

Tips for Using Reduce & Probe on the command-line

This is a brief introduction to off-line use of the programs Reduce (to add and optimize hydrogens) and Probe (to generate contact dots), in order to analyze the all-atom contacts either within a protein or between two molecules. They are easiest used on Linux or Mac OSX operating systems, although also possible on Windows or on other Unix systems (Probe does not run on MacOS9 or earlier). After downloading the programs, rename or alias them to "probe", "reduce", or "prekin", so the remote update commands will find them. To get help with format and parameters, just type "probe -h", etc.

Note that you can use any file names you like, but .pdb and .kin extensions help you keep track of file types. Reduce, Probe and Prekin all need PDB-format input files. Mage (or a text editor) runs on kinemage files. KiNG runs typically on kinemage files, but you can "import" a PDB file into KiNG's Molikin feature. On some systems, PDB file drag-and-drop onto Mage or KiNG will start up Prekin or Molikin.

Reduce, to add H atoms, is most easily done on the MolProbity web service at <http://kinemage.biochem.duke.edu/> and then downloading the resulting modified PDB file. It is possible to download the program Reduce and run it locally, but there is usually no reason to do that unless you are processing hundreds of files.

Next, you would either import the PDB file into KiNG using the Molikin dialog choices and save the resulting kinemage, or else run Prekin to get a kinemage of the structure, including the hydrogens with the command line:

```
prekin -lots 1xyzH.pdb > 1xyzHdot.kin
```

While the example here uses the command line interface with Prekin, a graphical interface is also available and is accessed via standard application start-up procedure for your computer. See the discussion for Probe for command line access on PC Windows or Mac OSX.

After creating the kinemage describing the molecular model, run Probe to generate contact dots for the protein and append on the end of the kinemage file, with this command line:

```
probe 1xyzH.pdb >> 1xyzHdot.kin
```

Above command is actually a short cut for:

```
probe -self "alta" 1xyzH.pdb >> 1xyzHdot.kin  
(the ">>" appends the command output to the target file.)
```

Interface Contacts

MolProbity has a section dedicated to producing all-atom contact kinemages for various types of molecular interfaces. Once you have added hydrogens to your structure, the main page will have an option called "Visualize interface contacts". On that page, you check boxes to pick the "source" pattern for one side of the contact and the "target" pattern for the other, and choose from a list of options for output type.

Probe on the command-line is even more versatile and can be used to generate contacts dots for many additional kinds of atom selections and many more output types. (To see the options, type "probe -h".) For example:

To get contacts between two helices in chain a of 1xyz, use the specific residue ranges:

```
probe -both "chaina 12-27" "chaina 59-73" 1xyzH.pdb >> 1xyzHdot.kin
```

To get just the H-bond and clash contacts between a ligand (non-water "het group") and protein, use:

```
probe -both -radius0.0 "protein alta" "het" 1xyzH.pdb >> 1xyzHdot.kin
```

If you are using all-atom contacts in your class project and want to do something more complex than the MolProbity options, first consult probe -h and then consult the TA or instructor.

Probe is available only from the command line. Users of "GUI-based" operating systems, may not be familiar with accessing the command line, so as a reminder:

PC Windows

To get a terminal window allowing command line input, select "Start", then "Programs", then "Accessories", then "Command Prompt". Some perhaps useful commands:

```
dir -- file listing of directory contents
cd directory-name -- change directory to that named
cd .. -- go up one level in heirarchy
```

To avoid setting up paths or typing long pathway names in commands, put the programs and the working PDB and .kin files in one directory.

Mac OSX

Use either an xterm window from X11 (installation described here) or the Terminal application found in the Utilities subdirectory of Applications. The app can be started by "double-clicking" its icon from the Finder window. Some perhaps useful commands:

```
ls -- file listing of directory contents
cd directory-name -- change directory to that named
cd .. -- go up one level in heirarchy
```

Finally, once you have appended your all-atom contacts by one method or another, look at the structure and the contact dots in KiNG (or Mage):

```
king 1xyzHdot.kin
```

The analysis tools of KiNG or Mage allow for an in-depth analysis of contacts.

To study a mutation or a sidechain conformational change in KiNG, choose Tools -> Structural biology -> Sidechain rotator, browse to find the pdbH file, then middle-click on an atom in the working residue. [If mutating, first use the Sidechain mutator tool, then switch to Sidechain rotator.] You will get dial widgets to rotate angles in the new, idealized sidechain, plus a list of good rotamers to choose by clicking. In the small model-manager window, choose "update Probe dots" to interactively update the all-atom contacts. Then try out rotamers and bond rotations.

To study a mutation or a sidechain conformational change in Mage, pick an atom in the residue, go to "remote update" under "Tools" and ask Prekin to mutate the residue. If actually mutating, then edit the amino acid name in the command line it produces; accept. You will get sliders to rotate angles in the new, idealized sidechain, and hypertext rotamers at the end of the text window. Choose "remote update" again, and ask Probe to interactively update the all-atom contacts. Then try out rotamers and bond rotations.

[Remember to put the pdb file in the same directory where you're working, or else you must edit the command line to find it.]