Internet On-Ramp

An Introduction to Molecular Visualization: Seeing in Stereo with RasMol

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Just as protein and nucleic acid sequences are collected in databases accessible on the Internet, so too are three-dimensional structures from X-ray crystallography and nuclear magnetic resonance studies. To appreciate the insights this data provide into macromolecular function, we need to visualize it structurally. Programs that translate mathematical coordinate values into interactive images on a computer screen can help us understand the relative positions and conformations of active, reactive or interactive sites. And besides that, molecules are really pretty.

Downloading the Software

This article focuses on RasMol (RAster MOlecules), a well-established molecular visualization program that runs on personal computers (1). Its development is currently supported by Glaxo-Wellcome Research and Development (Greenford, England, UK), and it can be downloaded at no cost from their Web site (<http://www.glaxowellcome.co.uk/netscape/software/>). More information and links, as well as a reference manual, can be found on the RasMol Home Page at <http://www.umass.edu/microbio/rasmol>. It is available for many platforms, including Windows®, Macintosh®, Unix and even the Acorn Archimedes.

RasMol can run on older computers, including 286-based IBM compatibles, or 680x0 Macintoshes, but on a newer Pentium® or Power Macintosh, it can really spin molecules around. Here we will demonstrate some useful features and tricks that can be done with Version 2.6b2, which allows stereo viewing. This is a "beta" version, so it is technically still in testing phase. While there are still a few bugs in the features that distinguish Version 2.6 from Version 2.5 (i.e., stereo), the program is quite useable.

Obtaining Structure Files

Molecular coordinates are archived at the Protein Data Bank (PDB) of Brookhaven National Laboratory at <http://www.pdb.bnl.gov/cgi-bin/browse>. A simplified interface is found at <http://molbio.info.nih.gov/cgi-bin/pdb>. The most basic way to use this data is to save a PDB structure file as text on your hard drive, then open it from within the RasMol program. Alternatively, your Web browser can be configured to automatically launch RasMol as a "helper application" whenever you download a file of the appropriate type. In Netscape, go to the options menu, choose "general preferences" and select the "helpers" tab. You need to create a new application MIME type with sub-type "x-rasmol". Set it to work with the extension "pdb" and to launch the RasMol application.

Setting the Viewing Variables

A variety of display modes can be chosen from the RasMol menu bar. For example, the molecule can be represented as "spacefilled" spheres, "wireframes" or "ribbons". Other options let you display the secondary structures or chains of a protein in different colors. The "specular" option (from the Latin specularis, meaning "of a mirror") adds (virtual) reflections of light from the molecule's surface.

Many additional commands and options can be typed in the command line window (This window may not be obvious; look

news.group.news

News.group.news goes to cyberspace and back to gather Net news for you. We emphasize practical, methods-related issues that are discussed in many newsgroups and also feature issues of general interest to biological scientists. But be warned! On this page of *BioTechniques*, we don't review it — we just report it!

To avoid contaminating RNase-free solutions while adjusting the pH, netters on bionet.molbio.methds-reagents recommend testing single drops on pH paper until you are within one unit of the desired value, then testing small aliquots with the electrode until the correct pH is obtained.

Would you like to know the theoretical **isoelectric point (pI)** and **molecular weight** of a particular protein? Enter the amino acid sequence or SWISS-PROT accession number at http://exPASy.hcuge.ch/ch2d/pi_tool.html and the values will be computed for you.

Do unyielding copyeditors at your favorite journal insist that you convert your rpm's to g's? Try this: $g = 1.12r \text{ (rpm/1000)}^2$ where r = radius of the rotor in mm.

Primer Primer. One of the best ways to avoid problems resulting from primer dimer formation during PCR is to carefully design primers that are unlikely to dimerize, according to netters on the METHODS newsgroup.

Do you find that you can't seem to remember the Kozak consensus sequence? See Kozak, M. 1981. Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. *Nucleic Acids Res.* 9:5233–5253. (Do you find that you can't seem to get to the library? It's AGXXAUGG.)

BioCitations

If you're still making up your X-gal in DMF instead of DMSO, you should check out Edwards and Taylor. 1993. Substitution of DMSO for DMF as a solvent for X-gal. *BioTechniques* 14:234 and Karlinsey and Hughes. 1993. A convenient and safe method for storing X-gal solutions, *BioTechniques* 15:292.

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for a "RasMol Command ..." button on your task bar in Windows 95® or a desktop icon in Windows 3.x. The command line window should be open behind the display window on a Macintosh.) For example, the command *set specpower n* (where n is a number between 1 and 100) changes the shininess of the molecule's surface. The command *select hydrophobic* selects all hydrophobic amino acids (aa), and the command *select (within(4.0,75-80)) and (not 75-80)* picks all atoms within the specified distance of aa 75–80, but not aa 75–80 themselves. The command *color blue* colors currently selected atoms blue. Information about commands can be found under "help" on the menu bar or in the RasMol manual.

Stereo Viewing

Clicking on "stereo" under "options" from the menu bar causes two similar pictures of the molecule to be displayed side by side. If the molecule appears off-center, it can be brought back to the middle of the viewing area by typing in the command *translate x -30*. Depending on the molecule, you might use a number from -25 to -35.

There are two basic methods for stereo viewing without special glasses: cross-eyed viewing and parallel viewing. Both rely on altering the normal relationship between the convergence of your eyes and the focal distance. Information on stereo viewing and tutorials to help you learn the methods can be found at <http://www.vision3d.com/3views.html>. By default, RasMol's "stereo" menu option gives you stereoscopic pairs set up for cross-eyed viewing, which means your right eye should see the left image, while your left eye sees the right image. Many people (including these authors) find the parallel method easier. To generate stereo pairs for parallel viewing, enter *stereo -8* on the command line. Again, the parameter -8 is an approximation; some people may be more comfortable with -4 or -10.

For parallel viewing, it is particularly important that the images not be set too far apart; you can change the size and shape of the display window by dragging the lower right corner with the mouse cursor. For parallel viewing, unfocus your eyes—imagine you are looking at something very far away—and allow the images to overlap. If you find one method of viewing is too difficult or causes too much eye strain, try the other. If you get the molecule in focus and it looks "inside out", your eyes are focused for the other viewing method, so

Erratum: The Look@Me collaboration software featured in the February 1997 **Internet On-Ramp** issue is really available from <http://www.farallon.com/www/look/ldownload.html>, not from wherever we said it was. Another Uniform Resource Locator (URL) in that article "Telecollaboration Software I: Reach Out and Show Someone" contained a slash-to-period transversion, which we leave as an exercise for the reader.

"set stereo" appropriately. Figure 1 should be viewed by the parallel method.

Command Scripts

RasMol commands can be stored in a file and run as a batch. Once you have your molecule displayed the way you like it, use the command *write script figure.txt* to automatically create a script file (in this case named figure.txt). Then, the current display configuration can be recreated by running the script with the command *script figure.txt*. Script files can be modified using a word processor, and saved as plain ASCII (American Standard Code for Information Interchange) text.

Exporting Images

RasMol can export images in several formats, including Windows bitmap (BMP, which can be labeled and edited with the Windows Paint program), and vectored postscript (for high-resolution printing). Note that vectored postscript works for some display modes, like ball-and-stick and spacefill, but not for others, like ribbons and cartoon. Also, it does not produce stereo images. This is, after all, a beta test version of the software.

Images can also be exported in Compuserve graphics interchange format (GIF) format, to use on a Web page. We have put several examples, including an animated stereo molecular movie, on our Web page at <http://128.120.169.130/Fairclough/Btx.html>.

The Internet Spirit

Another noteworthy thing about RasMol is that the source code is freely available, so other people can make modified versions or use parts of it in their own programs. For example, there is a University of California, Berkeley version of RasMol (you can find the link from the RasMol home page above) that allows you to load more than one molecule at a time, which is potentially useful for visualizing molecular interactions. The authors of the Netscape plug-in "Chemscape Chime" have also used some RasMol code to produce a remarkable piece of software for use with Web pages. This open-minded sharing of code exemplifies what many people feel is the true spirit of the Internet. These and other freely available molecular visualization programs are briefly reviewed at <http://www.umass.edu/microbio/rasmol/othersof.htm>, and a more comprehensive list of molecular modeling software is available at http://cmm.info.nih.gov/modeling/software_list/sw_index_document.html.

REFERENCES

1. Sayle, R.A. and E.J. Milner-White. 1995. RasMol: Biomolecular graphics for all. *Trends Biochem. Sci.* 20:374-376.
2. Basus, V.J., G. Song and E. Hawrot. 1993. NMR solution structure of an alpha-bungarotoxin/nicotinic receptor peptide complex. *Biochemistry* 32:12290-12298.

Figure 1. NMR structure of a snake neurotoxin, α -bungarotoxin, bound to a peptide from a nicotinic acetylcholine receptor (Reference 2; PDB ID code "1abt"). The peptide is shown in a modified ball-and-stick representation, and the amino acids from the toxin that interact with the peptide are shown in spacefill. The remainder of the toxin molecule is shown in "cartoon" format, with beta sheets depicted as broad arrows.