Tips for Using Reduce & Probe

This is a brief introduction to using 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 the Unix or Linux machines, although also possible on PCs or Mac OSX (Probe does not run on MacOS9 or earlier). To get help with format and parameters, just type the name of the program. 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; only Mage (or a text editor) runs on kinemage files.

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. On Unix or Linux computers, it is possible to download the program Reduce and run it locally. The command to locally run Reduce on a pdb file (e.g. 1xyz.pdb) is:

```
reduce - build 1xyz.pdb > 1xyzH.pdb
```

That will add all the hydrogens and optimize rotatable ones in the context of entire local H-bond networks, including flipping Asn, Gln, and His groups where needed. It generates a new PDB file including H-atoms, and comments in the header.

Next, you would 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 short for:

```
probe -self "alta" 1xyzH.pdb >> 1xyzH.kin
```

(the ">>" appends the command output to the target file.)
Probe is versatile and can be used to generate contacts dots for many pairs. For example:
To get contacts for a subunit interface, use:

```
probe -both "chaina" "chainb" 1xyzH.pdb >> 1xyzHdot.kin
```

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

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

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, look at the structure and the contact dots in Mage:

`mage 1xyzHdot.kin`

The analysis tools of Mage allows for an in-depth analysis of contacts.

To study a mutation or a sidechain conformational change, 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 the new, idealized sidechain, and hypertext rotamers at the end of the text window. Choose "remote update" again, and ask Probe to interactively updatee the all-atom contacts. Then try out rotamers and bond rotations.