takes an equilibrated structure as its starting point and produces a dynamics trajectory. In a typical simulation, the trajectory traces the motion of the structure through a specific period of time. As with energy minimization, provision is made to update the nonbonded and hydrogen bonded lists periodically.

Trajectory file analysis uses the same methods as analysis of a conformational search file. You can browse through all conformations. You can inspect the results of any stage of dynamics at any time. You can create any number of two-dimensional plots and save any conformation into an MSF.

When the results of the various stages of dynamics are analyzed, you can determine the effects of a particular stage on additional properties such as dipole moment and radius of gyration. Any number of properties can be plotted using any of the trajectory files.

Analysis is not limited to one trajectory file. Trajectory files can be concatenated to concurrently view the results of all three stages of dynamics. Concatenation creates a composite *.csr* file. Since a *.csr* file stores only energy and coordinates, some dynamics-related information such as time step is lost. In the plot of a concatenated file, the x axis shows the total number of conformations contained in the files, and the y axis shows values of the property selected for plotting.

Sample Procedure — Analyzing a Trajectory File

Complete the following exercise to become familiar with the process for analyzing trajectory files. For this exercise, you need the MSF mypeptide.msf, created in Chapter 4 *of QUANTA Generating and Displaying Molecule*s. You also need the dynamics files, mypep_h.DCD, mypep_e.DCD, and mypep_s.DCD created in Chapter 1 of this book.

1. Open mypeptide.msf.

Display the **File** menu and select the **Open** function. A File Librarian dialog box opens.

Select mypeptide.msf from the scrolling list.

Select the **Replace** button, then the **Open** button. The structure mypeptide.msf is displayed in the viewing area.

2. Start Analysis and open the heating dynamics file mypep_h.dcd.

Display the **Applications** menu. Select the **Analysis** function. A dialog box is displayed to permit selection of the type of input file to analyze.

Select the option:

Dynamics File (.DCD)

Select the **OK** button. A File Librarian dialog box is displayed.

Select mypep_h.DCD from the scrolling list, then select the **Open** button. The Analysis and Plots palettes replace the Modeling palette and supplement the display of the Geometry palette. The first conformation in mypep_h.dcd is displayed in the viewing area along with the tag mypep_h.DCD:1. The message line displays:

```
Rank=1 Time Step=0.010 ps 1st conf
```

If the file mypeph.ENE is present, it is automatically read. If the .ENE file is not present in the current directory, a File Librarian is displayed to permit selection of the .ENE file from a different directory. Since the .ENE file contains conformational energies (and pressures), energy analysis cannot be done if an .ENE file is not used.

3. Display all conformations in mypep_h.DCD.

From the Analysis palette, select **Auto Browse.** Each conformation in mypep_h.DCD is automatically displayed in the viewing area in sequential order, replicating the Dynamics Animation display. The automatic browsing procedure stops when the last conformation in the trajectory file is displayed.

4. Display a trace of temperature during heating.

From the Plots palette, select **Trace...** A dialog box is displayed, to permits selection of the property to plot.

Select the option:

Temperature

Select the **OK** button. The Trace plot is displayed, showing the number of conformations on the x axis and temperature values on the y axis. The plot shows a steady increase in temperature as kinetic energy is added to the structure during the heating stage.

5. Display a trace of potential energy during heating.

Display the **Trace Tools** menu. Select the **Next Property** function. The property plotted on the y axis changes to CHARMm potential energy. The plot shows a steady increase in potential energy as kinetic energy is added to the structure during the heating stage.

6. Display a trace of kinetic energy during heating.

Select **Next Item** again. The property plotted on the y axis changes to CHARMm kinetic energy. The plot shows the steady increase of kinetic energy added to the structure during the heating stage.

7. Display a trace of total energy during heating.

Select **Next Item** again. The property plotted on the y axis changes to CHARMm total energy. The plot shows a steady increase in total energy as kinetic energy is added to the structure during the heating stage.

8. Redisplay a trace of temperature during heating.

Select **Next Item** again. The y axis property returns to temperature.

Display the **Trace File** menu and select **Quit**. The plot is removed from the screen.

9. Open the equilibration dynamics file mypep_e.DCD.

From the Analysis palette select **File Options/Filters.** A dialog box is displayed to permit changing of the trajectory file in use.

Select the option:

Change File

Select the **OK** button. A dialog box is displayed, prompting for the selection of the type of input file.

Select the option:

Dynamics File (.DCD)

Select the **OK** button. A File Librarian dialog box is displayed.

Select mypep_e.DCD and then select the **Open** button. The first conformation in mypep_e.DCD is displayed in the viewing area along with the tag mypep_e.DCD:1. The message line displays:

```
Rank=1 Time Step=0.610 ps 1st conf
```

10. Display a trace of potential energy during equilibration.

From the Plots palette select **Trace.** A dialog box is displayed, permitting the selection of the property to plot.

Select the option:

Potential Energy

Select the **OK** button. The Trace plot is displayed, showing the number of conformations on the x axis and CHARMm potential energy values on the y axis. The plot shows a steady decrease in potential energy during the equilibration stage.

11. Display a trace of kinetic energy during equilibration.

Display the **Trace Tools** menu and select **Next Item**. The property plotted on the y axis changes to CHARMm kinetic energy. The plot shows fluctuations in kinetic energy during the equilibration stage.

12. Display a trace of total energy during equilibration.

Select **Next Item** again. The property plotted on the y axis changes to CHARMm total energy. The plot shows a drop in total energy as more stable conformations are found during the equilibration stage.

13. Display a trace of temperature during equilibration.

Select **Next Item** again. The property plotted on the y axis changes to temperature. The plot shows that although temperatures fluctuate during the equilibration stage, an overall decrease in temperature is realized.

Display the **File** menu. Select **Quit.** The plot is removed from the screen.

14. Open the simulation dynamics file mypep_s.DCD.

Display the Analysis palette. Select **File Options/Filters.** A dialog box is displayed to permit the trajectory file in use to be changed.

Select the option:

Change File

Select the **OK** button. A dialog box is displayed to permit the type of input file to be chosen.

Select the option:

Dynamics File (.DCD)

Select the **OK** button. A File Librarian dialog box is displayed.

Select mypep_s.DCD, then select the **Open** button. The first conformation in mypep_s.DCD is displayed in the viewing area along with the tag mypep_s.DCD:1. The message line reads:

```
Rank=1 Time Step=1.210 ps 1st conf
```

15. Display a trace of potential energy during simulation.

Display the Plots palette and select **Trace...** A dialog box is displayed to permit selection of the property to plot.

Select the option:

Potential Energy

Select the **OK** button. The Trace palette and the plot are displayed, showing the number of conformations on the x axis and CHARMm potential energy values on the y axis. The plot shows a steady decrease in potential energy during the simulation stage.

16. Display a trace of kinetic energy during simulation.

Display the **Trace Tools** menu and select **Next Property**. The property plotted on the y axis changes to CHARMm kinetic energy. The plot shows a steady increase in kinetic energy during the simulation stage.

17. Display a trace of total energy during simulation.

Select **Next Property** again. The property plotted on the y axis changes to CHARMm total energy. The plot shows a fluctuation in total energy during the simulation stage. Although these fluctuations appears quite dramatic when plotted, the scale of the Y axis shows these fluctuations are small.

18. Display a trace of temperature during simulation.

Select **Next Property** again. The property plotted on the y axis changes to temperature. The plot shows a steady increase in temperature during the simulation stage.

Display the **File** menu and select the **Quit** function. The plot is removed from the screen.

19. Define the Dipole Moment property for analysis.

From the Analysis palette, select **Calculate Properties.** A dialog box is displayed to permit selection of the properties to define. The list of options available varies, depending the structure being studied, for example, a polymer versus a monomer.

Select the option:

Dipole Moment

Select the **OK** button. The dialog box is removed from the screen and the dipole moment property is defined for all the conformations included in mypep_s.DCD.

20. Display a trace of dipole moment during simulation.

From the Plots palette, select **Trace**. The dialog box is displayed to permit selection of the property to plot. Since the dipole moment property is now defined, the **Dipole Moment** option has been added to the dialog box.

Select the option:

Dipole Moment

Select the **OK** button. The Trace palette and the plot are displayed, showing the number of conformations on the x axis and dipole

moment values on the y axis. The plot shows a decrease in dipole moment as more stable conformations are found during the simulation stage.

Display the **File** menu, and select the **Quit** function. The plot is removed from the screen.

21. Create a single file for analysis from the .DCD files.

From the Analysis palette, select **File Options/Filters.** A dialog box is displayed to permit changing of the trajectory file in use. Select the option:

Concatenate Files

Select the **OK** button. A File Librarian dialog box is displayed to permit creation of a new search file (.csr), containing the concatenated trajectory files.

Enter the name mypep_all and select the **Save** button. A dialog box is displayed to permit selection of the type of input file. Select the option:

Dynamics File (.DCD)

Select the **OK** button. A File Librarian dialog box is displayed to permit selection of the first file to be concatenated.

Select mypep_h.DCD, then select the **Open** button. A dialog box is displayed to permit selection of the type of input file. Select the option:

Dynamics File (.DCD)

Select the **OK** button. A File Librarian dialog box is displayed to permit selection of the second file to be concatenated.

Select mypep_e.DCD, then select the **Open** button.

Select the option:

Dynamics File (.DCD)

Select the **OK** button. A File Librarian dialog box is displayed to permit selection of the third file to be concatenated.

Select mypep_s.DCD, then select the **Open** button. A File Librarian dialog box is displayed to permit selection of the type of input file. Select the **Exit** button, indicating there are no other files to concate-

nate into mypep_all.csr. The new file is created and opened as the current analysis file.

22. Display all of the conformations contained in mypep_all.csr.

From the Analysis palette, select **Auto Browse.** Each conformation in mypep_all is automatically displayed in the viewing area in sequential order, starting with the first conformation of mypep_h.DCD. The automatic browsing procedure stops when the last conformation in mypep_s.DCD is displayed.

23. Define the radius of gyration property for analysis.

From the Analysis palette, select **Define Properties.** A dialog box is displayed to permit selection of the properties to define.

Select the option:

Radius of Gyration

Select the **OK** button. The dialog box is removed from the screen and the radius of gyration property is defined for all the conformations included in mypep_all.csr.

24. Display a radius of gyration trace of all dynamics stages.

From the Plots palette, select **Trace...** A dialog box is displayed to permit selection of the property to plot. Select the option:

Radius of Gyration

Select the **OK** button. The Trace palette and the plot are displayed, showing the total number of conformations on the x axis and the radius of gyration values on the y axis.

25. Exit Analysis.

From the **Trace File** Menu, select **Quit**.

From the Analysis palette, select **Exit Analysis**. The Analysis application is exited.

Summary

The Analysis application provides tools to read or compute physical properties of conformations generated during conformational search or dynamics simulations. It sorts, selects, and compares the structures using available physical properties data. Trends, correlations, and extremes of the data are revealed through a variety of plotting options. Additional tools are provided so that you may view the structures one at a time or view them overlaid after a least squares fitting procedure is applied**.** You may also cluster structures into subsets that have common conformational features.

You enter the Analysis applications either directly from the **Applications** menu or from the Conformational Search palette. Up to 6000 structures can be handled by the Analysis application, but performance is best when smaller datasets are used.

When you start Analysis**,** conformations in the input file are read and a table of properties is created. Each conformation has an identifying tag that consists of a meaningful prefix and a number that designates its order at the time it was created in the set of conformations.

Initially, the only defined property is energy. In some cases even energy values may not exist. If you only want to browse through the set of conformations, you need no additional properties. To sort structures or plot information about them, you must define additional physical properties such as torsions or distances.

When a set of physical properties is defined, you can use the plots facility and its powerful selection tools to look for structures that satisfy the criteria you specify. The power of the Analysis selections tools is enhanced when you use **Intersection** from the Analysis palette. This tool allows selections to be based on more than one property.

In the Analysis palette, the **File Options** selection provides access to further processing functions. In addition, this selection provides options for filing and tagging operations.

The Plots palette provides tools to generate plots of various types from the data supplied in the analysis file. Within the Analysis application, you can generate the following type of plots: property traces, histograms, box range maps, range maps, scatter plots, backbone plots, contour plots, and pair distribution histograms.

After you have specified the properties to be plotted, the plot appears in a new window with its own tools to change plot dimensions, inquire about specific conformations, make conformation selections, and create hard copy output.

When you select **Overlay Structures** from the Analysis palette, the Overlay Structures palette is displayed. This palette has selections that create and manipulate overlaid structures in the viewing area. Selections on this palette enable comparison of structures, clustering of the structures into groups, least squares fitting of structures using optimal multiple superposition, and simultaneous display of all the structures.

It is possible to compare all structures in the file under analysis with structures in other search or dynamics files. The files selected for comparison do not need to be generated from the same MSF.

When data in both files pertain to the same structure, the comparison can be performed on the basis of a set of defined distances, torsions, or least squares superposition. If the two files contain coordinates for different structures, the comparison may be done on analogous torsion angles. Distances cannot be compared.

In either case, each structure in the file under analysis is sequentially compared to each structure in the selected search or dynamics file. Pairwise root mean square differences between the structures in the file under analysis and the comparison file are computed and presented in an rmsd histogram when the calculation is completed.

From these results, it is possible to determine if the current analysis file contains any structures that are similar to any of those in the comparison file, or if the analysis file contains structures that are unique compared with all the structures in the comparison file.

The Analysis application also provides the ability to classify all conformations into families by calculating all pair-wise RMSDs among structures using torsion angles, interatomic distances, or least squares superposition. By selecting **Generate Clusters** in the Overlay Structures palette, you begin the process. When you make this selection, the Generate Clusters palette is displayed.

Trajectory file analysis uses the same methods as analysis of a conformational search file. You can browse through all conformations. You can inspect the results of any stage of dynamics at any time. You can create any number of two-dimensional plots and save any conformation into an MSF.

When the results of the various stages of dynamics are analyzed, you can determine the effects of a particular stage on additional properties such as dipole moment and radius of gyration can be accessed. Any number of properties can be plotted using any of the trajectory files.

Analysis is not limited to one trajectory file. Trajectory files can be concatenated to concurrently view the results of all three stages of dynamics. Concatenation creates a composite .crs file. However, in the process, some dynamics-related information is lost. In the plot of a concatenated file, the x axis shows the total number of conformations contained in the files, and the y axis shows values of the property selected for plotting.

7. Examining Molecular Similarity

This chapter describes methods available to compare two or more molecular structures by overlaying the structures in the viewing area. Read this chapter if you plan to evaluate molecules for structural similarity.

Structures needed for the exercises in this chapter are built using the 2D Sketcher (ChemNote) application and are minimized using CHARMm. If you are not familiar with ChemNote, read Chapter 2 of *QUANTA Generating and Displaying Molecules.* Read Chapter 3 of the same book for information on energy minimization procedures.

The Molecular Similarity application provides a visual comparison of two or more molecular structures by overlaying these structures in the viewing area. Comparisons can be made between:

- Different structures
- Different conformations of the same structure
- Different parts of the same structure

The only requirement is that each structure must be contained in a unique MSF. The application does not permit use of different generations of the same MSF.

The procedure used in Molecular Similarity to compare structures is divided into four basic steps:

1. Load the structures to be compared.
2. Define atom equivalences in these structures, that is, establish atom matches.
3. Perform a fitting operation.
4. Analyze the results.

Each structure is identified by the name of its corresponding MSF. One structure is identified as the target or fixed structure. All other structures are working or moving structures. Although only one structure can be designated as the target at a given time, the target definition can be

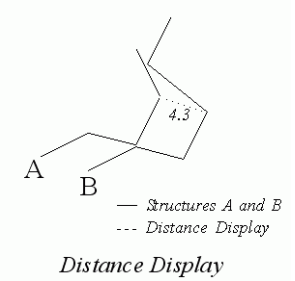
changed from within the application using **Define Target Molecule** in the Molecule Similarity palette.

The Molecular Similarity application makes no assumptions about the equivalency of atoms on different structures. Equivalency is defined through your input. When atom equivalencies are defined, they can be used for any fitting operation.

Fitting operations employ either a rigid or flexible method. If a rigid method is used, working structures are translated and rotated to obtain an optimum fit with the target structure. If a flexible fitting method is used, conformations of the working structures are changed to obtain an optimum fit.

Fitting operations use a least squares fitting algorithm. The least squares algorithm computes the optimum translation and rotation to be applied to the moving structure such that rmsd of the fit over the specified pairs of equivalent atoms is an absolute minimum. Although the algorithm deals only with a pair of structures at a time, fitting tools available in the application can handle a collection of molecules.

Several methods are provided for analyzing the results of a fitting operation. The target and working structures can be alternately displayed to examine the differences between the overlaid structures. Distance displays that include dashed lines and a numeric value between equivalent atoms on two structures provide indications of the quality of the fit.



Distance Display

Fitted structures can also be analyzed by computing the volume of overlap between the structures. If similarity calculations do not generate an optimal fit between structures, atom equivalences can be modified and fitting operations can be repeated.

Creating Structures

In this section, use the 2D Sketcher (ChemNote), accessed through the Applications menu, to create three structures: quinupramine, imipramine, and amitriptyline. Use these structures as test molecules in the exercises that introduce the Molecular Similarity application.

The minimum energy conformation of these structures is determined and saved before fitting operations proceed. The structures imipramine and amitriptyline are minimized using simple CHARMm minimization procedures. The structure quinupramine is a more rigid structure. Run Conformational Search using the Grid Search method to ensure that the lowest energy conformation of quinupramine is found. If you are not familiar with Conformational Search, see Chapters 3-5. When the Grid Search is completed, run Analysis to select and save to the MSF the lowest energy conformation**.** If you are not familiar with the Analysis applications see Chapter 6.

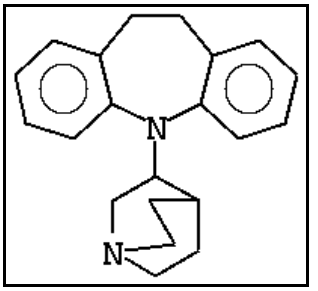
Sample Procedure — Building Molecules

Complete the following exercise to build and minimize the test molecules.

1. Build and save the quinupramine structure.

Display the **Applications** menu and select **Builders.** From the pull-right menu that opens, select **2D Sketcher**. The ChemNote window is displayed over the QUANTA window.

Sketch the structure quinupramine illustrated below.



Sketch of Quinupramine

Display the **ChemNote File** menu. Select the **Save** function. A File Librarian dialog box is displayed.

Enter the name quinpr *i*n the File Librarian name field. Select the **Save** button. A dialog box is displayed, offering several methods for the adjustment of partial charges.

Accept the default value:

```
The desired net charge is: 0.000
```

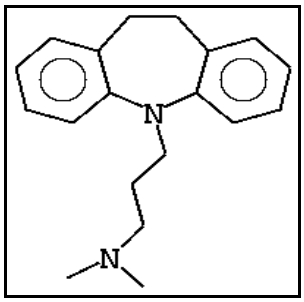
Accept the default option:

```
CT, CH1E, CH2E, CH3E, C5R, C6R, C5RE, C6RE, HA types
```

Select the **OK** button. The dialog box is cleared from the screen. The partial charges are changed, and the new charges are saved in the quinpr.mol file. The sketch remains in the ChemNote window, providing the opportunity to use it as a base for creating a .mol file for imipramine.

2. Build and save imipramine.

Sketch the structure imipramine illustrated below.



Sketch of Imipramine

Display the **ChemNote File** menu. Select the **Save** function. A File Librarian is displayed.

Enter the name imip in the File Librarian name field. Select the **Save** button. A dialog box is displayed, offering a list of methods for the adjustment of partial charges.

Accept the default value:

```
The desired net charge is: 0.000
```

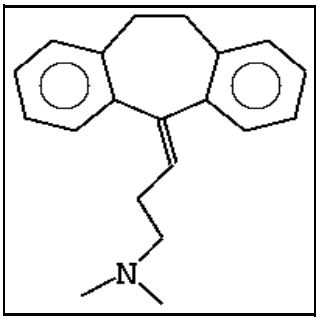
Accept the default option:

```
CT, CH1E, CH2E, CH3E, C5R, C6R, C5RE, C6RE, HA types
```

Select the **OK** button. The dialog box is cleared from the screen. The partial charges are changed, and the new charges are saved in the imip.mol file. The sketch remains in the ChemNote window, providing the opportunity to use it as a base for creating a .mol file for amitriptyline.

3. Build and save amitriptyline.

Sketch the structure amitriptyline illustrated below.



Sketch of Amitriptyline

Display the **ChemNote File** menu. Select the **Save** function. The File Librarian dialog box is displayed.

Enter the name *amit* in the File Librarian name field. Select the **Save** button. A dialog box is displayed, offering several methods for the adjustment of partial charges.

Accept the default value:

```
The desired net charge is: 0.000
```

Accept the default option:

```
CT, CH1E, CH2E, CH3E, C5R, C6R, C5RE, C6RE, HA types
```

Select the **OK** button. The dialog box is cleared from the screen. The partial charges are changed, and the new charges are saved in the amit.mol file. The sketch remains in the ChemNote window.

Display the **ChemNote File** menu. Select the **Return to Molecular Modeling** function. The ChemNote window is removed from the screen, revealing the Modeling window.

The file amit.mol is automatically converted to amit.msf in the Molecular Modeling mode. When amit.msf creation is completed, a dialog box is displayed offering options to save the structure.

Select the option:

```
Use the new molecule amit.msf only
```

Select the **OK** button. The structure amitriptyline is displayed.

4. Energy minimize amitriptyline.

Display the **CHARMm** menu and select **Minimization Options.** A dialog box is displayed in which you can select a minimization technique and specify associated parameters.

Select the option:

```
Steepest Descents
```

Enter the values:

```
Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. The new minimization parameters are established. The steepest descents method is chosen primarily to improve conformation.

From the Modeling palette, select **CHARMm Minimization**. The CHARMm calculation is started. The cursor changes from an arrow to a watch. Minimization is completed when the watch is restored to an arrow. The minimized energy results are displayed in the upper-right corner of the viewing area and in the textport.

5. Change parameters and continue minimizing *amitriptyline.*

Display the **CHARMm** menu and select the **Minimization Options** function. A dialog box is displayed in which you can select a minimization technique and specify associated parameters.

Select the minimization option:

```
Adopted-Basis Newton Raphson
```

Enter the values:

```
Number of Minimization Steps: 800
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. New minimization parameters are established.

Select **CHARMm Minimization**. The minimization process proceeds using the new parameters. It stops when the rms force reaches zero.

6. Save minimization results in a file.

From the Modeling palette, select **Save Changes.** A dialog box is displayed, permitting the new coordinates of amit.msf to be saved.

Select the option:

```
Overwrite amit.msf
```

Select the **OK** button. The new coordinates replace the original coordinates in amit.msf.

7. Convert imip.mol to an MSF.

Display the **File** menu and select **Import**. A File Librarian dialog box is displayed listing the available external file formats and the files available for the selected format

From the bottom scrolling list, select the data format:

```
ChemNote
```

The upper scrolling list displays the .mol files that are present in the current directory.

Select the file imip.mol from the scrolling list.

Select the **Import** button. When the creation of imip.msf is completed, a dialog box is displayed offering the option to use the structure just created.

Select the option:

```
Use the new molecule imip.mol only
```

Select the **OK** button. The structure imipramine replaces the display of amitriptyline in the viewing area.

8. Minimize imipramine.

Display the **CHARMm** menu and select **Minimization Options**. The dialog box containing minimization techniques and parameters choices is displayed.

Select the minimization option:

```
Steepest Descents
```

Enter the values:

```
Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. New minimization parameters are established.

From the Modeling palette, select **CHARMm Minimization**. The CHARMm calculation is started.

When minimization is complete, display the **CHARMm** menu again. Select **Minimization Options**. The dialog box containing minimization techniques and parameters choices is displayed.

Select the minimization option:

```
Adopted-Basis Newton Raphson
```

Enter the values:

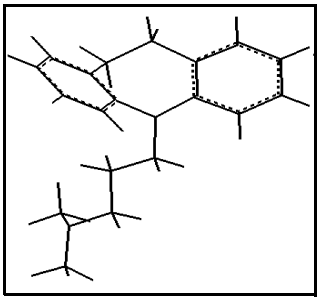
```
Number of Minimization Steps: 800
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. New minimization parameters are established.

From the Modeling palette, select **CHARMm Minimization.** The minimization process proceeds, using new parameters. It stops when the rms force reaches zero.

9. Save minimization results in a file.

From the Modeling palette, select **Save Changes.** A dialog box is displayed offering options to save the new coordinates of imip.msf.



Minimized Structure
Imipramine

Select the option:

`Overwrite imip.msf`

Select the **OK** button. New coordinates replace the original coordinates in the MSF.

10. Convert quinpr.mol to an MSF.

Display the **File** menu and select **Import**. A File Librarian dialog box is displayed listing the available external file formats and the files available for the selected format

From the bottom scrolling list, select the data format:

`ChemNote`

The upper scrolling list displays the *.mol* files that are present in the current directory.

Select the file quinpr.mol from the scrolling list.

Select the **Import** button. When the creation of quinpr.msf is completed, a dialog box is displayed offering the option to use the structure just created.

Select the option:

`Use the new molecule quinpr.mol only`

Select the **OK** button. The structure quinupramine replaces the display of imipramine in the viewing area.

11. Minimize quinpr.msf.

Display the **CHARMm** menu and select **Minimization Options**. The dialog box containing the minimization techniques and parameters is displayed.

Select the minimization option:

```
Steepest Descents
```

Enter the values:

```
Number of Minimization Steps: 50
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. The new minimization parameters are established.

From the Modeling palette, select **CHARMm Minimization.** The CHARMm calculation is started.

When the minimization is completed, display the **CHARMm** menu and again select **Minimization Options**. The dialog box containing the minimization techniques and parameters is displayed.

Select the minimization option:

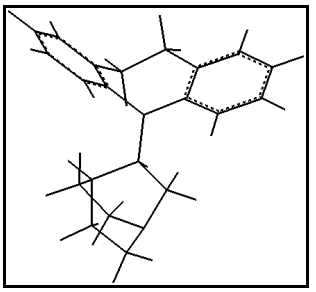
```
Adopted-Basis Newton Raphson
```

Enter the values:

```
Number of Minimization Steps: 800
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. New minimization parameters are established.

From the Modeling palette, select **CHARMm Minimization.** The minimization process proceeds using new parameters. It stops when the rms force reaches zero.



Minimized Structure
quinupramine

12. Save minimization results in a file.

From the Modeling palette, select **Save Changes.** A dialog box is displayed, permitting you to save the new coordinates of quinpr.msf. Select the option:

```
Overwrite quinpr.msf
```

Select the **OK** button. New coordinates replace the original coordinates in the MSF.

13. Start Conformational Search.

Display the **Applications** menu and select **Conformational Search**. The Conformational Search palette replaces the Modeling palette and supplements the display of the Geometry palette.

The textport reports:

```
Number of Fragments=1
Number of Ring Bonds=26
Number of Rotatable Bonds=1
```

14. Define torsions for the search.

From the Conformational Search palette, select **Torsions.** The Torsions palette supplements the display of the Conformational Search and Geometry palettes.

From the Torsions palette, select **Define All Torsions.**

The message line displays:

```
No of Torsions=1 Families=1
```

From the Torsions palette, select **Exit Torsions.** The palette is removed from the screen.

15. Select the Grid Scan search method and define specifications.

From the Conformational Search palette, select **Setup Search.** All of the search method selections become active. **Search Setup** is checked and highlighted**.**

From the Conformational Search palette, select **Grid Scan.** A dialog box is displayed allowing you to edit the values that define rotational range and torsion angle increments within this range.

Select the option:

```
Absolute Values
```

Accept the default values:

```
0.00, 330.0, 30.0
```

Select the **OK** button. A dialog box is displayed, offering options for processing each conformation.

Select the options:

```
CHARMm Minimization for each structure
Keep Minimized Conformation (do not reset)
Display Each Structure
```

Select the **OK** button. The dialog box is cleared from the screen. **Grid Scan** remains checked and highlighted. All other method selections are dimmed.

16. Setup minimization for the search.

Display the **CHARMm** menu and select **Minimization Options**. The dialog box containing the minimization techniques and parameters is displayed

Select the minimization option:

```
Adopted-Basis Newton Raphson
```

Enter the values:

```
Number of Minimization Steps: 100
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.01000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. New minimization parameters are established.

17. Start the Grid Scan search.

From the Conformational Search palette, select **Do Search.** The selection is checked and highlighted. A File Librarian dialog box is displayed.

Enter the name quinpr for the file that holds the search results.

Accept default values for tag specifications:

```
Tag prefix: quinpr.msf
Starting Tag Number: 1
```

Select the **New** button. The search procedure is started. New conformations are displayed in the viewing area every few seconds. When the search is completed, the last conformation is displayed in the viewing area and the message line displays:

```
GRID SEARCH Completed
```

18. Start Analysis.

From the Conformational Search palette, select **Analysis.**

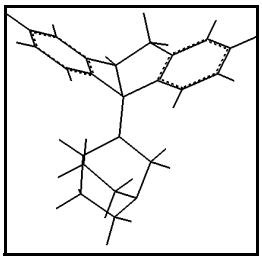
The Conformational Search application is temporarily exited. The Analysis and Plots palettes replace the Conformational Search palette. The Geometry palette remains in view.

The first conformation from the search is displayed. The conformation is labeled in the upper-left corner: quinpr_msf:1. There is also an informational line indicating the total number of conformations generated in the search and the number that is currently selected. The line reads:

```
Number Selected 0/12.
```

19. Display the conformation with the lowest potential energy.

From the Analysis palette, select **Lowest Energy Structure.** The conformation is displayed in the viewing area along with its associated energy and label. The actual conformation may vary, depending on the initial energy of the minimized structure.



Lowest Energy Conformation of Quinupramine

20. Save the conformation.

From the Analysis palette, select **File Options/Filters.** A dialog box is displayed offering the option to write the coordinates of the displayed conformation to an MSF.

Select the option:

`Write MSF File`

Select the **OK** button. A File Librarian dialog box is displayed.

Select quinpr.msf from the scrolling list.

Select the **OK** button. A dialog box is displayed asking if it is OK to overwrite the existing file.

Select the **Yes** button. The low energy conformation replaces existing coordinates in quinpr.msf.

21. Exit Analysis and Conformational Search.

From the Analysis palette, select **Exit Analysis.** The Analysis and Plots palettes are removed from the screen and the Conformational Search palette is redisplayed.

From the Conformational Search palette, select **Exit Conformational Search.** The Conformational Search palette is removed from the screen and the Modeling palette is redisplayed.

22. Reject the changes made during the search.

From the Modeling palette, select **Reject Changes**. All modeling changes made during the search and minimization are discarded. The lowest energy conformation as a result of the search remains saved in the file quinpr.msf.

Managing Structures

The Molecular Similarity palette provides selections to:

- Rotate and translate selected structures
- Control the display of structures
- Change target structure definitions
- Compare molecules

Rotation, translation, and display selections in the Molecular Similarity palette are used during atoms picking and after fitting procedures to obtain a clearer display of structures. If additional fitting operations are needed to resolve other similarity questions, superimposed structures can be separated and the target structure assignment can be changed.

Other palette selections are used to manage the files that are generated during fitting and comparison operations. Table 35 lists and briefly describes all palette selections.

The Molecule Management Table is used to change the activity and display of structures. Clicking on the activity column of a molecule in the table toggles the activity of the molecule. Molecule activity can also be changed by clicking on any atom of a molecule in the viewing area.

The activity toggle can be disabled by selecting **Options** in the Molecule Similarity palette, opening the Other Options dialog box, and deselecting **Enable Atom Picks to Control Molecular Activity**. If this feature is enabled, a click on an atom is equivalent to clicking on the activity column of the Molecule Management Table. The molecular display momentarily highlights whenever a molecule's activity changes.

Table 35. Molecular Similarity Palette

Option	Description
Move Molecules	Moves molecules using standard fragment notation and translation dials or mouse movements.
Rotate Molecules in Place	Rotates molecules using dials. Note that xyz rotations are done relative to the model coordinate system, as indicated by the axes in the upper-left corner of the viewing area.
Flash Molecules	Alternates the display of the target and working structures in rapid succession to assist you in visualizing structures after a fitting operation.
Define Target Molecules	Changes the target molecule to the molecule in the currently selected row of the Molecular Management Table**.** The default target molecule is the first structure displayed in the viewing area.
Use MSF Bundles	Applies MSF bundles to volume calculations. Bundles are collections of MSFs that are related through their names. All MSFs in a bundle are assumed to have the same covalent structure.
Match Atoms...	Displays the Match Atoms palette for atom selection and matching.
Torsions	Displays the Torsions palette for defining torsions.
CHARMm Minimization	Performs a CHARMm minimization on the displayed structures.
Rigid Body Fit to Target	Fits each active working structure to the target structure. The result is a superimposed display of structures in the viewing area.
Rigid Body Fit to Common Frame	Fits each structure to a common frame, consisting of a set of points corresponding to mean positions of all matched atoms.
Torsional Flexible Fit	Fits each active working structure to the target structure by varying the rotatable torsion angles.
Cartesian Flexible Fit	Fits each active working structure to the target structure using CHARMm energy minimization. Cartesian coordinates of all atoms in the working structures are treated as variables during optimization.
Options	Provides a series of preference settings and specifications that influence fitting operations.
Calculate Volume...	Calculates the Van der Walls volumes for target and working structures. Volume maps are displayed at the end of the calculation.
Compare Volume Maps...	Compares volume maps using logical operators. Two maps are combined with AND, OR, AND NOT, or EXCL to generated a third map.
Construct Volume Map Object...	Reads the specified volume file and displays a graphical object of the volume.

Table 35. Molecular Similarity Palette (Continued)

Option	Description
Undo Last	Reverts the coordinates and display back to the state prior to the last fitting operation, CHARMm minimization, or use of **Rotate Molecules in Place** or **Move Molecules**.
Undo Changes	Reverts the coordinates and display back to the state when last saved with **Save Changes.**
Save Changes	Saves all the current coordinates to an MSF. The method for creating the MSF can be selected using **Options...** from this palette.
Revert Changes	Reinstates the coordinates previously saved into a set of MSFs using **Save Changes**.
Exit Molecular Similarity	Exits Molecular Similarity and returns to the Modeling application.

The display of a molecule in the viewing area may be toggled from visible to invisible, or the reverse, by clicking on the appropriate cell of the **Visible** column for the molecule of interest.

Take time to note areas of the structures you are studying that appear to be similar. This will help you identify candidates for atom matching procedures. To make them easy to locate, label candidate atoms in each structure using **Label Atoms** in the **Draw** menu. Also use **Bond Style** in the **Draw** menu to alter color and vector representations of bonds, so that double and triple bonds are easy to identify.

Sample Procedure — Atom Matching Preparation

Complete the following exercise to prepare amitriptyline and quinupramine for atom matching.

1. Open amit.msf.

Display the **File** menu and select the **Open** function. A File Librarian dialog box is displayed.

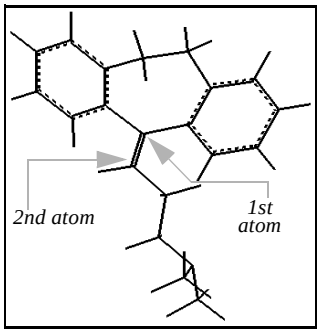
Select amit.msf from the scrolling list and then select the **Replace** button. Select the **Open** button. Amitriptyline replaces quinupramine in the viewing area.

2. Display the multiple bond in amit.msf.

Display the **Draw** menu and select **Bond Style**. From the pull-right menu that opens, select **Draw Half-bond Vectors**. Make a second selection, **Multi-vector Bonds**. The two aromatic rings and the double bond are now clearly visible in the viewing area.

3. Identify atoms at the double bond endpoints.

Pick the atom at each end of the double bond. An ID is displayed for each atom. Note the names of the two atoms, so that you can identify the double bond during fitting procedures.



Atom IDs in amit.msf

4. Open quinpr.msf.

Display the **File** menu and select the **Open** function. A File Librarian dialog box is displayed.

Select quinpr.msf from the scrolling list and then select the **Append** button. Select the **Open** button. Quinupramine and amitriptyline both appear in the viewing area. In the Molecule Management Table, the status of both structures is indicated as visible and active.

5. Start Molecular Similarity.

Display the **Applications** menu and select **Molecular Similarity**. The Molecular Similarity palette replaces the Modeling palette and supplements the Geometry palette.

6. Space structures in the viewing area so they do not overlap.

Display the **View** menu and select **Stamp**. This selection divides the viewing area into several smaller viewing areas. Each of the areas is stamped with only one molecule so that the structures in the viewing area no longer overlap.

7. Make amit.msf inactive.

In the Molecule Management Table, select the **Active** cell for amit.msf to make it inactive.

8. Rotate structures.

From the Molecular Similarity palette, select **Rotate Molecules in Place.** The dial emulators change to provide task-specific dials for x, y, and z rotations.

Select the **X Rotation** dial. Since quinupramine is the only active structure in the Molecule Management Table, it is the only structure to rotate about the x-axis.

Make quinupramine inactive by clicking on the structure's **activity** cell in the Molecule Management Table. Make amitriptyline active by clicking on its activity cell in the Molecule Management Table.

Select the **Y Rotation** dial. Amitriptyline rotates about the y axis.

Make quinupramine active again. Select the **Z Rotation** dial. Since both structures are now active, they both rotate about the z axis.

Select **Rotate Molecules in Place** to deselect it. The task-specific dials for x, y, and z rotation are replaced with Dial Set 1.

9. Control the display of quinpr.msf.

Click on the **Visible** cell for quinupramine in the Molecule Management Table. The structure is no longer displayed in the viewing area although it is still marked active in the table. Click again on the same **Visible** cell. Quinupramine is redisplayed.

10. Exit Molecular Similarity.

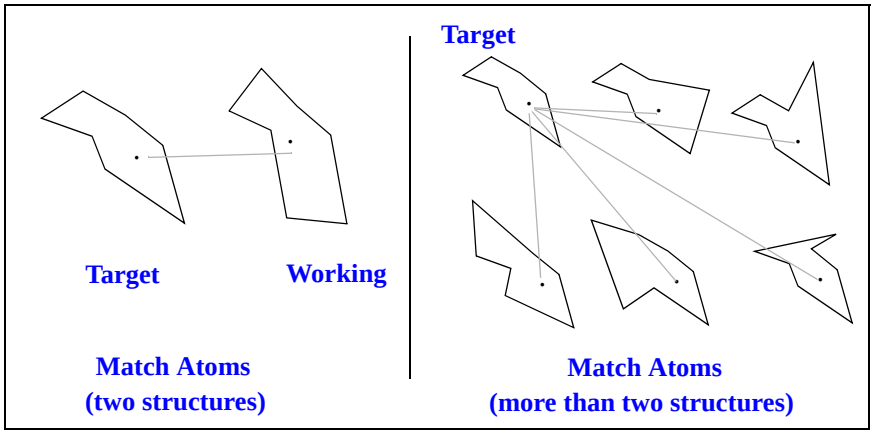
From the Molecular Similarity palette, select **Exit Molecular Similarity.** The program returns to Molecular Modeling mode.

Matching Atoms

In Molecular Similarity, the first-loaded structure is identified by default as the target structure. The target molecule has an * after its name in the Molecule Management Table. All other structures are working structures.

The target molecule is changed by selecting a row in the Molecule Management Table**,** then selecting **Define Target Molecule** on the Molecular Similarity palette.

The **Match Atoms** procedure links equivalent or near equivalent atoms on one or more working molecules to the corresponding atom in the target structure. Atom equivalences are always defined in pairs. When you use multiple working structures, equivalent atoms are individually paired with the target atom, not collectively grouped with each other. The following figure illustrates these matches.



With matches established, relationships between target and working atoms are defined, permitting sophisticated structural comparisons.

An atom equivalence list containing atom pairs is required before any type of fitting operation can be performed. A list is automatically created by picking equivalent atoms in the displayed structures. The tools needed for defining this list are contained in the Match Atoms palette. This palette opens when **Match Atoms** is selected from the Molecular Similarity palette. Table 36 lists and briefly describes the palette selections.

Table 36. Match Atoms Palette

Selection	Description
Pick Equivalent Atoms	Picks equivalent atoms in two or more structures that are not chemically identical.
Undo Last	Reverses the most recent atom pick.
End Atom Picking	Exits from the atom picking procedure with no picks saved.
Quit Atom Picking	Exits from the atom picking procedure and saves the picks for further work.
Select Residue Ranges	Selects the range of residues in structures that are chemically identical.
Select All Atom Pairs	Selects all atoms on structures that are chemically identical (same number of atoms, same atom names).
Select Subset of Atom Pairs	Selects a subset of atoms in a target structure to be matched with atoms in chemically identical working structures.
Display Atom Matches	Using dashed lines between atom pairs, displays the atom matches that have been selected for two or more structures. This is a default selection.
Display Distances	Displays as a label the distance (in angstroms) between matched atom pairs.
Remove Distant Atom Matches	Eliminates all atoms matches that are not within the cutoff distance for mutually close matches. Default cutoff distance is 0.50.
Select Mutually Close Matches	Eliminates all atom matches that are not within the cutoff distance for mutually close matches. Default cutoff distance is 0.50.
List Atom Matches	Lists atom match information in the Textport.
Read Atom Matches	Reads atom match data from an existing *.mch* file.
Save Atom Matches	Saves current atom matches to a *.mch* file.
Exit Match Atoms	Exits the Match Atoms palette, returning to the Molecular Similarity palette.

With two structure involved in a matching procedure, there are two methods available to establish atom matches:

- Alternating atom picks of equivalent atoms between target and working structures.
- First s electing all the target molecule atoms, then the corresponding atoms on the working molecule.

When more than two structures are involved in atom matching, a modification of the second matching method is used.

Sample Procedure — Matching Atoms in Two Structures

Complete the following exercise to become familiar with procedures for matching atoms in two structures. In this exercise, the structure amitriptyline is designated as the target structure and the structure quinupramine is designated as the working structure.

1. Start the Molecular Similarity application.

Display the **Applications** menu and select **Molecular Similarity**.

2. Set up Molecular Similarity options.

From the Molecular Similarity palette, select **Options.** A dialog box is displayed.

Select the options:

```
Other Options
Save the Options to File: lsq40.options
```

Select the **OK** button. The Set Other Options for Molecular Similarity dialog box is displayed.

Accept the defaults:

```
Enable Atom Picks to Control Molecular Activity
Automatically Generate New MSF File Names
Save Only Changed Molecules
Pick pairs of atoms.
Use one color for all atom matches
```

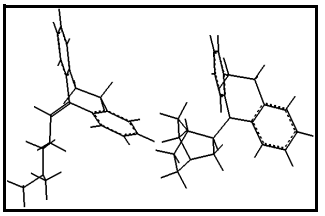
Use the default values:

```
Suffix (3 characters): LSQ
Start Counter: 1
Cutoff distance for mutually close matches: 0.5
Fraction of Upper Distances for Match Deletion: 0.7
```

Select the **OK** button.

3. Rotate structures until their views are parallel.

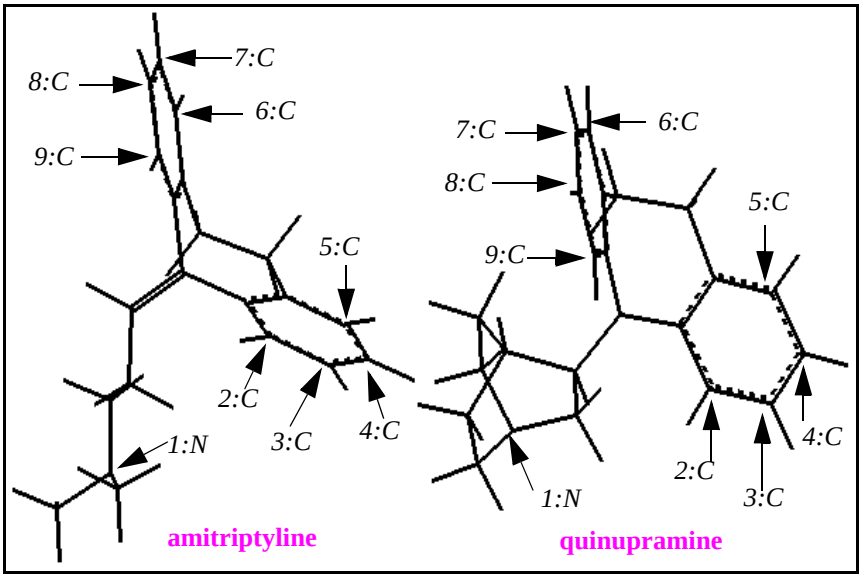
From the Molecular Similarity palette, select **Rotate Molecules in Place.** The Dial Emulator changes to provide task-specific dials for x, y, and z rotations for active molecules.



Parallel View of Structures Amitriptyline and Quinupramine

Use a combination of **Active** cell choices and x, y, and z dial rotations to adjust the position of each structure until the view of one structure is parallel to the view of the other, as in the illustration. The similarity of structure positions assists in locating equivalent atoms during atom matching procedures.

4. **Deselect Rotate Molecules in Place.**



5. Pick equivalent atoms.

From the Molecular Similarity palette, select **Match Atoms.** The Match Atoms palette supplements the display of the Molecular Similarity and Geometry palettes.

From the Match Atoms palette, select **Pick Equivalent Atoms**.

The textport displays instructions for picking atom matches:

```
All Matches Cleared!
Pick atom Pairs
Pick <End Atom/Molecule Picking> to end picking
```

From the amitriptyline molecule, pick the nitrogen atom indicated in the illustration above. The atom ID is displayed. Note that incorrect choices can be reversed by using **Undo Last** in the **Match Atoms** palette.

Pick the indicated equivalent nitrogen atom in quinupramine. The atom ID is displayed along with a dashed line to the nitrogen atom in amitriptyline.

Continue picking equivalent atoms until nine pairs are selected. Rotate structures as needed to see the atoms to be picked. Dotted lines are drawn to indicate all atom matches.

From the Match Atoms palette, select **End Atom Picking. Pick Equivalent Atoms** is turned off. The textport reports the number of atoms matched:

```
No of Matches 9
No of atom pairs= 9
```

6. List the atom matches.

From the Match Atoms palette, select **List Atom Matches**. A list of matches is displayed in the textport. As in the example below, the first column in this list indicates the number associated with that atom match. The second and third column indicate the atoms composing these matches. The fourth column indicates distance between matched atoms. The rms distance deviation over all atom matches is indicated at the end of the list.

```
1  N34:1  N30:29.28
2  C23:1  C20:29.54
3  C22:1  C21:29.56
4  C21:1  C22:29.35
```

```
5  C20:1 C23:29.20
6  C15:1 C12:29.47
7  C14:1 C13:29.78
8  C13:1 C14:29.94
9  C12:1 C15:29.79

Overall RMS deviation= 0.9548E+01
```

Actual atom names may differ, depending on the sequence in which bonds and atoms were placed when the structures were created in ChemNote. Actual rms deviation will differ if the degree of minimization for the structures was not the same.

7. Exit Match Atoms.

From the Match Atoms palette, select **Exit Match Atoms.** The Match Atoms palette is removed from the screen.

8. Exit Molecular Similarity.

From the Molecular Similarity palette, select **Exit Molecular Similarity.** The Molecular Similarity palette is replaced by the Modeling palette.

9. Reject the changes made from the Manage Atoms palette.

From the Modeling palette, select **Reject Changes.** The changes are discarded to prepare for matching atoms in three structures.

Matching Atoms in Three or More Structures

When there are more than two structures for atom matching, alternately selecting match atoms between target and working structures is no longer possible. When three or more structures are involved, matching atoms are selected in the following manner:

1. The target structure is displayed in the viewing area by itself in an enlarged size. Matching atoms on the target structure are picked.
2. The first working structure is displayed with the target structure. Corresponding atoms on the working structure are picked in the same order they were chosen on the target structure.

3. The second working structure is displayed. Corresponding atoms on this structure are picked in the same order they were chosen on the target structure.
4. The corresponding atoms on any additional structures are picked, one structure at a time.

Sample Procedure — Matching Atoms for Three or More Structures

Complete the following exercise to become familiar with the procedure for matching atoms on three or more structure. The equivalent atoms established in this exercise are used in the fitting method section of this chapter. You are automatically prompted through the steps in this procedure.

1. Open quinpr.msf, amit.msf, and imip.msf, changing the target.

Display the **File** menu and select **Mono.** When the files are opened, the structures are displayed one on top of the other.

Display the **Draw** menu and select **Open**. A File Librarian dialog box is displayed.

Select quinpr.msf, amit.msf, and imip.msf from the scrolling list. The structures will be displayed in the order selected. Quinupramine is the target molecule.

Select the **Replace** button and then the **Open** button. All three structures are displayed stacked on top of each other. The Molecular Management Table indicates all three structures are active and visible.

2. Start Molecular Similarity.

Display the **Applications** menu and select **Molecular Similarity**. The Molecular Similarity palette replaces the Modeling palette and supplements the display of the Geometry palette.

3. Setup Molecular Similarity options.

From the Molecular Similarity palette, select **Options.** A dialog box is displayed.

Select the options:

```
Other Options
Save the Options to File: lsq40.options
```

Select the **OK** button. A second dialog box is displayed.

Select the options:

```
Pick all target atoms first and then corresponding
equivalent atoms
```

Accept all other defaults selections and the default values:

```
Suffix (3 characters): LSQ
Start Counter: 1
Cutoff distance for mutually close matches: 0.5
Fraction of Upper Distances for Match Deletion: 0.7
```

Select the **OK** button.

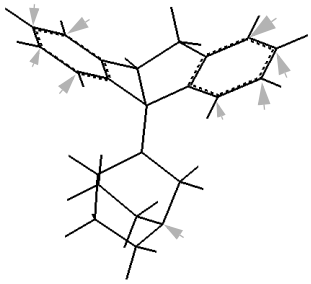
4. Select matching atoms.

From the Molecular Similarity palette, select **Match Atoms.** The Match Atoms palette appears.

From the Match Atoms palette, select **Pick Equivalent Atoms.** Only quinupramine is visible in the viewing area.

Use the Dials palette to orient and scale the structure for atom picking.

Click on each atom that is selected to match it with equivalent atoms on amitriptyline and imipramine. Refer to the illustration for designated atoms. As picking proceeds, a colored circle is placed on each picked atom.

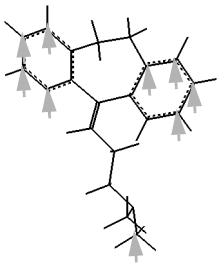


Quinupramine Atoms Picked for Matching

When all nine atoms have been picked, select **End Atom Picking** from the Match Atoms palette.

5. Select matching atoms from amitriptyline.

Amitriptyline reappears in the viewing area beside quinupramine. The first quinupramine atom requiring a matching atom has an enlarged crosshair placed on the circle that marks it.



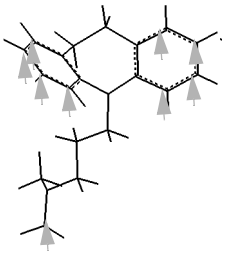
Amitriptyline Atoms Picked for Matching

Pick the corresponding atom from the amitriptyline structure. When the equivalent atom is picked, the large crosshair shifts to the next atom in the target molecule. Select the matching atom in the working molecule. Repeat this procedure until all nine matching atoms on amitriptyline have been picked. When the last matched pair is selected,

the quinupramine match atoms are no longer highlighted. Amitriptyline is removed from the viewing area and quinupramine and imipramine are both displayed.

6. Select match atoms from imipramine.

Select the imipramine match atoms in the same manner as for amitriptyline match atoms. When all match atoms are chosen, all three structures are displayed in the viewing area, with equivalent atoms on all structures connected by dashed lines.



Imipramine Atoms Picked for Matching

Pick Equivalent Atoms is turned off and the textport reports the number of atoms matched:

```
No of Matches 9
No of atom pairs= 9
```

7. List the atom matches.

From the Match Atoms palette, select **List Atom Matches.** A list of matches is displayed in the textport. The first column in this list indicates the number assigned to each match. The second and third columns indicate the atoms composing the matches The fourth column indicates the rms distance difference for each match, while the overall rms distance deviation is indicated at the end of the list.

```
1       N34:1   N30:2   9.28
2       C23:1    C20:2  9.54
3       C22:1   C21:2   9.56
4       C21:1    C22:2  9.35
5       C20:1   C23:2   9.20
6       C15:1    C12:2  9.47
7       C14:1   C13:2   9.78
8       C13:1   C14:2   9.94
9       C12:1   C15:2   9.79

Overall RMS deviation= 0.9548E+01
```

Actual atoms names may differ depending on the sequence in which bonds and atoms were placed in ChemNote when the structures were created.

8. Exit Match Atoms.

From the Match Atoms palette, select **Exit Match Atoms**. The equivalent atoms established in this exercise are used in the fitting methods section that follows.

Performing Structural Fitting

There are two types of fitting procedures available: rigid-body fitting and flexible fitting. Rigid-body fitting translates and rotates working structures to minimize the rms of the fit to the target structure. Flexible fitting modifies the conformations of the working structures to improve the quality of the fit.

Two methods of rigid-body fitting are available when an equivalence list is established: rigid body fit to common frame and rigid body fit to target.

Rigid Body Fit to Common Frame in the Molecular Similarity palette performs an optimal overlay of structures when no one particular structure has a unique role as target molecule. A common frame is created that consists of a set of points corresponding to mean positions of all matched atoms. The structures are then fit to this frame with the frame acting as the target structure. The fitting procedure is repeated each time, re-evaluating the common frame.

Rigid Body Fit to Target in the Molecular Similarity palette fits each active working structure to the target structure, providing a superimposed display of structures in the viewing area. The rms difference of the work-

ing structures, with respect to the target structure, is reported in the textport. The overall rms deviation (the sum of the rmsd of each working structure divided by the rmsd of the target structure) is reported.

There are also two methods of flexible fitting: torsional flexible fit and Cartesian flexible fit.

Torsional Flexible Fit in the Molecular Similarity palette manipulates the conformation of each working structure by suitably modifying its rotatable torsion angles. The target structure remains fixed. As the conformation is modified, it is rigid body fitted to the target, and the rms difference of the fit is evaluated. The rms difference is then minimized with respect to the torsion angles of the working structure. The rms minimization procedure is performed for one working structure at a time.

Cartesian Flexible Fit in the Molecular Similarity palette uses a CHARMm energy minimization with target constraints to achieve a fit of the working structures to the target structure. Through this process, the working structure is forced to conform to the conformation of the target structure, that is, the distances between matched pairs of atoms are effectively reduced to zero. After minimization, all target constraints are cleared.

Sample Procedure — Fitting

Complete the following exercise to become familiar with fitting procedures.

1. Fit amitriptyline and imipramine to the target structure quinupramine.

From the Molecular Similarity palette, select **Rigid Body Fit to Target.** The structures amitriptyline and imipramine are superimposed over the structure quinupramine, using picked equivalent atoms as the overlay points.

The textport reports the rms difference of each structure, with respect to the target structure, and the overall rms deviation.

```
Molecule2 RMS diff = 0.6271293
Molecule3 RMS diff = 0.7524440
No of Matches9

Overall RMS deviation= 0.6926E+00
```

2. Review results of the fitting procedure in the Textport.

Select the **Reset View** dial emulator. The structures are repositioned in the viewing area.

From the Match Atoms palette, select **List Atom Matches.** The textport reports the rms distances for each atom pair and the overall rms deviation.

The first column indicates the number of the atom pair. The second column indicates the picked atom in the target structure, and the third column indicates the equivalent atom picked in the first working structure. The fourth column (labeled 2) indicates the distance between the pair of atoms. The fifth column shows the equivalent atom in the next working structure, while the last column (labeled 3) indicates the distance of this atom from the equivalent atom in the target structure. The overall RMS deviation is the same value that was reported in the brief Textport summary at the completion of the fitting procedure.

```
No of Matches 9

        23
    1 C12:1 C15:2 0.72 C15:30.26
    2 C13:1 C14:2 0.49 C14:30.36
    3 C14:1 C13:2 0.17 C13:30.33
    4 C15:1 C12:2 0.36 C12:30.26
    5 C23:1 C20:2 0.20 C20:30.48
    6 C22:1 C21:2 0.17 C21:30.56
    7 C21:1 C22:2 0.42 C22:30.57
    8 C20:1 C23:2 0.50 C23:30.5
    9 N30:1 N34:2 1.45 N35:31.89

    Overall RMS deviation= 0.6926E+00
```

3. Analyze visual results of the fitting procedure.

Dashed lines between equivalent atoms are displayed by default. **Display Atom Matches** is checked and highlighted in the Match Atoms palette.

From the Match Atoms palette, select **Display Distances.** The distance between equivalent atoms is displayed next to these atom locations in the viewing area.

From the **Match** Atoms palette, select **Display Distances.** The distance between equivalent atoms is displayed next to these atoms in the viewing area. The Match Atoms palette is cleared from the screen.

4. Define torsions for the torsional flexible fit procedure.

From the Molecular Similarity palette, select **Torsions...** The Torsions palette is displayed. Check to see that **Define All Torsions** is displayed. From the Torsions palette, select **Define All Torsions.** The message line displays:

```
No of Torsions=13 Families=8
```

Torsion definitions are reported in the textport.

From the Torsions palette, select **Select Torsions.** The Torsion Selection palette is displayed.

From the Torsion Selection palette, select **Select a Subset of Torsions.** A dialog box containing a list of all defined torsions is displayed. All torsions are selected.

5. Exclude the torsion associated with the double bond in amitriptyline.

Select the option:

```
Include and Exclude all others
```

Deselect the torsion that includes the carbon atoms you marked in Step 3 of the exercise in Managing Structures on page 197.

Select the **OK** button. The dialog box is cleared from the screen.

Display the Torsion Selection palette and select **Finish.** The palette is cleared from the screen.

From the Torsions palette, select **Exit Torsions.** The palette is cleared from the screen.

6. Perform a torsional flexible fit procedure.

Display the Molecular Similarity palette and select **Torsional Flexible Fit.** Structures are displayed as the fitting procedure is performed. The rms distances between each structure and the target is reported in

the textport, and the overall rms deviation is shown, at the completion of the procedure.

7. Review the results of the fitting procedure in the textport.

Select the **Reset View** dial emulator. The structures are repositioned in the viewing area.

Display the Match Atoms palette. Select the tool **List Atom Matches.** The textport reports the rms distance for each atom pair and the overall rms deviation.

```
No of Matches 9
                      2        3
1   C12:1   C15:2   0.28 C15:3 0.26
2   C13:1   C14:2   0.33 C14:3 0.36
3   C14:1   C13:2   0.30 C13:3 0.33
4   C15:1   C12:2   0.24 C12:3 0.26
5   C23:1   C20:2   0.13 C20:3 0.48
6   C22:1   C21:2   0.11 C21:3 0.56
7   C21:1   C22:2   0.14 C22:3 0.57
8   C20:1   C23:2   0.16 C23:3 0.51
9   N30:1   N34:2   1.29 N35:3 1.89

Overall RMS deviation= 0.6311E+00
```

Comparing the results of the torsional flexible fitting with the results of the rigid body fitting shows the RMS values are the same or lower between quinupramine and imipramine (structure 2) and between quinupramine and amitriptyline *(*structure 3).

8. Setup CHARMm minimization conditions.

Display the **CHARMm** menu and select **Minimization Options**. A dialog box is displayed, permitting a minimization technique to be selected and associated parameters to be specified.

Select the option:

```
Adopted-Basis Newton Raphson
```

Enter the values:

```
Number of Minimization Steps: 200
Coordinate Update Frequency: 5
Energy Gradient Tolerance: 0.010000
Energy Value Tolerance: 0.000000
Initial Step Size: 0.020000
Step Value Tolerance: 0.000000
```

Select the **OK** button. The dialog box is cleared from the screen. The new minimization parameters are established.

9. Perform a Cartesian flexible fit procedure.

From the Molecular Similarity palette, select **Cartesian Flexible Fit.** The cursor changes to a watch during the fitting operation. The message line shows CHARMm status, and the structures are dynamically positioned in the viewing area as the fitting procedure is performed. The procedure is complete when the cursor returns to an arrow.

From the Molecular Similarity palette, select **Save Changes.**

10. In the Textport, review the results of the fitting procedure.

Select the **Reset View** dial emulator. The structures are repositioned in the viewing area.

From the Match Atoms palette, select **List Atom Matches.** The textport reports the rms fit of each atom pair and the overall rms deviation. An example of the data follows:

```
No of Matches 9

                2         3
1   C12:1 C15:2 0.00 C15:30.00
2   C13:1 C14:2 0.00 C14:30.00
3   C14:1 C13:2 0.00 C13:30.00
4   C15:1 C12:2 0.00 C12:30.00
5   C23:1 C20:2 0.00  C20:30.00
6   C22:1 C21:2 0.00  C21:30.00
7   C21:1 C22:2 0.00  C22:30.00
8   C20:1 C23:2 0.00  C23:30.00
9   N30:  N34:2 0.01  N35:30.00

Overall RMS deviation= 0.2852E-02
```

The results of the Cartesian flexible fit show that the rms values are reduced to zero or close to zero.

Performing Volume Fitting

In addition to the visual inspection of overlaid structures, it is possible to calculate the van der Waals volume of structures and display this information in a variety of volume maps.

Three types of volume maps can be displayed:

- Union volume
- Intersection volume
- Volume density

The union volume map displays all van der Waals volumes calculated for every structure. The intersection volume map displays only the area where structures overlap. The volume density map superimposes a grid over the cumulative union volume map and, through the use of color, displays the proportional number of structures occupying a given grid point.

Additional functions, such as making each structure a different color or alternating the display of structures, can help in the analysis of volume maps. Selections used to calculate, display, and compare volume maps are located in the Molecular Similarity palette.

Sample Procedure — Volume Calculations

Complete the following exercise to become familiar with volume calculations and display options.

1. Calculate volumes.

From the Molecular Similarity palette, select **Calculate Volumes.** A dialog box is displayed to permit adjustment of the grid resolution.

Accept the default value:

```
Grid Resolution (angstrom): 0.5
```

The dialog box is cleared from the screen and the cursor changes to a watch during the calculation. The textport reports the results of the calculation.

As the calculation proceeds, the Object Management Table is displayed to provide standard object information on each map display.

2. Display volume maps separately.

Select the density1 **Visible** cell. The cell entry changes from Yes to No. The map showing the total volume of all three structures is removed from the viewing area. The union and intersection maps remain displayed.

Select the union1 **Visible** cell. The cell entry changes from Yes to No. The map showing the van der Waals volumes calculated for every structure is removed from the viewing area. Only the intersection map remains displayed.

3. Determine the structures that have non-overlapping areas.

Display the **Draw** menu and select **Color Atoms**.

From the pull-right **Color Atoms** menu, select C**olor by Molecule**. Each molecule is displayed in a single color.

Rotate the intersection map and locate areas where bonds and atoms extend beyond the map boundaries. To help determine the molecule that has non-overlapping areas, toggle visibility of each atom on and off.

From the Molecule Management Table, select the amit **Display** cell. The cell entry reads No to indicate amitriptyline is not displayed. Examine the display of the remaining molecules and the color map.

From the Molecule Management Table, select the imip **Display** cell. Imipramine is removed from the viewing area. Only quinupramine is still displayed. Examine the structure and map to determine what portions of the molecule extends beyond the map boundaries.

From the Molecule Management Table, select the quinpr **Display** cell. Quinupramine is removed from the viewing area.

Select the amit **Display** cell again. The display of amitriptyline is restored. Examine it for portions of the molecule that extend beyond the map boundaries.

From the Molecule Management Table, select both the quinpr and imip **Display** cells. All structures are restored to the viewing area.

4. **Delete volume maps.**

   From the Object Management Table, select the heading cell for the **Delete** column. All objects are removed from the viewing area and from the table. The table is cleared.

5. **Exit Molecular Similarity.**

   From the Molecular Similarity palette, select **Exit Molecular Similarity.** The Molecular Similarity palette is removed from the screen and the Modeling palette is redisplayed.

6. **Reject the changes made during the fitting procedures.**

   From the Modeling palette, select **Reject Changes.** The changes made to quinpr.msf, imip.msf, and amit.msf are not saved. The coordinates saved after minimization of quinpr.msf, imip.msf, and amit.msf are redisplayed in the viewing area.

7. **Close the MSFs.**

   Display the **File** menu and select **Close**. A dialog box is displayed with options for what file to close.

   Select the **Close All** button between the two scrolling file lists. Amit.msf, quinpr.msf, and imip.msf are moved to the **Close** scrolling list of files.

   Select the **OK** button. The dialog box is removed from the screen and the structures are removed from the viewing area.

Using MSF Bundles for Multiple Conformers

Multiple conformers can be studied in the Molecular Similarity application using MSF bundles. An MSF bundle is a collection of MSFs which are related by the application through their names. All MSFs in a bundle are assumed to have the same covalent structure. Normally, the Molecular Similarity application works through a collection of MSFs, considering each MSF as a separate molecule. MSF bundles are defined to efficiently compare sets of multiple conformers as units.

Use MSF Bundles alters the way atom matching and volume calculations operate in Molecular Similarity**.** Normally, atom matching is specified for all active MSFs. When **Use MSF Bundles** is selected, matched atoms are only specified for the first MSF in each bundle.

For volume calculations, the normal procedure is to calculate an intersection map among all active MSFs. With **Use MSF Bundles,** volume maps are calculated for each MSF, then maps are combined by taking the union of the structures within each bundle. The resulting union maps are used to calculate the intersection between bundles.

Sample Procedure — Using MSF Bundles

Use the following exercise to become familiar with the use of MSF Bundles in volume calculations. This exercise uses the Conformational Search and Analysis applications. If you are not familiar with these, see chapters 3 through 6.

1. Generate six conformations of amitriptyline.

From the **File** menu, select **Open**. A File Librarian dialog box opens. Select amit.msf from the scrolling list, **Replace,** and the **OK** button.

Display the **Applications** menu, and select **Conformational Search**.

From the Conformational Search palette, select **Torsions.** The Torsions palette opens.

From the Torsions palette, select **Define All Torsions**.

Select **Exit Torsions.**

From the Conformational Search palette, select **Setup Search,** then select **Random Sampling.** The Random Sampling Setup dialog box is displayed.

Enter the values:

```
Number of Samples:5
Torsion Angle Window:50
```

Select the options:

```
Calculate CHARMm Energy for each Structure
Minimize each Structure
Display Each Structure.
```

Select the **OK** button.

From the Conformational Search palette, select **Do Search.**

A File Librarian is displayed.

Enter the text:

```
amit.
```

Accept the defaults:

```
Tag prefix: amit
Starting Tag Number: 1
```

Select the **New** button. The conformational search begins. When it is completed, the Message Line reads:

```
Random Search Completed
```

Six different conformations of amit.msf are created.

From the Conformational Search palette, select **Analysis.** The Analysis palette is displayed.

From the Analysis palette, select **Trace.**

The Select Property for Plotting dialog box is displayed.

Accept the default option:

```
Potential Energy
```

Select the **OK** button. A plot of CHARMm potential energy as a function of conformation number is displayed. A series of menus (**File, Edit, Display, Trace Tools**) are aligned across the top of the Trace Plot window.

Display the **Trace Tools** menu. Select the **Select Window** function. All six conformations plotted in the display are selected.

Select the **File** menu in the Plotting window. Select **Quit**. The Plotting window is cleared.

Display the Analysis palette. Select **File Options/Filters.** A dialog box is displayed.

Select the option:

```
Save Selected Conformations
```

Select the **OK** button. The Select the Output File Type dialog box is displayed.

Select the option:

```
msf file(s)
```

Select the **OK button.** Six MSF files are created with names the same as the tags for each conformation (amit.msf:1 through amit.msf:6).

Display the Analysis palette, and select **Exit Analysis.** The Plot and Analysis palettes are cleared.

From the Conformational Search palette, select **Exit Conformational Search.** The Conformational Search palette is cleared.

From the Modeling palette, select **Reject Changes** to reject the current conformation.

2. Generate and store six imipramine conformations.

Open imip.msf and replace the amit.msf conformation displayed in the viewing area.

Repeat the search and analysis procedure just completed for amit.msf. Substitute imip for amit where appropriate.

3. Start Molecular Similarity Calculations.

From the **File** menu, select **Open**. A File Librarian dialog box is displayed.

Open all six amitriptyline and six imipramine MSFs by selecting each file consecutively. The files are named amit^1.msf through amit^6.msf and imip^1 through imip^6.msf.

Select the **Replace** button and then select the **OK** button. All 12 conformations are displayed, stacked on top of one another, in the viewing area.

Display the **Applications** menu. Select **Molecular Similarity**. The Molecular Similarity palette is displayed.

Select **Use MSF Bundles** and **Match Atoms.** The Match Atoms palette is displayed.

Select **Pick Equivalent Atoms.** A single conformation (amit^1) of the target molecule amitriptyline is displayed.

Pick the 12 carbon atoms that are to be matched with atoms in the imipramine structure.

From the Match Atoms palette, select **End Atom Picking**.

Both amitriptyline and imipramine are displayed in the viewing area. The match atoms in amitriptyline are accentuated by colored circles with + marks inside.

Select the corresponding match atoms on imipramine in the same order the match atoms were chosen for amitriptyline. A large cursor is present on the amitriptyline atom that needs a corresponding imipramine match.

When all match atoms have been selected, the atom matches for all 12 conformations are automatically defined.

From the Match Atoms palette, select **Save Atom Matches.** A File Librarian dialog box appears, requesting a name for the .mch file.

Enter the text:

```
bundle
```

Select the **Save** button.

From the Match Atoms palette, select **Exit Match Atoms.**

From the Molecule Similarity palette, select **Rigid Body Fit to Target.** A fit is performed to fit all molecules to the target molecule.

From the Dial Emulator window, select the **Reset View** dial. The structures are realigned in the window for better viewing.

From the Molecule Similarity palette, select **Calculate Volumes.**

The Set Grid Resolution and Size dialog box is displayed.

Accept the default value:

```
Grid Size: 0.5
```

Select the **OK** Button.

An intersection volume map between union maps is calculated and displayed. For MSF bundles only this intersection map is displayed. The numerical volume is reported in the **Volume Maps** column of the Object Management Table and in the textport.

From the Molecular Similarity palette, deselect **Use MSF Bundles**.

Select **Calculate Volumes**. The Set Grid Resolution and Size dialog box is displayed.

Accept the default value:

Intersection Value, Without MSF Bundles

```
Grid Size: 0.5
```

Select the **OK** button.

A volume for each MSF is calculated and overall intersection and union maps are calculated and displayed, along with a density map. The reported volume calculated without MSF bundles is lower than the calculated volume for the MSF Bundles.

Because the intersection map is now the intersection among all the individual MSFs, the result is a lower intersection volume than that calculated between union volumes of each MSF bundle.

Summary

The Molecular Similarity application provides a visual comparison of two or more molecular structures by overlaying these structures in the viewing area. Comparisons can be made between different structures, different conformations of the same structure, and different parts of the same structure.

The only requirement is that each structure must be contained in a unique MSF. The application does not permit use of different generations of the same MSF.

The procedure used in Molecular Similarity to compare structures is divided into four basic steps:

1. Load the structures to be compared.
2. Define atom equivalences in these structures, that is, establish atom matches.
3. Perform a fitting operation.
4. Analyze the results.

Each structure is identified by the name of its corresponding MSF. One structure is identified as the target or fixed structure. All other structures are working or moving structures. Although only one structure can be

designated as the target at a given time, the target definition can be changed from within the application.

The Molecular Similarity application makes no assumptions about the equivalency of atoms on different structures. Equivalency is defined through user input. When atom equivalencies are defined, they can be used for any fitting operation.

Fitting operations employ either a rigid or flexible method. If a rigid method is used, working structures are translated and rotated to obtain an optimum fit with the target structure. If a flexible fitting method is used, conformations of the working structures are changed to obtain an optimum fit.

The Molecular Similarity palette provides selections to rotate and translate selected structures, control the display of structures, change target structure definitions, and compare molecules. Other palette selections are used to manage the files that are generated during fitting and comparison operations.

The Molecule Management Table is used to change the activity and display of structures.

The Match Atoms procedure links equivalent or near equivalent atoms on one or more working molecules to the corresponding atom in the target structure. Atom equivalences are always defined in pairs. When you use multiple working structures, equivalent atoms are individually paired with the target atom, not collectively grouped with each other. With matches established, relationships between target and working atoms are defined, permitting sophisticated structural comparisons.

With two structure involved in a matching procedure, there are two methods available to establish atom matches:

- Alternating atom picks of equivalent atoms between target and working structures.
- Selecting all the target molecule atoms first, then selecting the corresponding atoms on the working molecule.

When more than two structures are involved in atom matching, a modification of the second matching method is used.

In addition to the visual inspection of overlaid structures, it is also possible to calculate the van der Waals volume of structures and display this information in a variety of volume maps. Three types of volume maps can be displayed: union volume, intersection volume, and volume density.

Normally, the Molecular Similarity application works through a collection of MSFs, considering each MSF as a separate molecule. Alternatively, multiple conformers can be studied in the Molecular Similarity application using MSF Bundles. An MSF bundle is a collection of MSFs that are related by the application through their names. All MSFs in a bundle are assumed to have the same covalent structure. MSF bundles are defined to efficiently compare sets of multiple conformers as units.

8. Using Tables

This chapter describes the use of the Tables utility. QUANTA applications use tables for displaying data and allowing you to manipulate the data. Read this chapter for specific information on how to use these tables.

The Tables utility is used by the following QUANTA applications: Molecular Modeling and Molecular Similarity (Molecule Management Table), Maps (Maps Management Table), Graphical Objects (Object Management Table), Atom Property Editor (Edit Residue Table) and Constraint Options (Constraints Table). In addition, the utility is used by applications that may be purchased with QUANTA.

Tables present data appropriate for the application in which they are displayed. A table window opens when data to be presented in tabular form is generated.

Using Objects Specific to Tables

The Tables utility is composed of the following objects:

- Table — Consists of a collection of cells organized as an array of rows and columns. You can control the font used to render cell data, the background color, foreground color, and table title. You also can control the way grid lines are displayed.
- Column — A vertical slice through the table, one cell wide. Columns have a default data type and can have user-assignable labels. Cells of a different type can be inserted into a column. You can control column width, font, format, and foreground color, overriding table-wide defaults.
- Row — A horizontal slice of the table, one cell high. Rows can have user-assignable labels. You can control row height and foreground color, overriding table and column-wide defaults. Unlike columns, rows have no data type.

- Cell — An individual data item at the intersection of a specific row and column. Table cells contain user-assignable and application-assignable data consisting of numbers, character strings, file names, and Boolean values. You can control cell font, format, and foreground color, overriding table, column, and row-wide defaults.
- Group — A named set of rows and/or columns. Tables can contain multiple groups, and different groups can share rows and columns with other groups.
- Derivation — An equation that can be associated with an entire column. Derived columns are automatically recalculated when a cell on which the derivation depends changes.
- Table Selection — You select rows, columns, and cells in a table where subsequent actions will be performed. These selections are called table selections.
- Current Row, Column, and Cell — Tables track a current row, column, and cell, using them to control cell editing as well as to anchor operations like row and column insertion. The current cell defines the current row and the current column. If the current cell is being displayed, a rectangular border encloses the cell. By default, the current cell is always selected. Similarly, every table selection always contains a current cell.

Operating Within the Table Window

The Table window consists of a title bar, table display, message line, and scroll bars. In some tables, there is also a menu bar. You move through the table using scroll bars or cursor keys.

Table operations fall into two categories: operations over an entire table, and operations over a portion of the table. Examples of operators that affect the entire table include **Open, Save, Save As, Revert,** and **Sort**. No explicit table selection is required to perform these operations.

To apply operators of the second type, table selections are required to identify a region of a table. Examples of these operations include **Formatting, Fonts, Alignment, Color, Cut, Copy, Paste,** and **Clear**.

Table selections are made using the mouse and keyboard. Visually, table selections appear in the highlight color.

The following section describes mouse and keyboard actions permitted in the table window.

Mouse Actions

When the cursor is within the table window, the following mouse actions are supported:

- Cell Click — Single clicking within a cell causes the cell cursor to move to the cell. The cell is the selected and current cell. The row containing this cell is the current row, and the column containing the cell is the current column.
- Column Click — Single clicking within a cell heading a column selects the column. Visually, the entire column (except for the current cell) is displayed in the highlight color. The selected column is the current column, and the first visible cell in the column is the current cell. The row containing this cell is the current row.
- Row Click — Single clicking within a cell to the far left of a row selects the row. Visually, the entire row (except for the current cell) is displayed in the highlight color. The selected row is the current row, and the first visible cell in the row is the current cell. The column containing this cell is the current column.
- Shift Click — If the shift key is held down when a row or column is selected, the selection is added to the selection set.
- Cell Double Click — Double clicking on a cell causes it to become selected and current. The command associated with the cell is sent to the command dispatcher. Double clicking on a cell that has no command associated with it is treated as a cell click operation. In either case, the cell becomes the current cell.
- Column Double Click — Double clicking on a column causes the column to be selected and the command associated with the column to be sent to the command dispatcher. Double clicking on a column that has no command associated with it is treated as a column click operation.
- Row Double Click — Double clicking on a row causes the row to be selected and the command associated with the row to be sent to the command dispatcher. Double clicking on a row that has no command associated with it is treated as a row click operation.

- Ctrl Click — If a row or column has been double-clicked to select it, you can then move the mouse to a new row or column and select it by clicking while you hold down the control key. This action causes all rows or columns between the selected two to be selected also. This is an rapid method for selecting a range of rows or columns.

Keyboard Actions

The following keyboard actions are supported:

- Cursor keys — Using the left, right, up, and down keys, you can move the cell cursor to a new cell. The new cell is the selected and current cell. If the cursor is moved beyond the bottom or right edge of the table, a new row or column is added if **Expand Table** in the **Edit** menu has been enabled.
- Tab — Pressing the tab key causes the cursor to advance to the right by one cell.
- Return — Pressing the return key causes the cursor to move down one cell.
- Escape — Pressing the escape key cancels cell editing and returns an edited cell to its previous value.
- Text keys — If a cell is not read-only, you may type new information into the current cell.

Using Table Menus

The **File, Edit, Format,** and **Data** menus are common to tables that include a menu bar. In addition, some tables may include a **Table Tools** menu that contains selections specific to the table that is displayed. Menus may contain some or all of the selections described below.

The **File** menu provides selections to save, export, and graph table data. You also quit the Tables utility through this menu. Table 37 lists and briefly describes the selections.

The **Edit** menu provides facilities to modify the contents of cells and the general organization of the table. Users can insert and delete rows and columns, clear table cells, and select table components. Most of the items

Table 37. Tables Utility File Menu

Selection	Description
Save	Writes the contents of the table into the file named in the title bar of the Table window. Selecting **Save** always causes the application to write a table file even if there have been no changes to the displayed table. If no file name has been specified for the table, the **Save As** operation is performed to prompt for a filename.
Save As	Writes the contents of the table into a new file. The standard Save As dialog box is displayed to prompt for the name of the file. All modifications are written into the disk file.
Export	Allows tables to be written to files using a variety of formats. From the Export dialog box, you select the file to be used for export, the file format (ASCII, DIF), and a table group. The group designation controls the rows and columns exported. The **Options** button allows you to customize the selected file export method. If no options exist for a method, the **Options** button is disabled.
Quit	Terminates the Table utility and removes the table from the screen. If the table has been modified, the Save As dialog box is displayed, allowing you to preserve table changes.

on the **Edit** menu operate on the current table selection. Table 38 lists and briefly describes selections.

Table 38. Tables Utility Edit Menu

Selection	Description
Select Group	Allows you to select table cells based on the contents of a named group. The operation displays a dialog box that contains a scrolling list of the groups defined within the table. When a group is selected, the cells within the group are redrawn using the highlight color. The table window is scrolled, as appropriate, to show the upper left cell of the group. This cell is the new current cell. If there are no groups defined, **Select Group** is disabled.
Select All	Selects all cells in the table.
Insert Row	Inserts a new row before the row containing the current cell. The number of rows selected prior to selecting **Insert Row** determines the number of rows that are added. Cells in the new rows are initialized to null and appear empty. The table is reorganized by this operation (new rows are inserted before old ones). New rows are always added before the row containing the current cell.

Table 38. Tables Utility Edit Menu (Continued)

Selection	Description
Insert Column	Inserts a new column before the column containing the current cell. The number of columns selected prior to selecting **Insert Column** determines the number of columns that are added. The cells in the new columns are initialized to null and appear empty. New columns are assigned the default column data type, which can be modified using the Column Properties dialog box. New columns are always added before the column containing the current cell.
Delete Row	Deletes the selected rows and data from the table. Rows below those being deleted are moved up.
Delete Column	Deletes the selected columns and data from the table. Columns next to those being deleted are moved to the left.
Label	Displays a dialog box through which you can modify row and column labels and units. Rows and columns can have alphanumeric labels that can be displayed as well as used to reference rows and columns for various operations. Columns can have an optional Units field for display purposes only. If more than one row (or column) is selected, then the entered label (and units) is applied to all rows (columns). Labels and units can contain letters, numbers, spaces, punctuation characters, and any other special characters supported by the base display. Carriage returns can also be inserted using the UNIX special character convention "\n".
Expand Table	Controls whether new rows and columns can be added to the table. If **Table Expands** is on (checked), then the **Insert Row** and **Insert Column** menu items are available. New rows and columns are created if you attempt to go beyond the current bounds of the table. If **Table Expands** is off, then **Insert Row** and **Insert Column** are disabled, and the cell cursor moves to the next line if you attempt to go beyond the bounds of the table.

The **Format** menu controls the appearance of the data displayed in a table as well as attributes of the table display itself. You can specify the format of cell data and can control the font and alignment used to render numbers and text, the row, column, and cell coloring, and the appearance of the grid lines drawn between cells. Selections in this menu also control the visibility of table components such as row and column labels and the table title. Table 39 lists and briefly describes selections.

The **Data** menu provides facilities to manipulate, organize, and find data stored in tables. You can create and delete groups, go to specific cells or to a group, search for data within a table, sort rows and columns, and perform calculations. Table 42 lists and briefly describes selections.

Table 39. Tables Utility Format Menu

Selection	Description
Numeric	Displays the available numeric and Boolean formatting options, and allows users to set the format of numeric columns and cells. The format you choose is applied to all columns and cells in the current selections. See Table 40 for available numeric and Boolean formatting options
Molecule	Displays the available molecule formatting options, and allows users to set the format of columns and cells that contain molecules. The format you choose is applied to all columns and cells in the current selection. See Table 41 for formatting options.
Data Type	Modifies the data type of a set of columns or cells.
Font	Selects the font family, size, and style used to render textual and numeric table data, column and row labels, and the table title. The font families supported are Times Roman, Helvetica, Courier, and Symbol. The sizes supported are 8. 10, 12, 14, 18, and 24 points. The styles supported are bold, italic, and underline.
Alignment	Specifies the alignment of textual and numeric data inside table cells.
Width/Height	Changes the display width and height of table rows and columns as well as the width and height of the column labels and row labels. In order for these fields to be active, the table selection must contain complete rows or complete columns. If it contains a simple cell range, the fields are disabled.
Color	Selectively colors entire rows, columns, and/or cells. The color value entered in the dialog box is applied to the current table selections. The color value must be between 1 and 14.
Grid	Controls the appearance of the grid lines drawn between table cells. It is not possible to control grid lines on a per cell, per row, or column basis.
Display	Controls the visibility of various table components such as row and column numbers and labels, the table title, and the edit window. When a component is disabled, it is removed from the display, and the table cell display is expanded to fill the vacant space.

Table 40. Numeric and Boolean Format Options

Format	Description
0	Displays numbers as integers.
0.00	Displays numbers as floating point.
0.00E+00	Displays numbers using scientific notation.
True/False	Displays zero and FALSE values as False, all others as True.
Yes/No	Displays zero and FALSE values as No, all others as Yes.

Table 41. Molecule Display Options

Format	Description
Structure	Displays a pictograph of the structure.
Formula	Displays a molecular formula such as $C_9H_7ClO_2$.
File Name	Displays the name of the base file that contains the structure.
Chemical Name	Displays the chemical name of the structure.

Table 42. Tables Utility Data Menu

Selection	Description
Groups	Creates, manipulates, and deletes table groups, and collections of related rows and columns designated by the user. Groups can be used to limit sorts and queries and to view a subset of a table. They can be created manually through the **Groups** menu item, through a query, or through an application.
Restore View	Causes the table display to show the entire table rather than a specific group.
Find	Allows the table to be queried by row or by column. Relational operators can be specified to perform comparison operations. Options are listed in Table 43. If you select **Case Sensitive** in the box, character case is considered for string comparisons. If **View Results** is selected, the rows and columns that meet the query are displayed, and the **Restore View** menu item is activated. The results of a query can be saved as a group
Sort	Reorganizes table rows and columns by performing multi-level sorts on table columns or rows. All data in the table is reorganized.
Derivation	Derives an equation to be associated with a column. The equation is used to fill the column with data. Derivations are simple mathematical equations that can include math operators, math functions, and subroutine calls. Derivations can reference other table columns either by name or index. They are evaluated by selecting **Recalculate**. The derivation is checked for correct syntax and applied to the column. The table is redisplayed to show the results. If the derivation can not be parsed, an error message is displayed, and the user is returned to the Edit Derivation dialog box. It is not possible to store an incorrect derivation in a column. Supported binary math operators, unary math operators, trigonometric functions, scientific constants, table functions, and 2D molecular functions are listed in Table 44.
Recalculate	Recalculates all derived columns. Recalculate must be selected for derived columns and cells to be updated.

Table 43. Relational Operators for Find

Option	Description
=	Equality
~	Approximate equality using user-defined tolerance value
!=	Inequality (not equals)
>	Greater than
>=	Greater than or equal
<	Less than
<=	Less than or equal
in	Query is contained within a value (text comparison)
!in	Query is not contained within a value (text comparison)

Table 44. Math Operators for Table Equations

Operators	Description
Binary Math Operators	
+	Addition
-	Subtraction
*	Multiplication
/	Division
%	Modulus (also “mod”)
^	Exponentiation
Unary Math Operators	
-	Negation (also “neg”)
abs	Absolute value
sqrt	Square root
log10	Log base 10
exp	Exponentiation (inverse of log)
ln	Natural log (also “ln”)
rand	Random number
Trigonometric Functions	
sin	Sine (radians)
cos	Cosine (radians)
tan	Tangent (radians)
asin	Arc-sine (radians)
acos	Arc-cosine (radians)
atan	Arc-tangent (radians)

Table 44. Math Operators for Table Equations (Continued)

Operators	Description
sind	Sine (degrees)
cosd	Cosine (degrees)
tand	Tangent (degrees)
asind	Arc-sine (degrees)
acosd	Arc-cosine (degrees)
atan	Arc-tangent (degrees)
Scientific Constraints	
pi	Pi (3.1415926)
k	Boltzmann constant (1.98713 E-3 kcal/mol deg)
h	Planck constant (9.53709 E-14 kcal sec/mol)
r	Gas constant (0.08206 liter atm/mol deg)
e	Base of the natural logarithm (2.71828)
n	Avogadro's number (6.02217 E+23 particles/mole)
Table Functions	
row#	Current row index
col#	Current column index
2D Molecular Functions	
mw	Molecular Weight

Summary

QUANTA applications use tables for data display and manipulation. The Tables utility is used by the following QUANTA applications: Molecular Modeling and Molecular Similarity (Molecule Management Table), Maps (Maps Management Table), Graphical Objects (Object Management Table), Atom Property Editor (Edit Residue Table) and Constraint Options (Constraints Table). In addition, the utility is used by applications that may be purchased with QUANTA. Tables present data appropriate for the application in which they are displayed.

A Table window opens when an application generates data to be displayed in tabular form. The Table window consists of a title bar, table display, message line, and scroll bars. In some tables, there is also a menu bar. The **File, Edit, Format,** and **Data** menus are common to tables that include a menu bar. In addition, some tables may include an application-

specific menu that contains selections specific to the table that is displayed.

Table operations fall into two categories: operations that impact an entire table, and operations that impact only a portion of the table. Table selections are made using the mouse and keyboard. Visually, table selections are rendered in the QUANTA highlight color.

9. Generating Graphs

This chapter describes the QUANTA Graph Plotting Utility that provides tools and capabilities for displaying and manipulating data in graphic form. Read this chapter if you are running applications where you want use graphs.

Each application with graphic capabilities opens a graph plotting window to display graph data compiled by the application. In the graph plotting window, you have a number of operations and tools available to manipulate the appearance of the graph and to interact with the application's program requests.

Alternatively, when you select **XY Graphs** from the **File** menu, you have access to the Graph Editor**.** Although this application does not create graphic data, it allows you to select graph files that have previously been created by other applications, load the files for display, edit the appearance of the graphs, and save the changes. A base set of tools is provided using a menu bar and pulldown menus in a plot window. This set of tools is what makes up the Graph Editor.

When the Graph Plotting utility is invoked by an application other than the Graph Editor, only a subset of the functions are available. A number of items in the pulldown menus are dimmed to prevent changing application data. If you want to do more editing, save the graph using **Save As**... in the **File** menu, then open it again in the Graph Editor by selecting **XY Graphs** also in the **File** menu.

Using Objects Specific to Graph Plotting

The graph plotting interface includes the following objects:

- Plot window — the window where graphs are plotted displayed, and manipulated. It is a standard rectangular window with window manager function similar to all other QUANTA windows. It is composed of a menu bar, a viewing area, and a message line. The viewing area

includes a plot title, one or more graphs views, and a number of legends.

In QUANTA the window is created when an application invokes the Graph Plotting Utility or when you request the Graph Editor.

- Graph View — Area within the plot window where a graph is displayed. Each graph view contains one graph and has a border that frames the graph view area. The border may or may not be displayed. There may be more than one graph view in a plot window.
- Graph — Elements include datasets, axes, annotations, a graph title, and a border.
- Dataset Object — Defines all coordinates of the data to be graphed. Each object can be displayed in more than one graph view.
- Axes Object — Elements include the x axis and the y axis. Each axis includes a leg and its label, tic marks, and tic labels.
- Annotation Object — Text string or a symbol displayed in a plot window in the graph view or legend view.
- Legend View — Area within the plot window where a legend is displayed. Each legend view contains one legend and has a border that frames the legend view area. The border may or may not be displayed. There may be more than one legend view in a plot window.
- Legend — Area made up of annotation objects, a legend title, and a border.
- Title Object — Text string that is specifically tied to a plot window, a graph view, or a legend view.
- Border Object — Line that outlines the edge of a plot window, a graph view, or a legend view.
- Graph File — Archived definition of all the objects displayed in the plot window. The native format file (*filename*.graph) for the Graph Editor. Maintains all the data necessary to reproduce the same image the next time the file is opened.
- XY File — Two-column file created by QUANTA applications (*filename*.plt). Used in creating 2D plots.
- CHARMm Energy File — *Filename*.ENE created by CHARMm to store energy values calculated during a dynamics calculation.

- User-Specified Format File — File that specifies columns the user wants to use for x,y data.
- PostScript File — File that defines objects displayed in the plot window in PostScript format.

Using Graph Plotting Menus

File, Edit, and **Display** menus are displayed on the menu bar regardless of the access point to the Graph Plotting Utility.

The **File** menu is made up of selections that provide access to graph data in files and allow storage of graph data in different formats. Table 45 lists and briefly describes the menu selections.

Table 45 . Graph File Menu

Selection	Description
Open	Opens a list of existing graph (*filename*.graph) files that can be selected, loaded, and displayed. If a graph is currently displayed, the system will provide the user with a chance to save the current environment to a graph file.
Close	Removes the currently displayed graphs from the graph plotting environment. If the displayed graphs have been changed since they were loaded, the Save Changes First dialog box is displayed.
Save	Writes the currently displayed graphs to a file. The filename used will be the last specified filename. If there is none, the **Save As** function is invoked to prompt the user for a filename. The .graph extension is automatically added to the filename. The displayed graphs remain active and displayed.

Table 45 . Graph File Menu (Continued)

Selection	Description
Save As...	Writes the currently displayed graphs to a new file. The standard file access dialog box is displayed to allow specification of the filename. The .graph extension is automatically added to the filename. The displayed graphs remain active and displayed.
Revert	Rereads the graph file. All changes made since the file was last read are removed. A confirmation dialog box will be displayed before the action is performed.
Import	Allows you to select a file with a foreign format to be loaded and displayed. A number of file formats are supported: x y format (.plt), CHARMm Energy file format (.ENE), and user-specified column format.
Export	Allows creation of files that can be output to printers that accept PostScript format. Three file formats are supported: image PostScript (.ps), inverted image PostScript (.ps) and x,y format (.plt).
Quit	Terminates graph plotting. The plot window is removed from the screen. If graphs are displayed and have been changed since they were loaded, the Save Changes First dialog box is displayed.

The **Edit** menu consists of functions that allow you to change and add objects displayed in the plot window. Objects to be edited must be selected before an **Edit** selection is applied. If no object is selected, a new object will be added automatically to the graph.

To select an object or a specific location in a graph, move the mouse so the cursor is over the object or location, and click the left mouse button. To select a text string, position the cursor at the lower-left corner of the text string.

Selections are displayed as follows:

- A selected object is displayed in the highlight color (purple).
- A selected location is denoted by a small crosshair, also displayed in the highlight color.
- Selected data sets are not highlighted. If a mouse click is performed over a section of the data set and a location crosshair symbol is not displayed, the data set is selected.

Table 46 lists and briefly describes the menu selections.

The **Display** menu consists of selections that manipulate the display of objects and viewing of graphs. Table 47 lists and briefly describes the menu selections

Table 46 . Graph Edit Menu

Selection	Description
Title...	Modifies selected titles or adds a new title if no title is selected.
Border...	Modifies selected borders or adds a new border if no border is selected.
Data Set...	Modifies the appearance of selected data sets. If no data set is selected, you are prompted in the message line to select one. When selection is complete, one of four dialog boxes (depending on the dataset type) is displayed.
Text...	Modifies selected annotation text or adds a new text object if no object is selected.
Symbol...	Modifies selected annotation symbol or adds a new symbol object if no object is selected.
Axes...	Modifies the color and visibility of selected axes or adds a new axis if none is selected. If you are adding a new axis object, entering OK in the initial dialog box causes each of the axis legs, the axis tic marks, and the axis tic labels dialog boxes to be displayed.
Axes Legs...	Modifies the legs and labels of the x and y axes. If no axis is selected, you are prompted to select an axis object. When selection is complete, a dialog box is displayed. When this dialog box is approved, a similar dialog box for the y axis is displayed. The only difference between the x and y axis dialog box is the reference to the legs. For the y axis these would be ‘leg at left’, ‘leg at right’, and ‘both legs’.
Axis Tic Marks...	Modifies tic marks of the x axis and y axis.
Axis Tic Labels	Modifies the tic labels of the x and y axes. If no axis is selected, you are prompted to select one. When selection is complete, a dialog box is displayed. When this dialog box is approved, a similar dialog box for the other axis is displayed. The only difference between the x and y axis dialog boxes is the reference to the legs. for the y axis these would be leg at left, leg at right, and both legs.
Delete Object	Removes selected objects from the display.

Depending on the point of access to the Graph Plotting utility, an additional menu may be accessed from the menu bar. This menu consists of tools for manipulating the display of specific types of graphs. For example, if you are plotting data from a dynamics calculation on a trace plot, the additional menu will be the **Trace Tools** menu. The tools in this menu are specific for adjusting data display for a trace plot.

For a number of examples and exercises that use the Graph Plotting utility, see Chapter 6, *Creating 2D Plots*. Other sections of Chapter 6 also contain examples and exercises that use this utility.

Table 47 . Graph Display Menu

Selection	Description
Display All	Makes all invisible objects visible.
Display Handles	Toggles on and off the display of text strings that identify the plot window, graphs, and legends. When the display is turned on, the text string that identifies the plot window is displayed in the upper-left corner of the window. The text string that identifies each graph is displayed in the lower left corner of each graph's view. The text string that identifies each legend is displayed in the lower-left corner of each legend's view.
Echo Location	Toggles on and off the display of the cursor location. When echo is turned on and the cursor is within a graph area, the X and Y location is displayed in the plot window's message line.
Select Cursor Type...	Displays a dialog box with choices for the appearance of the cursor.
Zoom In	Extends the current graph to expand a small range of the data (80% of what is viewed) into the graph's viewing area. You are prompted for the center of the zoom area. Clicking the left mouse button on a location in a graph view causes the view to be centered around the selected location.
Zoom Out	Extends the current graph to include a larger range of the data around a selected location in the graph's viewing area. You are prompted for the center of the zoom area. Clicking the left mouse button on a location in a graph view causes the view to be centered around the selected location and decreased in range.
Zoom Window	Prompts the user to select two points within a graph's viewing area to define the rectangular range of data to be viewed within that area. The two points are selected by holding down the left mouse button over the first location, sliding the mouse to the second location, and releasing the button. When the second point is selected, the viewing area is updated to display the new range.
Reset View	Resets the graph to display the original data.

Summary

The QUANTA Graph Plotting utility provides tools and capabilities for displaying and manipulating data in graphic form. Each application with graphic capabilities opens a plotting window to graphically display data compiled by the application. In the graph plotting window, a number of operations and tools are available to manipulate the appearance of the graph and to interact with the application's program requests.

When you select **XY Graphs** from the **File** menu, you have access to the Graph Editor and the full range of utility capabilities. When the Graph Plotting utility is invoked by an application other than the Graph Editor, only a subset of the functions are available.

File, Edit, and **Display** menus are displayed on the menu bar regardless of the access point to the Graph Plotting utility. Depending on the point of access to the Graph Plotting utility, an additional menu may be accessed from the menu bar. This menu consists of tools for manipulating the display of specific types of graphs.

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